



Original Paper

## Changing Consumption Patterns of Bamboo shoots: A Case Study of Traditional Food-Related Knowledge Systems

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**Abstract**— Bamboo shoots form an integral part of the local diet of the Northeastern states of India. Fermented shoots have been consumed by tribal communities since ancient times. However, with increasing globalization and fast-food consumption, the traditional knowledge and skills of processing bamboo shoots seem to be changing. This paper highlights the changes in processing ways over the years in the younger generation. Data were collected using an unstructured interview schedule and observation through home visits from *Adi* women of four villages namely Balek, Sibut, Runne, and Mirbuk in Pasighat Block of Arunachal Pradesh. It was observed that some newer practices of processing bamboo shoots had been adopted while ignoring the traditional practices which emphasized eliminating toxicity in bamboo shoots.

**Keywords**— Bamboo shoots, traditional knowledge, globalization

### I. INTRODUCTION

Northeastern India is one of the richest regions in India, not only for its plant diversity but also in terms of cultural, social, and linguistic variability conserved by tribal people. These regions constitute a treasure of indigenous knowledge systems pertaining to agriculture, food, medicines, and natural resources. Among these resources, wild plants have been selected by rural women as the food staple in the local diet [1].

Bamboo shoots are one of the non-timber forest products (NTFPs) chosen by the tribal people in their diets to maintain food and nutritional security. Bamboos are a group of giant arborescent grasses and belong to the family *Poaceae* and sub-family *Bambuseae*. More than 1,250 species belonging to 75 genera have been reported to be distributed worldwide, out of which 125 species have been grown in India spreading over an area of 9.57 million hectares. This has made India the richest bamboo resource, after China. The tender shoots have ample food potential in countries like China, Japan, and Thailand. The tender shoots of a few species are consumed either as vegetables or in curries or as pickles in fermented form by various tribes since ancient times. The method of processing shoots varies across regions and tribes. Bamboo shoots are nutritionally and medicinally rich [2][12]. This has been acknowledged in recent studies demonstrating a positive correlation between the

consumption of bamboo shoots and the prevention of cholesterol and diabetes [3][4].

The consumption of traditional foods by tribes of the Northeastern region is virtually connected to socio-cultural aspects, spiritual well-being, and health. The new generation has undergone a rapid change in their diets over the past 30 years due to the intervention of modern crop varieties, fast-moving life, and the trend towards consuming commercially processed foods. The transformation has taken place from traditional food rich in nutrients to a more generic processed diet. Changing diet patterns, farming systems, and fast foods through globalization has resulted in many metabolic syndromes, heart diseases, diabetes, stroke, cancer, etc. [5][11]. As an attempt to address these adverse impacts, this paper tries to focus on the adoption of newer practices of processing bamboo shoots by the younger generation and erosion of the traditional knowledge system led by the older generation.

### II. METHODOLOGY

#### A. Study Area

Arunachal Pradesh is situated between 26°28' and 29°30' North latitudes and 97°30' and 97°30' East Longitudes covering an area of 83,743 km<sup>2</sup>. Rainfall varies from 1000 mm in higher reaches to 5,750 mm in the foot-hill areas, spread over 8-9 months, except during the drier days in the winter. The population of Arunachal is 1,091,117 according to the 2001 census and is scattered over 12 towns and 3649 villages. The State has the lowest density of 13 persons per km<sup>2</sup>. The knowledge and skills of consuming and preparing bamboo shoots were studied among the *Adi* women of different age groups in randomly selected villages. The study was conducted in July when the bamboo shoot was abundant. The study was divided into two phases.

#### B. The Selection of Villages and Research Subjects

In the first phase, the data on different types of preparation and consumption pattern of bamboo shoots used by *Adi* women was collected purposively in the East Siang district of Arunachal Pradesh. In the second stage, Pasighat Block in Arunachal Pradesh was selected randomly. A list of villages was obtained

from the Block Development Office, and four villages namely, Balek, Sibut, Runne, and Mirbuk were selected randomly for the study. A list of *Adi* women who collected bamboo shoots from their *jhum* land (slash and burn agriculture) and community forest was prepared with the help of village *Gaon Burha* (customary chief). From that list, 30 women were chosen from each village, and thus a total of 120 women from four villages were sampled randomly. Out of these 120 women, 30 women were young (16-35 yrs), 50 were middle-aged (36-45 yrs) and 60 were old (46 to 75 years) group. Prior consent was obtained from the women included in the study and their respective village level customary chief (*Gaon Burha*) to use their information for publication.

### C. Data Collection

An unstructured interview with open-ended questions was employed to garner the data from sampled population. The questions made were designed to capture the knowledge and skills of the women. Before the final application, the interview guideline was tested in a pilot study with 30 *Adi* women in non-sampled villages to refine and validate the types of questions (for qualitative and quantitative data). Data on the knowledge of food processing was collected on a four-point continuum of efficiency: ‘full knowledge’ ‘partial knowledge’, ‘limited knowledge’ and ‘no knowledge’ [6,7]. A score of 3 was assigned for ‘full knowledge’, while 2, 1, and 0 were assigned to ‘partial knowledge’, ‘limited knowledge’, and ‘no knowledge’ respectively. The skill was also tested by observing the actual food processing to determine the efficiency. Before recording the final data, the women were asked to collect bamboo shoots and prepare the required recipe in front of the researchers.

### D. Statistical Analysis

The significance of knowledge variation was tested by applying the ‘Z’ test.

## III. RESULTS AND DISCUSSION

### A. Foods prepared from a bamboo shoot and their ethnomedicinal usage

It was observed that the bamboo shoot was used in the boiled form, and the frequency of using fresh bamboo shoots was found relatively low amongst *Adi* women. The women selected new emerging shoots from a bamboo garden to peel out the bark and then chop the slices for preparing *ekung* (fermented bamboo shoots) and *eup* (dried bamboo shoot) for further use (Fig. 1). These slices were separated from selected emerging new bamboo shoot (Fig. 2) and kept in a bamboo basket after being wrapped with *ekkam* (*Phyrium pubenerve*) leaves. After 2-3 days, this pack of slices was transferred into a plastic bucket where it would be left for over 10-15 days. After this period, the bamboo shoot would be ready to consume (Fig 3). The second category of bamboo-based food consumed at a large scale was called ‘*eup*’. Green slices of bamboo shoots were prepared as mentioned for *ekung* and dried in the sun. This powder was then packed in a poly bag and stored for use throughout the year. *Eup* was used in variety of ethnobotanicals harvested from community forests and *jhum* land (Fig 4). Depending on the food habits, locations, and types of food resources available *ekung* and *eup* were mixed with variety of indigenous vegetarian and non-vegetarian foods (Table 1). The market demand for *eup*

was relatively lower than *ekung*, though it was easier to store *eup* than *ekung*.

TABLE I. TYPES OF FOODS AND MIXTURE OF EKUNG AND EUP ADDITION

Name of foods	Mixture of <i>ekung/eup</i>	Percentage*
Leafy vegetables	<i>Ekung</i> and or <i>eup</i> both	89.8
Pork	<i>Eup</i> only	78.5
Mithun ( <i>Bosfrontalis</i> ) meat	<i>Eup</i> only	92.4
Rice granules	<i>Ekung</i> and or <i>eup</i>	71.2
Local fishes	<i>Eup</i> and or <i>ekung</i>	68.7

\* Multiple percentages



Fig. 1. Bamboo shoots collected to be peeled and sliced



Fig. 2. Sliced and un-sliced bamboo shoots ready for fermentation



Fig. 3. Fermented bamboo shoots in a green bamboo cylinder ready to serve





Fig. 4. Boiled eup mixed with ethnobotanicals ready to consume

The fermented preparation was also found to have some ethnomedicinal usage and was given for constipation and indigestion when mixed with green leaves of *ongin* (*Clerodendrum colebrookianum*). About 30 ml extract of 'ekung' was mixed with a local fresh green leafy vegetable called 'ongin' or 'pakum saag' and local ingredients like ginger, chilly, salt, and local onion called 'dilap'. This mixture was boiled for about 15-20 minutes and given for constipation and indigestion. This food was given twice a day generally for lunch and dinner regularly for 3 to 4 days. It helped to cure constipation, and indigestion and also controlled high blood pressure.

#### B. The Loss of traditional knowledge system

It was observed that in the abovementioned process a quicker pre-processing technique had been adopted by the people. The preparation of *ekung* through a classical method using green bamboo stem was observed among only 2-3 elder women (80 yrs). Conical bamboo baskets lined with banana (*ekkam*) leaves used traditionally by the tribes had been replaced by plastic jars. Traditionally, a hole was made at the bottom of the basket and a bamboo stick was inserted for draining the sap [8]. Nowadays, people do not make any holes and the water remains accumulated in the jars. Earlier processing techniques during fermentation aided in reducing the cyanide percentage. Cyanide causes several disorders

related to the neural system, miscarriage, abnormal childbirth, and goiter problem. Fresh bamboo shoots contain high cyanogenic glycoside (551mg/kg), followed by thiocyanate (24mg/kg) and glucosinolate (9.57mg/kg), and chronic consumption can lead to hypothyroidism by inhibiting thyroid peroxidase activity (TPO) activity [9]. Due to these problems, aged women still advise pregnant women not to eat any bamboo-based product unless it is rigorously processed. For reducing the cyanide content during processing, 2-3 small holes were made inside the *edung*. During the fermentation, these *edung* are kept near the water stream in such a manner that water touches the bottom of *edung*. In this way, the toxic compound is leached out and it becomes safe for consumption [10]. Nevertheless, it was observed that the practice of leaching out the toxic chemical in the water stream seemed to be fading out in cities with more modern and quicker techniques. Local people followed processing steps like dipping shoots in water, removing the tip, slicing into gratings, etc. These were known to help remove bitterness and toxic compound.

#### C. Knowledge and skills of processing using bamboo shoots across the generations

We managed to measure the knowledge status across the generation to see the rate of knowledge erosion on processing and use of *ekung*. Scores obtained by women of different age groups in the interview on possessing traditional knowledge and skills for processing bamboo shoots are given in Table 2 and Table 3. It was found that older women were found to have significantly higher traditional knowledge status in preparing *ekung* (fermented bamboo shoots) as they scored high in all the parameters defined as the basis for having traditional knowledge. It was observed that there was a significant gap in 'traditional knowledge' among young, middle, and old age *Adi* women leading to diverse preparations of *ekung*. Older women also possessed a greater degree of skills as compared to the young generation with regard to processing, storing, packaging, and marketing skills. These findings revealed a massive erosion in traditional knowledge and skills required for the collection, fermentation, processing, marketing, and use of traditional foods prepared from bamboo shoots.

TABLE II. STATUS OF TRADITIONAL KNOWLEDGE OF ADI WOMEN IN PREPARING EKUNG (FERMENTED BAMBOO SHOOTS)

Particulars	Age groups			'Z' value		
	Old	Middle age	Young	Old vs middle age	Old vs young age	Middle vs young age
Identification of species for preparation of <i>ekung</i>	2.89	2.18	0.78	7.89**	10.24**	8.74**
Identification of appropriate bamboo shoots	2.72	1.75	0.90	6.71**	9.47**	9.87**
Selection of portions for making slices	2.85	1.92	0.68	8.90**	11.87**	6.98**
Selection of utensils for fermenting bamboo shoots	2.69	1.45	0.60	4.57**	12.41**	10.14**
Processing techniques for fermentation	2.75	1.31	0.45	6.80**	13.47**	9.45**
Use method	2.80	1.46	0.58	5.60**	8.97**	6.74**
Precautions to be taken for use: like avoiding malaria, acidity, etc.	2.80	1.10	0.40	6.87**	10.64**	9.84**
Health-related knowledge	2.90	1.21	0.39	8.67**	14.47**	7.80**

\*\* Significant at  $p < 0.01$

TABLE III. SKILLS STATUS IN FERMENTATION AMONG DIFFERENT AGE GROUPS OF ADI WOMEN

Particulars	Age groups			'Z' value		
	Old age	Middle age	Young age	Old vs middle age	Old young age	vs Middle vs young age
Processing techniques for fermentation of <i>ekung</i>	2.87	1.48	0.42	8.87**	10.24**	10.34**
Making mixture with various food resources	2.82	1.61	0.55	9.45**	9.31**	7.98**
Preparation techniques of <i>ekung</i>	2.60	1.34	0.61	7.90**	9.45**	8.84**
Storage method	2.65	1.45	0.58	6.32**	10.32**	6.48**
Package skill	2.70	1.25	0.40	5.50**	7.84**	7.41**
Marketing skill	2.18	1.95	0.63	7.62**	6.78**	5.24**

\*\* Significant at  $p < 0.01$

#### IV. CONCLUSION

Traditional knowledge systems and practices of food preparation engraved in tribal communities have relevance to the recent measures on maintaining food and nutrition security. The preparation of fermented bamboo shoots, which is not only rich in nutrition but also reflects rich traditional knowledge, in the Northeast seems to be vanishing. With the increase in fast food consumption and changing food choices coupled with the fear of nutritional crises, we need to strengthen our existing traditional knowledge system among the youths through educational programs emphasizing the importance of these foods in terms of health, social, cultural, and nutritional security.

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#### ABSTRACT:

Artificial intelligence (AI) can be utilized in numerous exercises in Education Systems, for example, evaluating, in school, reviewing schoolwork and tests for courses can be monotonous work, instructors find that evaluating takes up a lot of time, that could be utilized to cooperate with students, get ready for class, or work on proficient turn of events, educators can robotize reviewing for a wide range of different decision and fill-in-the-clear testing. Computer based intelligence can call attention to places where online courses need to improve, when countless students are found to present an off-base response to a schoolwork task, the framework cautions the instructor and presents future students a modified message that offers indications to the right answer. Students can get extra help from AI guides, while human mentors can offer that machines can't, few coaching programs dependent on man-made reasoning exist and can help students through essential science,

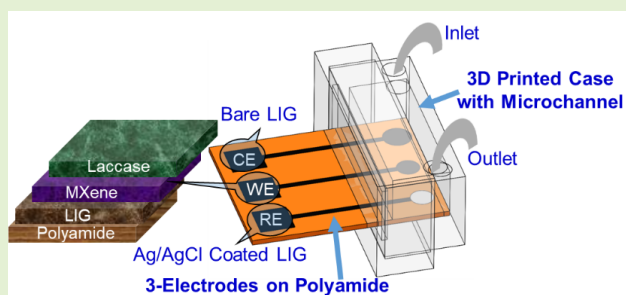
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# Integrated Microfluidic Device With MXene Enhanced Laser-Induced Graphene Bioelectrode for Sensitive and Selective Electroanalytical Detection of Dopamine

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**Abstract**—Microfluidic Electrochemical biosensors present promising means for many critical biosensing applications such as neurotransmitter monitoring, pathogen detection, and molecular diagnostics. However, such biosensors are prone to many challenges like enzyme degradation being the major concern with electrode materials while testing multiple times, hindered detection limit range, low sensitivity due to direct contact of enzyme with the environment, and poor shelf life of the electrodes patterned by conventional techniques. Herein, a microfluidic multi-sensitive biosensor is developed using a layer-by-layer process, incorporating a novel Laccase/MXene/LIG (L-Ti<sub>3</sub>C<sub>2</sub>-G) composite designed for selective detection of dopamine and other biochemicals with applicability in human blood serum and synthetic urine. 2D nano-material, MXene, gave an excellent conductivity, more volumetric capacity, flexibility, surface hydrophilicity, and temperature stability, leading to its use in various applications. It was found that laccase immobilized electrode (L-Ti<sub>3</sub>C<sub>2</sub>-G) showcased a significant electrocatalytic activity towards biomolecules such as uric acid (0.12 V), L-cysteine (0.62 V), xanthine (0.7 V), ascorbic Acid (0.1 V), and dopamine (0.42 V). For dopamine alone, the proposed biosensor exhibits a LOD of 0.47 nM with a linear concentration range of 1 nM – 10 μM and 6.37 mA nM<sup>-1</sup> cm<sup>-2</sup> sensitivity. Moreover, real sample analysis indicated that spiked dopamine can be determined accurately by the electrode with a recovery ratio of more than 97% in synthetic urine and human blood serum samples. The fabricated bioelectrode had high stability and reproducibility with an exceptional selectivity and negligible interference with various biochemicals.

**Index Terms**—Microfluidics, electroanalytical detection, MXene, laser-induced graphene, dopamine, layer by layer.



## I. INTRODUCTION

**D**OPAMINE is a catecholamine neurotransmitter found in almost all species, including humans. Disruptions in dopamine create a role in the process of some severe central nerve illnesses. Thus, monitoring and maintaining required levels of these neurotransmitters are essential not only for interpreting signal routes and nervous system functions but also for diagnosing and treating a wide range of brain-related

diseases [1]. Electroanalytical detection, particularly based on voltammetry, colorimetry, electrochemiluminescence, and amperometric approaches [2], [3] lead to various disadvantages due to numerous aspects [4]. The selectivity of the devices, however, is a crucial issue that must be addressed because of the overlapping oxidation potential of dopamine with numerous different chemicals found in the central nervous system [5].

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Because of their enormous availability in blood serum and urine samples (much higher than dopamine) and similarity to dopamine, xanthine (Xn), L-cysteine (L-C), uric acid (UA), and ascorbic acid (AA) have been identified as the major interfering species [6], [5]. To selectively sense dopamine, bioelectrode materials, including the development of 2D nanomaterials, with the required attributes are needed. However, there are many challenges to overcome, such as interference from other biochemicals and low dopamine peak oxidation signals [7]. When developing electrode surfaces, the



sensitivity and selectivity of electrochemical biosensors might be strengthened by using nanomaterials with superior electrical and large surface area characteristics [8], [9]. Karim *et al.* proposed the sensitive detection of the catechol by layer-by-layer (LBL) assembly of negatively charged poly(sodium 4-styrene sulfonate) with enzyme tyrosinase and positively charged hexadecyltrimethylammonium coated gold nanocubes. Such LBL assembly of nanomaterials or metallic and polyelectrolytes, with positively and negatively charged nanoparticle layers, might be a feasible alternative [10]. Various functional materials and compounds, specially various 2D nanomaterials, have been used to enhance the active surface area of the bioelectrode to improve its performance for multianalyte analysis. However, most of the 2D nanomaterials use randomly structured immobilizing matrices, resulting in poor electrical connections with the current collector of the electrode and low 2D nanomaterial utilization efficiency for immobilization of enzymes [11].

2D metal carbides or nitrides, also called as MXenes ( $\text{Ti}_3\text{C}_2$ ), have suitable morphology for sensing. MXene nanosheets have acquired attention owing to the fact that it can give an effective method for enhancing the porosity of matrix, interfacial coupling, diffusivity of ion, and improved electrode performance, depicting its key role for usage as an immobilization matrix [12], [13]. Recent studies showed that the immobilization with exfoliated MXene electrodes is suitable to obtain a significant distinguishing of oxidation potential for both dopamine and other biochemicals, which could improve the selective sensing of dopamine (increased voltammetric current signals) [14]. Jin X *et al.* reported that to facilitate charge transport and reduce constriction resistance at the interface between MXene and current collector (polyamide sheet), laser-induced graphene (LIG) was used as conductive bridges [15], [16].

In this work, the LBL biosensor consists of an as-synthesized MXene coating with a dense bottom porous graphene layer and a highly porous intricate top matrix network layer. This configuration provides an ideal structure for a highly sensitive effective surface area and perfect entrapment for laccase enzymes (i.e., the active  $\text{Ti}_3\text{C}_2$  network is inextricably linked to the conducting LIG support). Because laccase is sensitive to its environment, an enclosed microfluidic platform was required to maintain the sensitivity of enzymes with dopamine [17]. One of the important parameters is to control the surface properties and spatial arrangement of the functional biomolecules inside a microchannel for designing neurotransmitter diagnostics. Ali *et al.* used the lithography technique for fabricating the microfluidic environment, which is a very expensive technique and a multistep process [18].

In order to resolve his limitation, a simple desktop 3D printer has been utilized here. It is a low-cost method for preparing a 3D printed microfluid case in a polymer substrate. The method has advantages in terms of designing and preparing 3D complicated structures economically and rapidly [19]. Therefore, herein, the LBL biosensor was then encased in a 3D Printed microfluidic enclosure. The sensing properties of MXene are due to its metallic conductive nature, biocompatibility, several adsorption sites, and excellent homogenous

dispersion in the aqueous medium [20]. Following this, a reduced electrical signal to noise ratio, lower detection limit, and improved signal toward dopamine is obtained.

The L- $\text{Ti}_3\text{C}_2$ -G biosensor has conducive biosensing capacity with lower LOD along with a larger linear range due to the following factors. First, a large specific surface area of the MXene matrix potentially enhances the effective surface area of the LIG bioelectrode and its accessibility to enzyme immobilization, resulting in a higher enzyme surface loading amount. Evidently, redox-active sites in the enzymes are effective for immobilization of matrix, and L- $\text{Ti}_3\text{C}_2$ -G and  $\text{Ti}_3\text{C}_2$ -G are conducive for improving the electrochemical performance of the redox-active site in the plane. Nafion aids in the solubility of  $\text{Ti}_3\text{C}_2$ -G composites, ensuring that the MXene is well-dispersed and develops a homogeneous and stable coating on the graphene surface even after long durations. The enhanced activity of laccase in the existence of MXene nanoparticles is associated with the sensing capability of L- $\text{Ti}_3\text{C}_2$ -G. The modified LBL biosensor was used to monitor dopamine concentrations in blood serum and synthetic urine. The peak current changes involved with the surface site-specific electrocatalytic reaction of the substrate dopamine and the enzyme laccase assembled on  $\text{Ti}_3\text{C}_2$ -G lead to the detection of dopamine up to 0.47 nM. Further, the co-existing biochemicals, that are likely to interfere with dopamine, were also tested. It was found that the fabricated device gave a selective response for the various biochemicals at distinct  $E_0$  values. Hence, the fabricated device can be applied for simultaneous sensing of multiple analytes. In addition to this, real-time blood serum and synthetic urine samples were also tested for dopamine sensing that displayed appreciable recovery values.

## II. EXPERIMENTAL SECTION

### A. Chemicals and Materials

Biochemicals, like dopamine (D), xanthine (Xn), L-cysteine (LC), uric acid (UA) and ascorbic acid (AA), buffer salts like sodium phosphate monobasic dehydrate ( $\text{NaH}_2\text{PO}_4$ ) and sodium phosphate dibasic anhydrous ( $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ ), nafion (5% (w/w) in mixed alcohols), ethanol,  $\text{Ti}_3\text{AlC}_2$  powder Isopropyl alcohol (IPA), laccase (EC 1.10.3.2 from *Trametes Versicolor*, 1.34  $\text{U} \cdot \text{mg}^{-1}$ ) were procured from Sigma-Aldrich Co. Ltd. The solution of hydrofluoric acid (HF, 57 wt%) was obtained from Macklin Co. Ltd. Double-distilled water ( $18.2 \text{ M}\Omega \cdot \text{cm}^{-1}$ ) was used throughout the experiment.

### B. Solution and Sample Preparation

0.1 M of phosphate buffer solution (PBS) with pH ranging from 4.0 to 7.0 was prepared. The stock solution of laccase enzyme ( $0.3 \text{ mg} \cdot \text{mL}^{-1}$ ) was made in 0.1 M PBS of 6.0 and was stored at  $2^\circ\text{C}$  till use. For MXene synthesis, Titanium Aluminium Carbide powder ( $\text{Ti}_3\text{AlC}_2$ , 99% purity, Nanoshell LLC, Punjab), Lithium fluoride (SRL India), hydrochloric acid were used as starting materials and without any further purification. 0.20 g of KCl, 5.0 g of NaCl, 1.1 g of  $\text{Na}_2\text{HPO}_4$ , 0.20 g of  $\text{NaH}_2\text{PO}_4$ , and 21 g of urea were dissolved in 500 mL of double distilled water to make synthetic urine

then the pH was calibrated to 3.5 using  $\text{H}_2\text{SO}_4$  (1 M) and blood serum was taken from Medical Center, BITS Pilani Hyderabad campus. A polyamide sheet (PI) of  $254\ \mu\text{m}$ , used as a current collector, was procured from DuPont, India. The LIG conductive electrodes were fabricated using a 30 W  $\text{CO}_2$  Laser (VLS 3.60 from Universal Laser Systems, USA). Scanning Electron Microscope (SEM) and EDX were performed with a Scanning Electron Microscope (SEM) (Apreo from Thermo Fisher Scientific, USA). All the CV and SWV measurements were performed utilizing an electrochemical work-station (SP-150 from Biologic, France) in the 3-electrode arrangement.

### C. Synthesis of MXene ( $\text{Ti}_3\text{C}_2$ ) and Fabrication of Microfluidic L- $\text{Ti}_3\text{C}_2$ -G Biosensor

A 6 M solution was prepared by diluting 4.927 mL of concentrated HF in 2.5 mL DI water and adjusting the final volume to 10 mL by adding more DI water. To prevent an exothermic reaction, about 1 g of  $\text{Ti}_3\text{AlC}_2$  (MAX phase) powder was mixed into the combined solution after the salts were completely dissolved and the resultant suspension was stirred for 24 h slowly at ambient temperature. Consequently, the resultant solution was washed and centrifuged (4000 rpm for 10 min) repeatedly with DI water followed by centrifugation and decanting of the supernatant solution until the pH reached a value of 6. Finally, the obtained slurry was air-dried after filtering and then delaminated in Dimethylsulfoxide (DMSO) using the probe sonication method under inert conditions for 40 min. The obtained stable  $\text{Ti}_3\text{C}_2$  colloidal solution was used for further processing.

A schematic for the LBL synthesis process of bioelectrodes is illustrated in Fig. 1. First, a design with a three-electrode system was created which is similar to the one earlier reported. These three electrodes were engraved on the PI sheet as a current collector using a  $\text{CO}_2$  laser engraver as shown in Fig. 2(a)-(b). One of LIG electrodes with a modified surface was used as a working electrode, a bare LIG electrode as an auxiliary counter electrode, and Ag/AgCl coated LIG electrode as a reference electrode as can be seen in Fig. 2(c).

The L- $\text{Ti}_3\text{C}_2$ -G as a working electrode (shown as final bioelectrode in Fig. 2(d)) was fabricated as follows. First, LIG was synthesized by the optimized laser parameters ( $1.8\ \text{W}$  and  $112.5\ \text{mm}\cdot\text{s}^{-1}$ ) as reported in the previously published work by our group [21]. Herein, the PI sheet was kept in the  $\text{CO}_2$  laser engraver and LIG electrodes were patterned at the positions as designed using the parameters mentioned above. The prepared MXene delaminated solution was coated over LIG as shown in Fig. 2 (e)-(f).  $0.2\ \mu\text{L}$  of Nafion, was used for the formation of the  $\text{Ti}_3\text{C}_2$  layer over the LIG surface.  $5\ \text{mg}$  of laccase enzyme was dispersed in 1 ml of the phosphate buffer solution kept at  $4^\circ\text{C}$ . Later,  $2\ \mu\text{L}$  of the laccase enzyme solution was cast onto the dried  $\text{Ti}_3\text{C}_2$ -G sheet. Thereby, the L- $\text{Ti}_3\text{C}_2$ -G bioelectrode has been fabricated using LBL assembly of a positively charged MXene coated on LIG complexes ( $\text{Ti}_3\text{C}_2$ -G) and a negatively charged laccase enzyme, as shown in Fig. 2 (g) [3].

As shown in Fig. 2 (h), the modified electrode L- $\text{Ti}_3\text{C}_2$ -G was utilized for the oxidation of dopamine as discussed in a

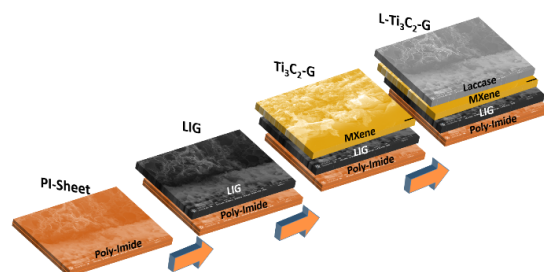


Fig. 1. Schematic representation for LBL Synthesis Process for L- $\text{Ti}_3\text{C}_2$ -G Bio-Electrode.

further section of reaction mechanism. The  $\text{Ti}_3\text{C}_2$ -G electrode (without the laccase enzyme) was synthesized by a similar technique for comparative study. The L- $\text{Ti}_3\text{C}_2$ -G as the working electrode was subsequently enclosed in the microchannel of a microfluidic device fabricated via stereolithography (SLA) based 3D printing technique for establishing a leakage-proof microfluidic environment. The sensing area of the miniaturized device was  $3\ \text{mm} \times 10\ \text{mm}$ . As a proof of concept, for sensitivity and selectivity toward modified bioelectrode, this device was evaluated for different biofluids including untreated blood serum and synthetic urine for detecting dopamine, l-cysteine, uric acid, xanthine, and ascorbic acid. The microfluidic device with integrated bioelectrodes is shown in Fig. 2 (i)-(k). A 3D CAD modeling software (Solid works 2021) was used to design  $5 \times 15\ \text{mm}^2$  rectangular boxes. The SLA 3D printed design was used to create a microfluidic device with an inlet and outlet as shown in Fig. 2 (j) with a sample volume requirement of  $150\ \mu\text{L}$ .

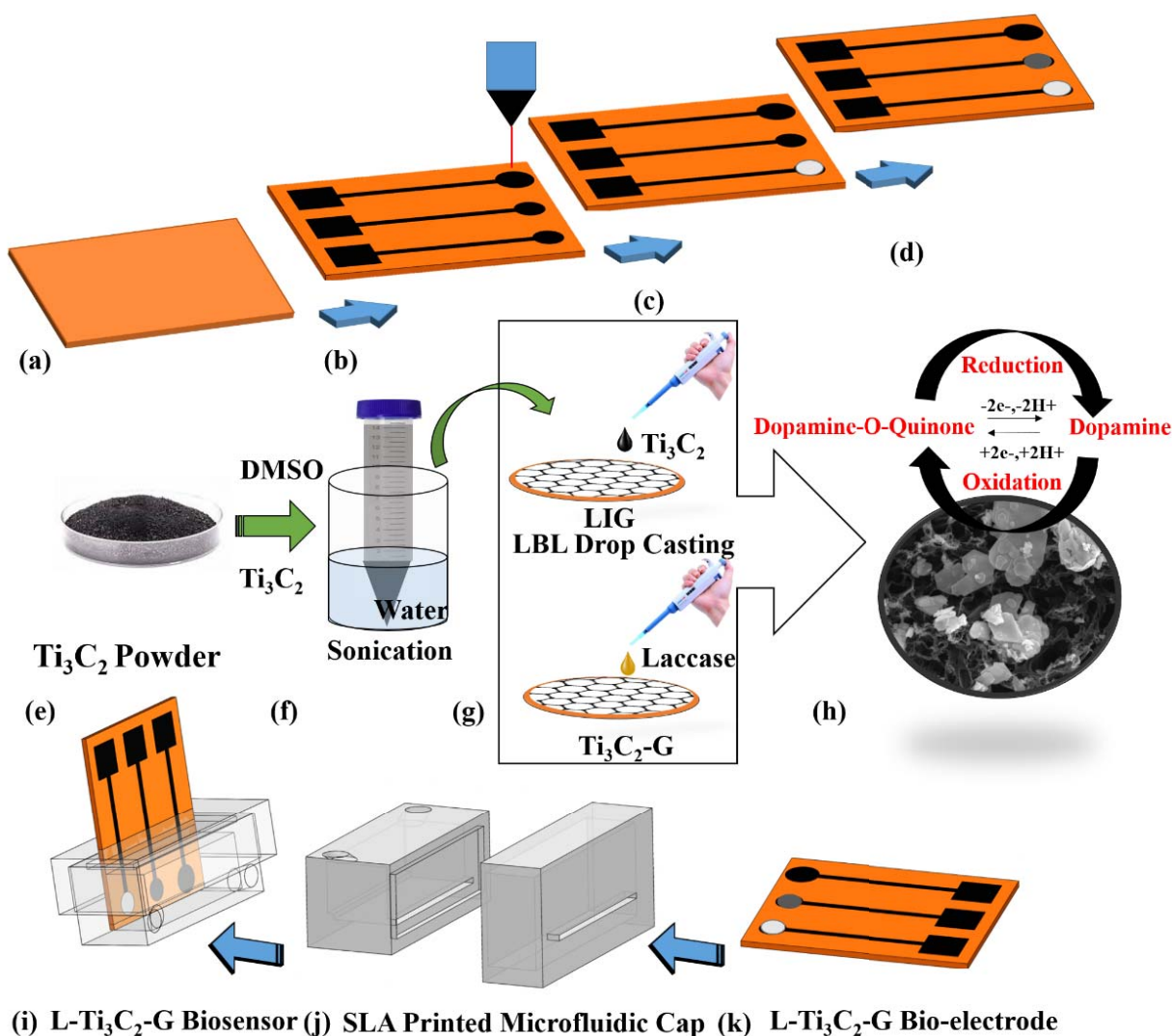
## III. RESULTS AND DISCUSSION

### A. SEM, XRD, UV Spectroscopy and EDX Characterization of L- $\text{Ti}_3\text{C}_2$ -G Bioelectrode

Fig. 3 depicts the morphology of the synthesized and modified surface of MXene ( $\text{Ti}_3\text{C}_2$ ). Layers over honeycomb structure of the LIG, like multilayer nanostructure  $\text{Ti}_3\text{C}_2$ , are observable in synthesized  $\text{Ti}_3\text{C}_2$ -G crystallites (Fig. 3(a)). The unique 2D graphene structure provides conductive bridges to facilitate charge transport and mitigate the constriction resistance at the interface between the  $\text{Ti}_3\text{C}_2$  and the current collector (PI sheet).

Laccase molecules are trapped by  $\text{Ti}_3\text{C}_2$ -G due to its large specific surface area, which accumulated on  $\text{Ti}_3\text{C}_2$  surface functional groups (Fig. 3(c)). Nafion was preloaded onto  $\text{Ti}_3\text{C}_2$ -G to provide matrix stability while also regulating the accuracy and sensitivity of the device for dopamine sensing [17]. As depicted from Fig. 3 (b)-(c), on the LIG electrode, a thin layer of laccase was developed. The high porosity of the uniform film of laccase on  $\text{Ti}_3\text{C}_2$ -G enhanced the improved bioelectrodes specific surface area, resulting in enhanced performance characteristics of fabricated biosensors. Also, such a 2D matrix is suitable for allowing trapped enzymes to sustain their biological properties and for enzymatic molecules to be transported efficiently. The spectra of energy dispersive spectroscopy (EDX) and XRD are shown in Fig. S1 (a-c). Fig S5 depicts for UV spectra the rise in the absorbance for the L- $\text{Ti}_3\text{C}_2$ -G as compared to the L-G. It confirmed the





**Fig. 2.** Schematic illustrates the synthesis of Bioelectrode and fabrication flow of Biosensor (a) Polyamide Sheet (b) Laser engraved Electrodes (c) Ag/AgCl coated Reference Electrode (d) L-Ti<sub>3</sub>C<sub>2</sub>-G Working Electrode. (e) Ti<sub>3</sub>C<sub>2</sub> Powder (f) Sonication (g) LBL Drop Casting (h) L-Ti<sub>3</sub>C<sub>2</sub> Bio-electrode catalytic activity (i) 3-Electrodes on polyamides, (j) 3D printed Microfluidic cap, and (k) Complete prototype of the fabricated biosensor.

enhanced structure of L-Ti<sub>3</sub>C<sub>2</sub>-G for the functional assessment over the surface of LIG. The maximum absorbance of L-Ti<sub>3</sub>C<sub>2</sub>-G lies in the range of the UV visible light.

### B. Reaction Mechanism Dopamine-o-Quinone

Fig. 2 (h) illustrates the oxidation mechanism of dopamine catalyzed using the laccase enzyme. The charge transfer method for the L-Ti<sub>3</sub>C<sub>2</sub>-G electrode is as follows: in the presence of oxygen, the laccase oxidizes dopamine to its particular dopamine-o-quinone form (laccase-catalyzed oxidized product from dopamine), which is subsequently electrochemically reduced back to its dopamine at the electrode surface, transferring electrons to the electrode surfaces and returned as an electrical current anodic peak current ( $I_{pa}$ ) [15].

### C. Electrocatalytic Behavior of the Biosensor

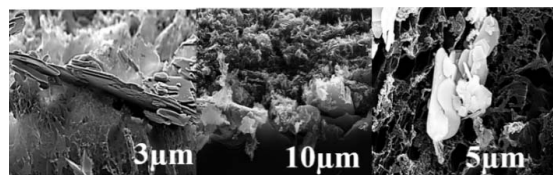
The L-Ti<sub>3</sub>C<sub>2</sub>-G bioelectrode was tested for electrocatalytic activity towards dopamine sensing. Fig. 4(a) is a typical comparative CV of the optimal modified electrode in 1 mM dopamine solution with the blank electrodes for  $n=2$ , at

20 mV.s<sup>-1</sup> in pH 7 PBS. The oxidation and reduction peaks of redox probe potentials were more reversible when L-Ti<sub>3</sub>C<sub>2</sub>-G was used, showing effective electron transport in the presence of laccase immobilized over MXene. In terms of electrocatalysis, the oxidation peak current of dopamine on L-Ti<sub>3</sub>C<sub>2</sub>-G was 2.5 and 3.26 times higher than Ti<sub>3</sub>C<sub>2</sub>-G and L-G, respectively, demonstrating superior catalytic activity of MXene and a higher proportion of catalytically active sites.

This effect can be explained by the fact that the electrostatic interactions of copper ions (present in laccase enzyme) with Ti<sub>3</sub>C<sub>2</sub>-G sheets neutralize the originally negative charges of the MXene surface-terminating groups (-OH, -O, -F), decreasing electrostatic repulsion and resulting in accumulation [13]. Adsorption of laccase ions is easily possible on the titanium molecules because of the erect position of the hydroxyl (-OH) group. The scan rate effect was used to demonstrate the stability of the electrode with that peak potential by sweeping at multiple scan rates from 20 mV.sec<sup>-1</sup> to 150 mV.sec<sup>-1</sup> with peak current at a constant potential as depicted in Fig. 4(b). As can be seen, the oxidation current increases linearly when the scan rate is increased. In the inset of Fig. 4(b), the plot of

**TABLE I**  
COMPARATIVE ASSESSMENT OF ANALYTICAL PERFORMANCES OF ELECTRO-CHEMICAL BIOSENSORS OBTAINED FOR DOPAMINE DETERMINATION BASED ON 2D NANOMATERIALS

#	Electrode	Detection Technique	Limit of Detection ( $\mu\text{M}$ )	Linear Range ( $\mu\text{M}$ )	Reference
1.	MoS <sub>2</sub> /Laccase	CV	0.01	NA	[22]
2.	Graphene Ink	CV	0.1	1000	[23]
3.	GO/PEDOT: PSS	CV	1	1-1000	[24]
4.	PDA/ErGo/GCE	CV	2.3	10-200	[25]
5.	Cu/CuOx/NP	DPV	1.07	0.3-53	[26]
6.	Laccase-EPS-MWCNTs	SWV	0.127	2.99-38.5	[27]
7.	L-Ti <sub>3</sub> C <sub>2</sub> -G	SWV and CV	0.00047	0.001– 10	This work



**Fig. 3.** Scanning Electron Morphology of (a) Ti<sub>3</sub>C<sub>2</sub>-G (b) L-Ti<sub>3</sub>C<sub>2</sub>-G: Immobilization of laccase on Ti<sub>3</sub>C<sub>2</sub>-G (c) L-Ti<sub>3</sub>C<sub>2</sub>-G bioelectrode.

**TABLE II**  
CATHODIC/ANODIC PEAK CURRENT AND CATHODIC/ANODIC PEAK POTENTIAL FOR THE ELECTRODES FOR DOPAMINE

Bioelectrodes	E <sub>pc</sub> (V)	I <sub>pc</sub> (mA)	E <sub>pa</sub> (V)	I <sub>pa</sub> (mA)
L-Ti <sub>3</sub> C <sub>2</sub> -G	-0.05	-3.22	0.11	6.37
Ti <sub>3</sub> C <sub>2</sub> -G	-0.09	-2.14	0.018	2.54
L-G	-1.49	-0.1	0.02	1.95

anodic peak current ( $I_{pa}$ ) vs scan rate ( $\text{mV}\cdot\text{s}^{-1}$ ) was observed to be linear. The obtained regression coefficient was  $R_2 = 0.9972$  from the inset graph, and the diffusion coefficient value was calculated with the Randles Sevcik equation.

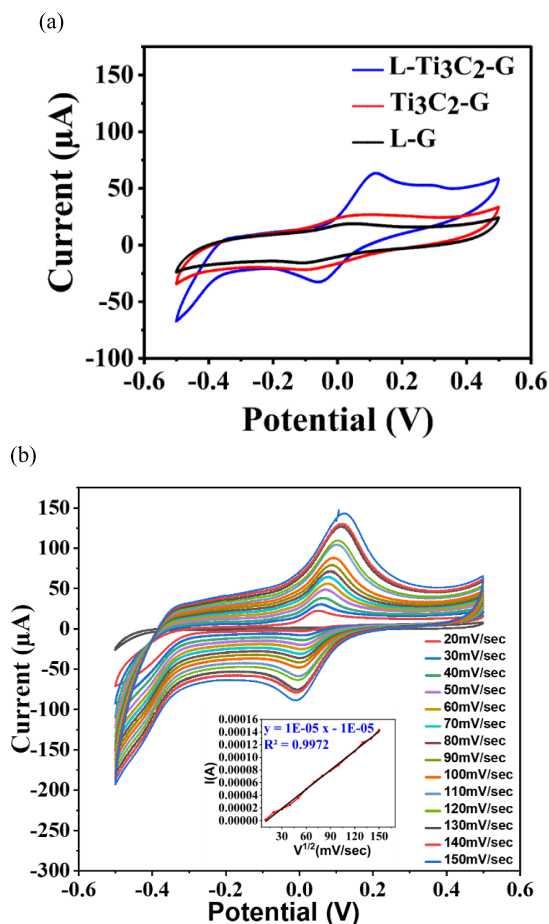
$$I_{pa} = 2.69 \times 10^5 \times n^{\frac{3}{2}} A C D^{\frac{1}{2}} V^{\frac{1}{2}}$$

wherein,  $I_{pa}/V^{(1/2)}$  is the slope obtained from the inset linear plot of Fig. 4(b),  $D$  is the diffusion coefficient =  $4.38 \times 10^{-12} \text{cm}^2\cdot\text{s}^{-1}$ ,  $C$  is the 1 mM concentration of dopamine,  $A$  is an active electrochemical surface area of ( $3 \text{mm}^2$ ),  $n$  = the total number of electrons in the reaction are assumed as 2 based on reported reaction mechanisms,  $I_{pa}$  is the anodic peak current value (mA). This device has shown considerably better sensitivity and selectivity than previously reported dopamine biosensors, as shown in summary Table I. The functional characteristics of the dopamine biosensor are similar to the finest reported results.

According to these CV scans, oxidation peaks, in particular, correspond to the potential 0.11 V, which is amplified, and these peaks had higher currents in the presence of MXene (Ti<sub>3</sub>C<sub>2</sub>) than in the absence of it such as in the case of bare LIG.

Electrochemical characteristics of these bioelectrodes were compared in PBS solution. The cathodic or anodic peak voltage ( $E_{pc}$  or  $E_{pa}$ ) and cathodic or anodic peak currents ( $I_{pc}$  or  $I_{pa}$ ) are given in Table II.

1) *Effect of Varying Concentration:* Square wave voltammetry (SWV) is an excellent approach for the detection of



**Fig. 4.** Cyclic voltammetry of (a) Optimal L-Ti<sub>3</sub>C<sub>2</sub>-G and control electrodes with 1 mM dopamine in pH 7 PBS at 20  $\text{mV}\cdot\text{Sec}^{-1}$  for  $n=2$ . (b) Scan Rate effects of L-Ti<sub>3</sub>C<sub>2</sub>-G in 1 mM dopamine concentration from 20-150  $\text{mV}\cdot\text{sec}^{-1}$  with inset showing correlating trendline calibration graphs of  $I_{pa}$  versus  $V^{1/2}$  oxidation peak currents.

different analytes as it is more precise and sensitive than Cyclic Voltammetry (CV).

As a result, SWV was chosen to examine the analytical capabilities of the L-Ti<sub>3</sub>C<sub>2</sub>-G for dopamine detection in PBS. Fig. 5 depicts SWV profiles of the L-Ti<sub>3</sub>C<sub>2</sub>-G in PBS for different concentrations of dopamine (1 nM – 10  $\mu\text{M}$ ). Fig. S3 shows SWV for L-G for different concentrations of dopamine (1  $\mu\text{M}$  – 10 mM). The current response and its linear relationship with dopamine concentrations are compared and depicted in Table III. Herein, a very low LOD = 0.476 nM and high current sensitivity ( $6.37 \text{mA}\cdot\text{nM}^{-1}\cdot\text{cm}^{-2}$ ) were achieved. The



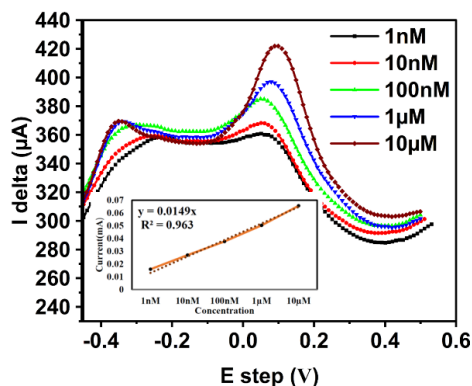


Fig. 5. Square wave voltammogram of (a) L-Ti<sub>3</sub>C<sub>2</sub>-G in PBS with different concentrations of dopamine (1 nM -10 μM).

LOD is determined by the equation  $LOD = 3 \sigma / m$ , where  $\sigma$  denotes standard deviation depicting peak current values for three measurements and  $m$  represents the slope for linear calibration plot shown in Fig. S3 (c)-(d). The LOD of the L-Ti<sub>3</sub>C<sub>2</sub>-G biosensor is lower than the other two fabricated dopamine biosensor devices presented in Table III.

2) *Simultaneous Determination of L-Cysteine, Ascorbic Acid, and Uric Acid at L-Ti<sub>3</sub>C<sub>2</sub>-G Towards Dopamine Detection*: The individual oxidation peak potential for L-Ti<sub>3</sub>C<sub>2</sub>-G is shown by the CV plot depicted in Fig. S4 (a)-(e), each for uric acid, L-cysteine, and ascorbic acid.

The mixture of these analytes was deposited on the bioelectrode, and the individual peak in the SWV plots, shown in Fig. 6, was distinguished by the bioelectrode, demonstrating the simultaneous sensing ability of the device. This plot obtained, for ascorbic acid, L-cysteine, uric acid, and dopamine in a mixture at the L-Ti<sub>3</sub>C<sub>2</sub>-G bioelectrode with 0.1 M PBS solution, define distinctive oxidation peaks for the concentration of 1 M of each analyte at 0.50, 0.12, 0.62, 0.70, 0.10, and 0.42 V respectively [5]. For the sensitive and selective detection of dopamine at lower concentrations values in human blood serum, amplification of dopamine electrochemical response and distinction from quasi surface adhesion of interferences are required. [6]. The increased peak current of 1 mM dopamine after mixing with 1 mM concentrations of uric acid, L-cysteine, and ascorbic acid at L-Ti<sub>3</sub>C<sub>2</sub>-G is shown in Fig. 6. The synergistic impact of MXene nanoparticles adsorbed on the graphene electrode surface can be used as a general technique to resolve the aforementioned flaws while also strengthening analytical performance. In comparison to individual CV, the oxidation potentials of all analytes contained in the SWV were not significantly shifted. These findings corroborate that the L-Ti<sub>3</sub>C<sub>2</sub>-G device is well suited for detecting five distinct analytes simultaneously.

3) *Reproducibility, Anti-Interference (Selectivity), and Stability Properties of Biosensor*: The repeatability of L-Ti<sub>3</sub>C<sub>2</sub>-G was evaluated before biological quantification by evaluating SWV responses of 5 different biosensors prepared independently at 1 mM concentration of dopamine as shown in Fig. 7(a).

The selectivity was determined by observing the impact of the interfering biological samples such as including 1 mM concentration of various biochemicals that co-exist in biological fluids. Selectivity of the developed bioelectrode was

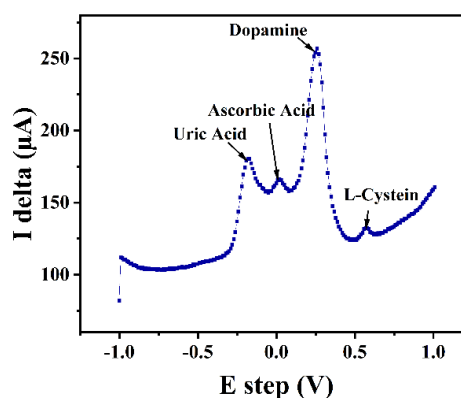


Fig. 6. SWV (I delta Vs E step) for Simultaneous detection analysis of common interferent.

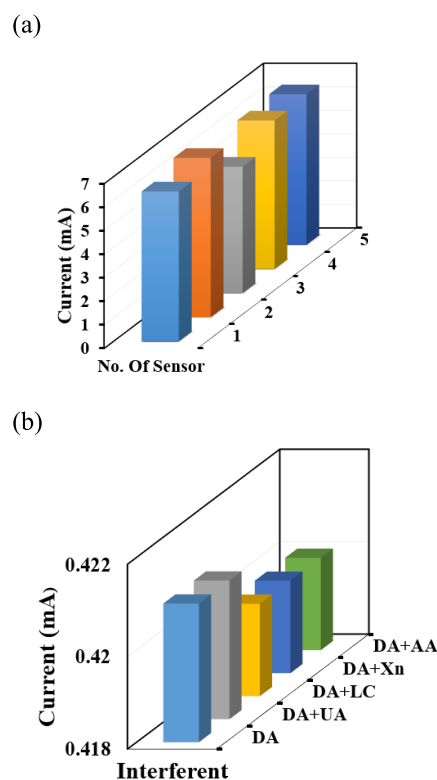


Fig. 7. (a) Reproducibility of L-Ti<sub>3</sub>C<sub>2</sub>-G biosensors for dopamine detection with fixed concentrations of 1mM were used in 0.1 M PBS (b) Anti-interference property of the biosensor (\*Do=Dopamine, UA=uricAcid, LC=L-cysteine, Xn=Xanthine, AA=Ascorbic Acid).

evaluated for dopamine detection in the existence of equal concentrations of certain five common interfering agents such as L-cysteine, ascorbic acid, uric acid. As depicted in Fig. 7 (b), in the presence of strong concentrations of the interfering species, no substantial changes in the current were observed, demonstrating dopamine has superior selectivity over the other analytes. In both serum and synthetic urine samples, the maximum concentration values for the proportion ratios of ascorbic acid and dopamine, uric acid and dopamine were 1:1. Because these species may persist in blood serum and urine, SWV data were analyzed for possible interference with dopamine by uric acid and ascorbic acid. When the SWV responses were compared, it was evident that neither uric acid nor ascorbic acid interfered with the dopamine response depicting

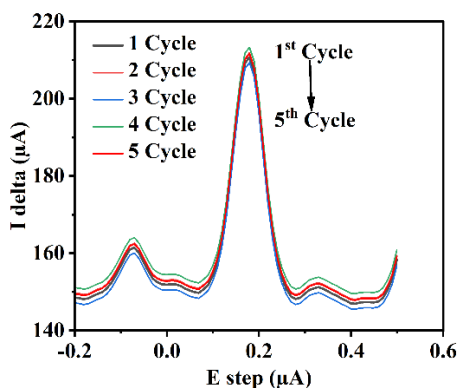


Fig. 8. Stability analysis using SWV plots of L-Ti<sub>3</sub>C<sub>2</sub>-G for continuous scanning of 5 cycles.

TABLE III  
SUMMARY OF LIMIT OF DETECTION OF BIOELECTRODES  
TOWARDS DOPAMINE

Electrode	Limit of Detection for dopamine (Calculated)	Limit of Detection for dopamine (Experimental)	Linear Range
L-Ti <sub>3</sub> C <sub>2</sub> -G	0.476 nM	1 nM	1 nM to 10 $\mu$ M
Ti <sub>3</sub> C <sub>2</sub> -G	1.03 $\mu$ M	1 $\mu$ M	1 $\mu$ M to 10 mM
L-G	1.81 $\mu$ M	1 $\mu$ M	1 $\mu$ M to 10 mM

TABLE IV  
REAL SAMPLE SENSING OF DOPAMINE IN BLOOD SERUM (N = 3)  
AND SYNTHETIC URINE

Analyte	Added (nM)	Found (nM)	Recovery (%)	Average recovery (%)
Dopamine in human blood Serum	100	91.35	91.35	97.08%
	50	48.35	96.7	
	10	10.32	103.2	
Dopamine in Synthetic Urine	100	93.36	93.36	97.81%
	50	51.29	102.58	
	10	9.75	97.5	

the fabricated L-Ti<sub>3</sub>C<sub>2</sub>-G biosensor is more selective towards dopamine [7]. It can be inferred that the electrocatalytic activity of the bioelectrode was efficient for the detection of dopamine with a linear ranging from 1 to 10<sup>6</sup> nM, a LOD of 1.03 nM, excellent stability, repeatability, and reproducibility.

Fig. 8 demonstrates SWV for the use of L-Ti<sub>3</sub>C<sub>2</sub>-G in dopamine more than 5 consecutive times and the response peak current of L-Ti<sub>3</sub>C<sub>2</sub>-G attenuates negligibly. Finally, in this microfluidic biosensor, a continuous and steady reference signal was obtained. Due to the protective influence of the microfluidic cover, the activity of the laccase was intact and the biosensor exhibited zero surface leaching [21].

#### 4) Real Sample Analysis in Blood Serum and Synthetic Urine:

Different interferences were used to evaluate human blood serum. Dopamine detection in human blood serum and synthetic urine was performed to determine the evidence-based effectiveness of the proposed biosensor. The real sample blood serum was first evaluated to determine if there any dopamine

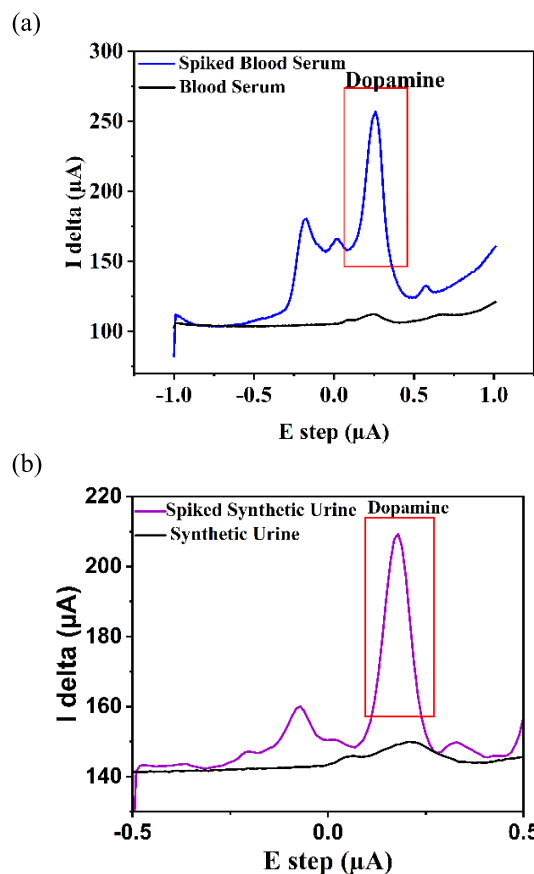


Fig. 9. SWV Graph for (a) Blood serum with and without spikes, and (b) Synthetic urine with and without spikes.

was present, and it was observed that there was only one oxidation peak for dopamine in the blood serum, with a 2% shift in the potential as shown in blood serum and synthetic urine samples of the SWV graph in Fig. 9 (a) and (b) respectively. The samples were analyzed before and after spiking with known concentrations of dopamine standard. For cross-verification, the samples were developed by diluting the blood serum in PBS and spiking with a required concentration of dopamine using the dilution technique. The spiking levels were based on the biological range in the human body with the electroanalytical quantification of dopamine in biological matrices.

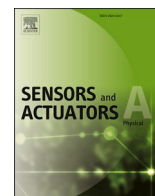
The relative average recovery is presented in Table IV. The dopamine recovery percentage is in the corresponding range of 93.36 %, 102.58 %, and 97.5 % with an average recovery of 97.81 %. The modified bioelectrode was also tested for the real-sample detection of the dopamine content (100 nM) of synthetic urine samples. Before experiments, 100 nM, 50 nM, 10 nM of dopamine were directly diluted 20 times with 0.1 M PBS electrolyte. The results of the recovery tests, which were used to evaluate the reliability and accuracy, are shown in Table IV. The dopamine concentration in the diluted solution was detected to be 93.36 nM, 51.29 nM, 9.75 nM, and the corresponding recoveries were 91.35–103.2 % with an average recovery of 97.08 %. In further, to prove the selectivity in real samples, various other interferences are added in the blood serum and synthetic urine, and no significant response for them is seen as shown in Fig. S6.

#### IV. CONCLUSION

In this work, a novel Laccase-MXene-LIG (L-Ti<sub>3</sub>C<sub>2</sub>-G) bioelectrodes were fabricated on a polyamide sheet by immobilizing laccase over Ti<sub>3</sub>C<sub>2</sub> modified LIG electrode. The resultant bioelectrodes were encased on a 3D printed microfluidic device and the integrated platform was utilized to electroanalytical sense dopamine in clinical and biological samples. When applied in a dopamine biosensor, Ti<sub>3</sub>C<sub>2</sub> proved to be an effective matrix for laccase immobilization, providing a biocompatible microfluidic environment without enzyme leaching from the fabricated biosensor with good analytical properties (sensitivity, anti-interfering, reproducibility, and stability) inferring that device has analytical aspects with potential to be used as point-of-care (PoC) applications. Peak current and dopamine concentration were shown to be linearly related using SWV in the linear range of 1 - 10<sup>3</sup> nM and obtained limit of detection was 0.47 nM. The sensitivity of the electrode was found to be 6.37 mA.nM<sup>-1</sup>.cm<sup>-2</sup>. This biosensor was precisely used to determine dopamine levels in blood serum and synthetic urine and presented good selectivity towards dopamine. Further, simultaneous detection of the existence of ascorbic acid, L-cysteine, and uric acid was carried out to prove the selectivity of the platform. As the MXene configured over LIG provides superior selectivity and specificity with a useful LOD and should be preferable in real-time bio-sensing applications.

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## Rapid, sensitive and specific electrochemical detection of *E. coli* using graphitized mesoporous carbon modified electrodes

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### ABSTRACT

*Escherichia coli* (*E. coli*) is a highly pathogenic bacterium causing infections to the human body primarily from a variety of sources including food intake. As per the World Health Organization (WHO), the mortality and morbidity rate due to *E. coli* is significantly high, approximately 8%. Therefore, a real-time, rapid, user-friendly and interference-free *E. coli* detection system offers great clinical importance. In this work, a highly sensitive, specific, and simple method of electrochemical detection of *E. coli* has been demonstrated. The electrochemical system employs the conventional three-electrode configuration, wherein glassy carbon (GC), chemically modified with graphitized mesoporous carbon (GMC), has been employed as the working electrode. While using platinum and Ag/AgCl as counter and reference electrodes respectively, voltametric techniques were applied to obtain the interference mitigated response of *E. coli* detection. The GCE/GMC electrode showed excellent sensitivity and selective response towards *E. coli* in a linear detection range of  $2.52 \times 10^3$  CFU/mL to  $25.2 \times 10^4$  CFU/mL and 252CFU/mL to 2268CFU/mL respectively with a limit of detection (LOD) of 50.40CFU/mL. Further, the developed system was tested for interference with other bacteria and real samples, such as pond water, tap water, and deionized water. Appreciable recovery values and negligible interference were observed. The prepared electrode demonstrated promising results towards efficient, real-time, and rapid *E. coli* detection.

### 1. Introduction

*Escherichia coli* or *E. coli* bacterium is a rod-shaped bacteria class having a wide presence in the environment [1]. These bacteria are not only present in the environment but are also found i.e. contaminated food and untreated water, and even in the human intestine and animal gut. Although most of the *E. coli* bacteria are good bacteria and are considered to be symbiotic yet a few adversely affect human and animal health [2]. It can cause severe illnesses, such as urinary tract infections, respiratory illness and, bloodstream infections leading to complaints like stomach pain, cramps, fever, diarrhea, etc. [3]. Therefore, detection of the bacterial load is vital for human and animal health and surroundings like water-bodies, etc. [4]. The conventional method of viable cell counting takes more than 48 h for the detection of bacteria [5], whereby the probability of errors and inaccuracy is quite significant. Thus, a simple, rapid approach with high accuracy is required for qualitative and quantitative detection of *E. coli*.

The Electrochemical(EC) technique is the oldest and well-proven technique and offers a wide range of analytical possibilities. Because of quick response time, simple procedure, high sensitivity, and selectivity, EC-based sensing is widely employed for diversified applications. Different EC techniques used for the detection of bacteria include such as square wave anodic stripping voltammetry(SWASV), cyclic voltammetry(CV), and square wave voltammetry (SWV). Various advantages of the voltammetry technique include the possibility to identify the concentration of the *E. coli* without any separation or pre-treatment, high specificity and sensitivity, and amenability to miniaturization. The SWV technique [6] inculcates the features of other techniques and proves to be the most reliable and effective technique for electrochemical sensing of *E. coli* bacteria [7].

The available literature reports have demonstrated a three-electrode-based method for bacterial detection. Viswanathan et. al. reported an immunosensor based on nanocrystal bio conjugates and multi-walled carbon nanotube (MWCNT) over a screen-printed electrode for

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detection of food-borne pathogens such as *E. coli*, campylobacter, and salmonella [8]. Qi et al. suggested a signal amplification technique based on PDA (polydopamine)-mediated nanomaterial modification for the detection of *Desulfohalobacter*, and dopamine was enhanced by adding Fe<sub>3</sub>O<sub>4</sub>@MnO<sub>2</sub> nanoplates [9]. Li et al. described the synthesis of AUT (amine-terminated alkanethiol 11-amino-1-undecanethiol hydrochloride) as an immunosensor on a gold electrode surface and CHIT-MWNTs-SiO<sub>2</sub>@THI was synthesized utilizing LBL (Layer by Layer) assembly for *E. coli*O157:H7, somatic (O), and flagellar (F) detection (H) [10], Zhao et al. developed an immunosensor to detect *Vibrio parahaemolyticus* foodborne pathogens using a screen-printed electrode covered with agarose/Nano-Au membrane and horseradish peroxidase (HRP) [11], Li et al. developed detection system for *E. coli*O157:H7 using Au-SiO<sub>2</sub> embedded on C<sub>60</sub>/Fc/CHI-SH [12]. A summary of various reported works, electrochemical detection of *E. coli* using different electrodes, and voltammetry methods are presented in Table 1 for better illustration.

There are certain research gaps observed in the reported literature, such as smaller limit of detection (LOD), the additional need for immunosensor, longer assay time, complex surface modification, etc. Motivated with this, the present work demonstrates a conventional glassy carbon electrode (GCE) modified with graphitized mesoporous carbon (GMC) (designated as GCE/GMC), used for the detection of *E. coli* in pH 7 phosphate buffer solution (PBS) with a working volume of 5 mL. The GCE/GMC electrode is found to be an effective and reliable method in sensing *E. coli* bacteria. Various important parameters, such as repeatability, sensitivity, and stability, for the present system with *E. coli* bacteria, were perfectly achieved. The demonstrated sensor is easily scalable owing to the short preparation time, simplicity of the electrochemical method, and low preparation cost. Furthermore, interference from other bacteria was also examined such as *Shewanella putrefaciens*, *Lactobacillus*, *Helicobacter pylori*, and *Bacillus anthracis* [15] were considered. Real sample analysis with a tap, pond, and deionized water were also carried out. The platform exhibited excellent interference mitigated electro-catalytic oxidation of *E. coli* with good stability and

**Table 1**

Comparison of various reported works showing electrochemical detection of *E. coli* using different electrodes and voltammetry method.

Method	Electrode	Linear ranges (CFU/mL)	LODs (CFU/mL)	Ref.
SWASV	MWCNT-PAH/SPE	$1 \times 10^3$ to $5 \times 10^5$	$4 \times 10^2$	[8]
SWV	PDA/Fe <sub>3</sub> O <sub>4</sub> @MnO <sub>2</sub> -Fc-conA	$1 \times 10^3$ to $1 \times 10^8$	–	[9]
CV	AUT/AuNP/CHIT/MWNTs/SiO <sub>2</sub> @THI	$4.12 \times 10^2$ to $4.12 \times 10^5$	$2.50 \times 10^2$	[10]
CV	Agarose/AuNP	$10^3$ to $10^9$	$7.37 \times 10^4$	[11]
CV	CHI-SH/Fc/C <sub>60</sub> /Au-SiO <sub>2</sub> /GOD/PtNCs	$3.2 \times 10^1$ to $3.2 \times 10^6$	15	[12]
DPV	Cu-β-CD-GO/GCE	$10$ – $10^7$	5	[13]
Amperometric	HRP-TMB/ H <sub>2</sub> O <sub>2</sub> /AuNP	$0.99 \times 10^4$ to $3.98 \times 10^9$	50	[14]
SWV	GCE/GMC	$2.52 \times 10^3$ to $25.2 \times 10^4$	50.40	This work

SWASV: square wave anodic stripping voltammetry, MWCNT: multiwalled carbon nanotube, PAH: polyallylamine, SPE: screen printed electrode; SWV: square wave voltammetry, PDA: polydopamine, Fc: ferrocene, conA: concanavalin A, CV: cyclic voltammetry, AUT: amine-terminated alkanethiol 11-amino-1-undecanethiol hydrochloride, CHIT: chitosan, SiO<sub>2</sub>: silica, THI: thionine, AuNP: gold nanoparticles, CHI-SH: thiolated chitosan, C<sub>60</sub>: fullerene, Au-SiO<sub>2</sub>: Au nanoparticle coated SiO<sub>2</sub> nanocomposites, GOD: glucose oxidase, PtNCs: platinum nano chains. DPV: Differential Pulse Voltammetry, Cu-β-CD-GO: Copper-β-cyclodextrin-graphene oxide, HRP: horseradish peroxidase, TMB: 3,3',5,5'-Tetramethyl benzidine, H<sub>2</sub>O<sub>2</sub>: Hydrogen peroxide, GCE: glassy carbon electrode, GMC: graphitized mesoporous carbon.

reproducibility. Moreover, the LOD reported using this technique was observed to the lowest, amongst other techniques reported to date. Except for other features, and excellent linearity and sensitivity, to the best of the authors' knowledge, *E. coli* detection using such GCE/GMC electrode has not been reported yet.

## 2. Experimental

### 2.1. Chemicals and reagents

The sodium phosphate dibasic dihydrate (Na<sub>2</sub>HPO<sub>4</sub>·2 H<sub>2</sub>O) and sodium phosphate monobasic anhydrous (NaH<sub>2</sub>PO<sub>4</sub>) were purchased from Avra chemicals. These two chemicals were used for making the PBS buffer of 0.1 M ionic strength which was used as a supporting electrolyte. GMC (50 nm and 99.95% purity) were procured from Sigma-Aldrich. *Shewanella putrefaciens*, *Lactobacillus*, *Helicobacter pylori*, and *Bacillus anthracis* were procured from the Biological Department of the BITS Pilani Hyderabad Campus and Deionized water was obtained from the Central Analytical Laboratory of our Institute.

### 2.2. Apparatus

A benchtop potentiostat (SP-300 from Biologic, France) was used for performing the CV and SWV experiments. A three electrodes system was used which comprises of disk-shaped glassy carbon electrode (3 mm diameter and 0.0707 cm<sup>2</sup> working surface area) as the working electrode. Platinum wire was used as the counter electrode and Ag/AgCl was used as the reference electrode. All of the experiments were carried out in a 5 mL cell [16].

### 2.3. Bacteria Preparation

*E. coli* strain DH5α was provided by the Department of Biological Sciences of our Institute. The cultured medium used was Lysogeny Broth (LB), which was prepared by taking 5 mL of LB media in 200 mL distilled water and autoclaved for 20 min at 15 psi at 121 °C. The 0.5 gm yeast extract, 1 gm tryptone, and 1 gm NaCl were the constituents of the LB media. *E. coli* was cultured by inoculating 200 μL of strain in 20 mL of LB media and incubated for 36 h with shaking at 180 rpm at 37 °C. The turbidity was checked and optical density (OD) measurements were carried out at a wavelength of 600 nm using the UV visible spectrophotometers [17].

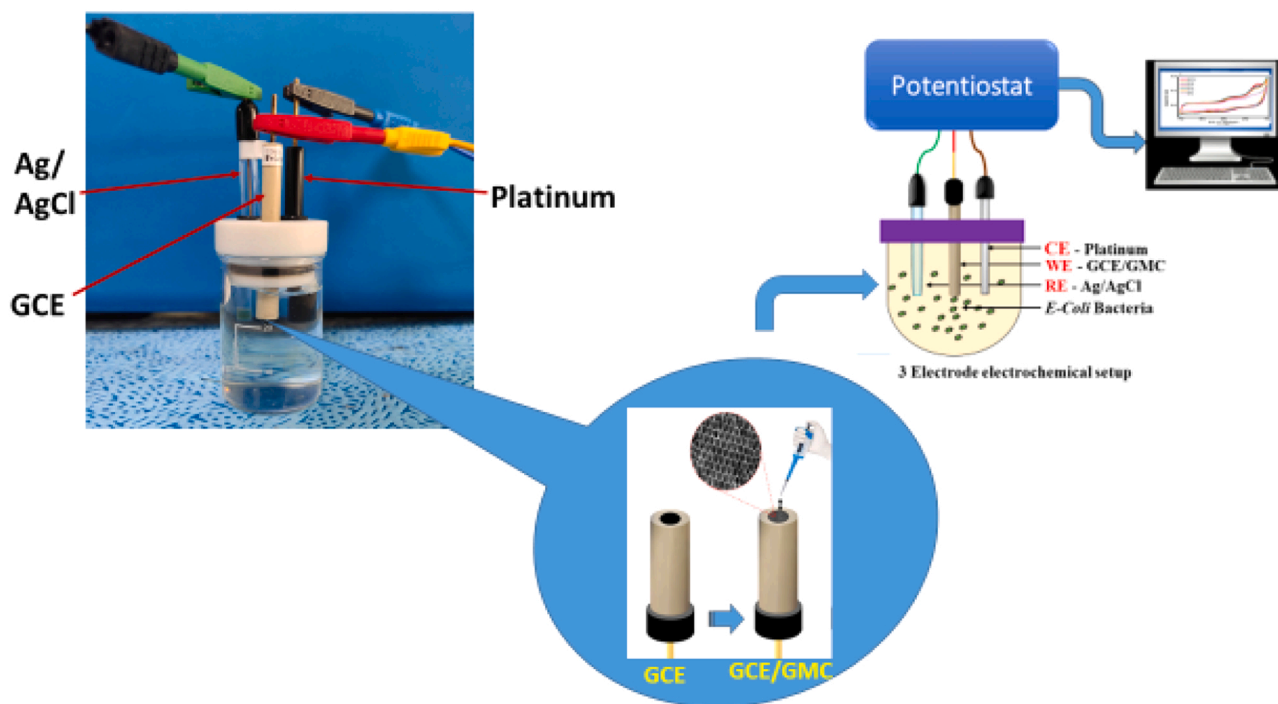
### 2.4. Electrode preparation

The GCE was mechanically and electrochemically pretreated and cleaned before modification. For the mechanical polishing, Al<sub>2</sub>O<sub>3</sub> of 0.05 μm was used to polish the surface and then cleaned with distilled water. For electrochemical pretreatment, the polished GCE was kept in pH 7 phosphate buffer solution (PBS) and CV was run in a potential window – 1 to + 1.2 V to ensure no peak is obtained. 2 mg of GMC was dispersed in 500 μL of ethanol, sonicated for 15 min was used. Then,

5 μL of the dispersed solution was drop-casted onto the surface of GCE. Finally, the electrode was air-dried for 1 h. The electrochemical measurements were performed within the potential window of (–1 to 1) V and a scan rate of 50 mV/s using the modified electrode immersed in a neutral pH electrolyte of PBS, LB media, and *E. coli*. Scheme 1 illustrates the experimental procedure.

### 2.5. Bacteria concentration

The colonies of *E. coli* were taken from the plate and inoculated into 1 mL of LB media centrifuge tubes. The tubes were placed in the incubator at 37 °C and were left overnight with shaking at 180 rpm. Then, 200 μL of *E. coli* was inoculated into the 20 mL of LB media centrifuge tubes. This culture was placed in a shaking incubator of 180 rpm at



**Scheme 1.** Real diagrammatic view and schematic representation of the electrode modification and electrochemical setup.

37 °C for 36 h. To count the cultured plate, the resulting cell suspension of *E. coli* bacteria has been used. The supernatant was discarded by centrifugation at 10000 rpm. Various *E. coli* bacteria concentrations (from  $5 \times 10^1$  CFU/mL to  $2 \times 10^2$ CFU/mL) were made in the PBS buffer. Under the optimized conditions, the square wave voltammetry (SWV)

technique was used for the determination of *E. coli* bacteria using the GCE/GMC electrode.

Generally, the unit used to denote *E. Coli* bacteria concentration is CFU/mL, (colony-forming unit/mL). CFU stands for colony-forming units, and it refers to the number of live bacteria found in a sample. A



**Scheme 2.** Schematic representation of the Serial dilution and the plating process.

viable bacterium means the bacteria that are growing or dividing in a sample. Due to higher bacteria concentration, first, the bacteria were diluted in the PBS to get the OD of 0.6. Then, the method known as Serial dilution or 10-fold dilution followed by the plating method was used to find the *E. coli* bacteria concentration. (Scheme 2).

Five centrifuge tubes were labeled with the dilution factor of 1:10, 1:100, 1:1000, 1:10000, 1:100000. The 1 mL pipette was used to introduce 9 mL of LB media into five labeled centrifuge tubes. Then inoculated 1 mL of cultured bacteria was placed into the first tube of LB media and the mixer was vortexed for 15 s. Again 1 mL of media from the first tube was transferred into the second tube and the mixer was vortexed for 15 s. The same process was repeated for the second, fourth, and fifth centrifuge tubes.

The plating method was carried out to count the number of colonies present in a sample. Five plates were prepared in a petri dish from nutrient agar media. 1 mL of liquid culture was taken from each diluted tube and spread into the labeled five plates of nutrient agar. All five plates were incubated overnight for 37°C. The colonies were counted from all these plates. The ideal range of colonies to be counted as (30–300) colonies in a sample. Less than 30 colonies resulted in the disguised result which was unable to justify and more than 300 colonies resulted in the merger of too many colonies that were difficult to count [18].

The formula used was,

Number of bacteria (CFU)/mL = (number of colonies on plate × reciprocal of the dilution of the sample) / volume of the culture plate

$$\text{CFU/mL (experiment)} = (252 \times 1000) / 1 = 252000 \text{ CFU/mL} = 2.52 \times 10^5 \text{ CFU/mL}$$

## 2.6. Scanning electron microscopy (SEM) sample preparation

Firstly, 70 mg Glassy Carbon powder was taken on the petri dish and 30  $\mu\text{L}$  of baby oil was poured into it. Stirring was done to make a thick paste out of it. A small amount of paste was taken and put into the Glass substrate. To dry the paste, the glass substrate was put into the oven at 100 Celsius for around 2 h. After then, 10  $\mu\text{L}$  of GMC solution was drop cast on the Glass substrate having Glassy carbon paste. Finally, it was left for the air to dry for 1 h. Thus, the sample was ready to carry out the SEM study.

## 3. Results and discussion

### 3.1. Electrocatalytic activity of the *E. Coli*

To understand the electrochemical oxidation of *E-Coli*, CV and SWV was observed in PBS and *E. coli* solution, whereby a distinct oxidation peak was observed using the CV and SWV techniques for *E. coli* bacteria. Using the CV technique, an anodic peak at  $E^0 = 0.749 \text{ V}$  was observed. Similarly, using the SWV technique an oxidation peak at  $E^0 = 0.742 \text{ V}$  vs Ag/AgCl was observed. Fig. 1(a) illustrates the CV response of GCE/GMC in various concentrations of *E. coli* in 0.1 M PBS buffer at a scan rate of 50 mV/s. The electrochemical characterization of LB media and *E. coli*

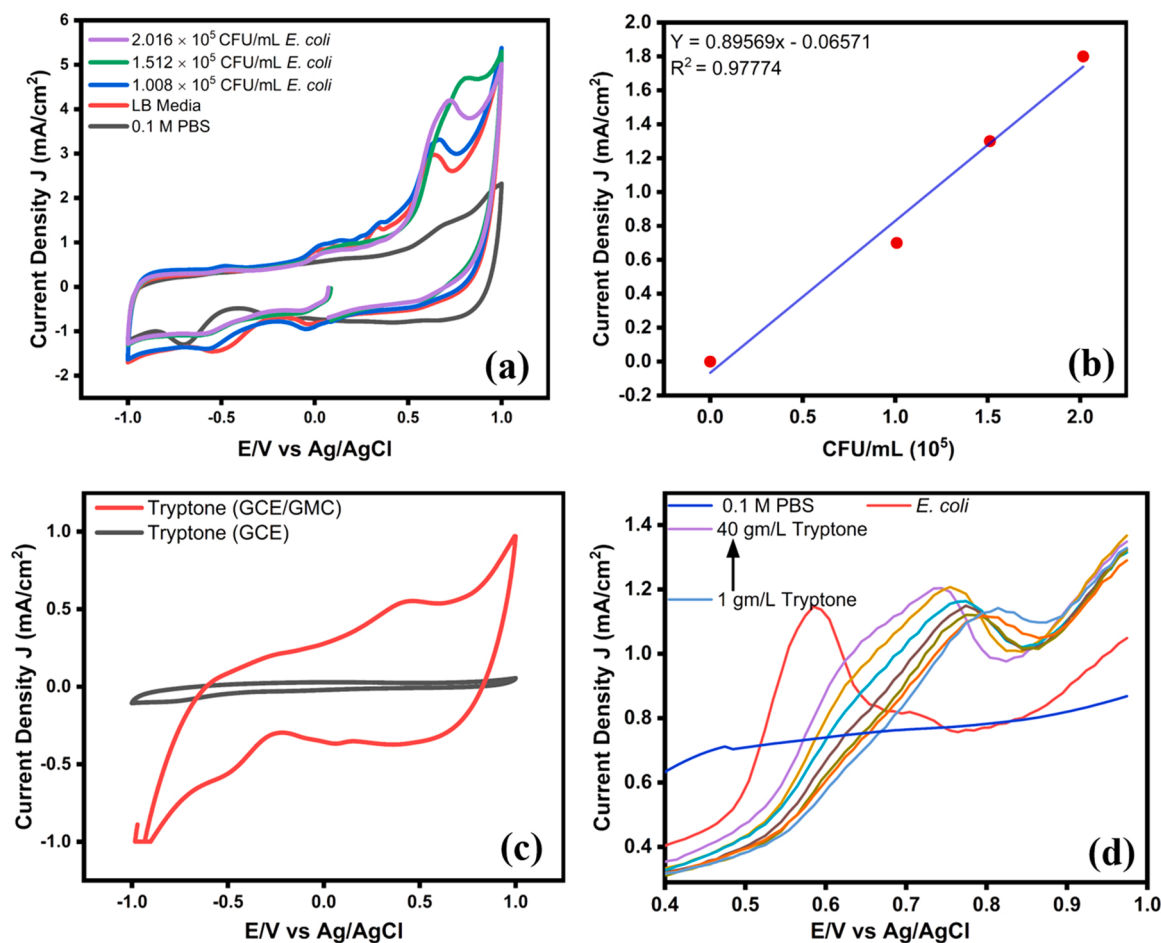


Fig. 1. (a). CV Response of GCE/GMC in pH 7 PBS, LB and various concentrations of *E. coli* 50 mV/s vs. Ag/AgCl. (b) Corresponding linear plot of peak current density vs *E. coli* concentration from  $1.008 \times 10^5 \text{ CFU/mL}$  to  $2.016 \times 10^5 \text{ CFU/mL}$  by subtracting the background current density. (c) CV Response of GCE/GMC of Tryptone 50 mV/s vs. Ag/AgCl. (d) SWV Response of varying Tryptone concentration from 1 gm/L to 40 gm/L with a constant concentration of the *E. coli*.

was performed and no significant peak was observed for GCE/GMC electrode in PBS. However, GCE/GMC in a mixture of PBS and LB media gave a feeble peak. Upon addition of bacterial culture of various concentrations, a significant increase in the oxidation peak current density was observed authenticating that the peak current density was directly proportional to the bacterial concentration.

The electrochemical sensing [19] of *E. coli* gets enhanced when the GCE was modified with GMC resulting in a larger peak current density, better peak shape, and lower potential because of the good electron transfers ability. While during reverse scanning, a negligible amount of peak was observed, the electron transfers between the electrode surface and the probe molecule were hampered by the poorly conductive electrode. The continuous scanning was carried out on increasing the *E. coli* concentration to study the mechanism involved in the electrochemical sensing of bacteria. On increasing the *E. coli* concentration, the peak current density gets increased and moved to a higher potential. Fig. 1(b) is the corresponding linear fit graph manifesting that the increase in anodic peak current density is directly proportional to the increase in concentration.

The LB media has a composition of tryptone, yeast extract, and NaCl which is possibly contributing to the peak obtained. The addition of bacteria in the solution is enhancing the peak due to an increase in the electron transfer mechanism by the influence of bacteria. The increase in current density value is due to the conductivity of bacterial cell membranes and cytoplasm [20]. For a better understanding, a control experiment with tryptone alone is performed with GCE/GMC and a feeble peak was observed. However, this peak slightly shifts plausibly

due to yeast extract and NaCl.

The effect of Tryptone concentration has been studied with constant *E. coli* concentration as shown in Fig. 1(d). Fig. 1(d) depicts the effect of varying the Tryptone concentration with a constant concentration of the *E. coli*. The concentration of the Tryptone was varied from 1 gm/L to 40 gm/L and Square Wave Response was recorded. As the concentration of tryptone is varied, the current is linearly increased with constant *E. coli*. A shift in peak is observed. However, when *E. coli* concentration is increased, the peak current increases linearly at the potential slightly different from tryptone alone.

### 3.2. Effect of scan rate

The electron transfers process of the working electrode GCE modified with GMC was observed by carrying out the relationship between the peak current density and scan rate in the range of (10–150) mV/s. The reduction peak observed around  $-0.5 \pm 2$  V is due to GMC and dissolved oxygen in PBS. The same peak is observed in the plain PBS as well. By using the following equation,

$i_{pa} = 2.99 \times 10^5 n[(1 - \alpha)n_a]^{1/2} ACD^{1/2} v^{1/2}$ , [21] and it is found that the diffusion-controlled reaction takes place with the diffusion coefficient is calculated as  $12.6164 \times 10^{-28} \text{cm}^2 \text{sec}^{-1}$ .

wherein  $n$  = total no of electrons,  $A$  = electrode surface area ( $0.07065 \text{cm}^2$ ),  $C$  = concentration of *E. coli* ( $2.016 \times 10^5 \text{CFU/mL}$ ),  $v$  = scan rate (150 mV/s),  $n_a$  = no of electrons involved in rate-determining step,  $\alpha$  = transfer coefficient (0.5),  $I_p$  = peak current (0.25) mA.

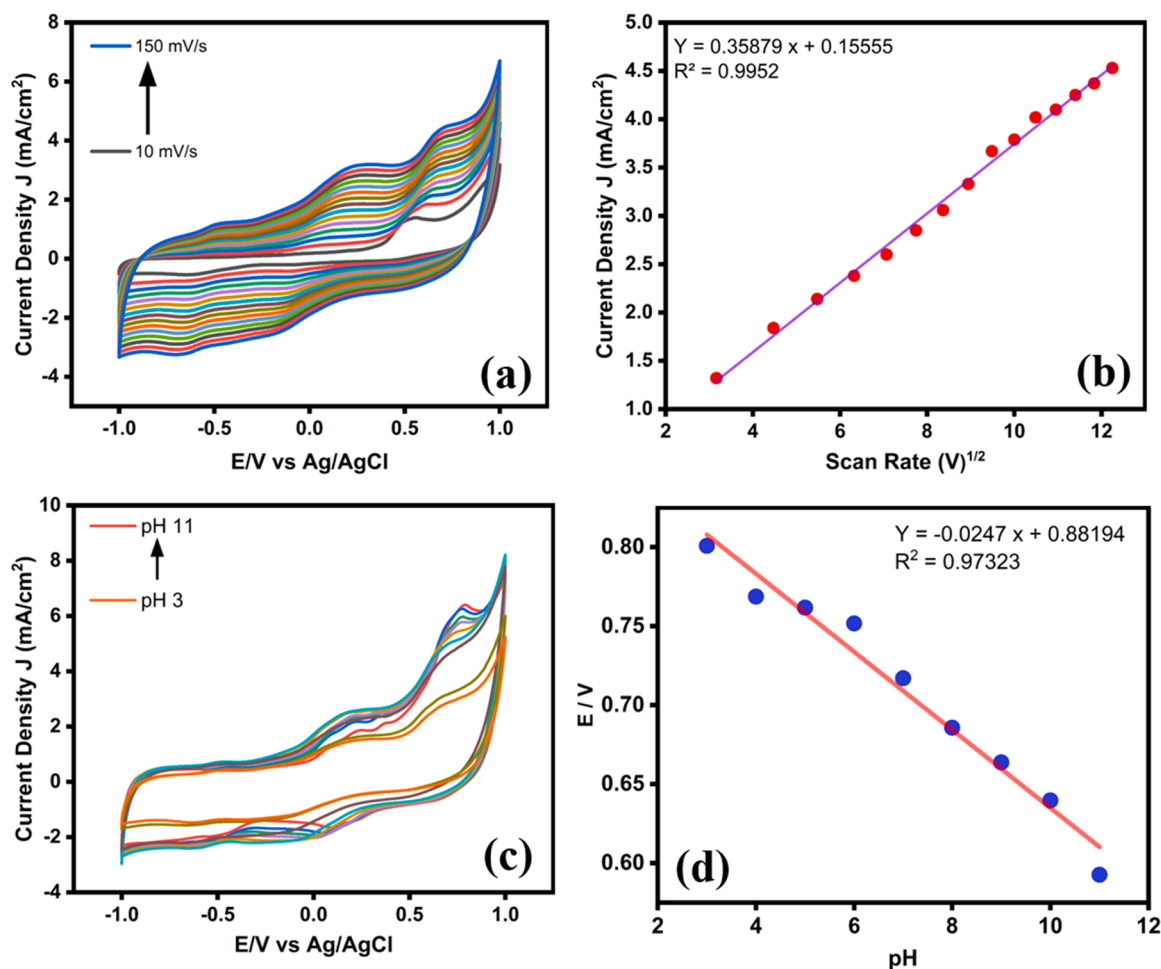


Fig. 2. (a) Effect of varying scan rate in *E. coli* PBS solution (10–150) mV/s; (b) Corresponding plot of  $I$  vs  $v^{1/2}$ ; (c) Comparative CV response of *E. coli* in various pH (3–11); (d) Corresponding plot of  $E^0$  vs pH.



### 3.3. Effect of pH

The effect of solution pH was analyzed on the CV response of various pH (3–11) PBS buffer was examined. Fig. 2(c) illustrates the CV

response of GCE/GMC in pH (3–11) bacterial solution. The response depicts that the increase in oxidation peak current density on increasing the pH from 3 to 11. The plot of  $E^0$  vs. pH was linearly observed with a negative slope of  $0.0247 \text{ mV pH}^{-1}$ . Here, the participation of electrons

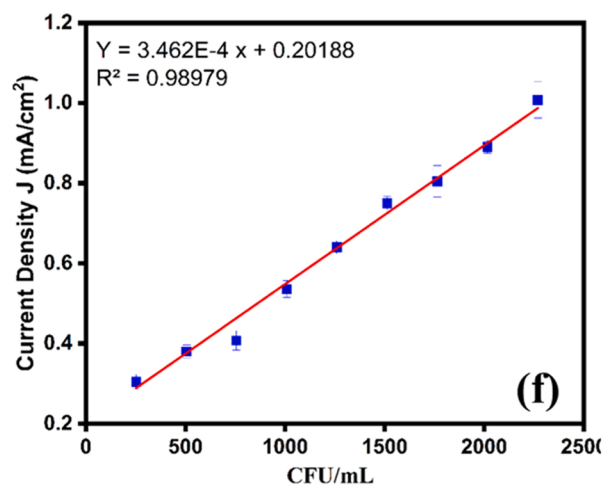
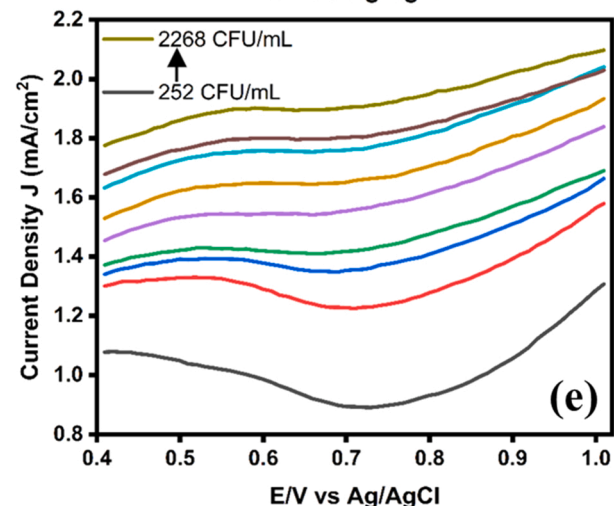
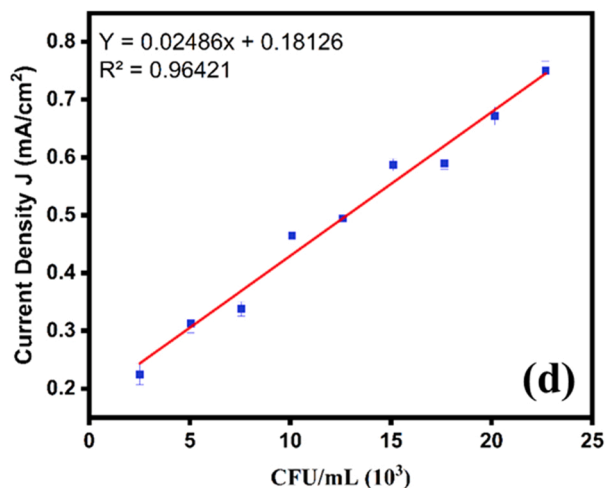
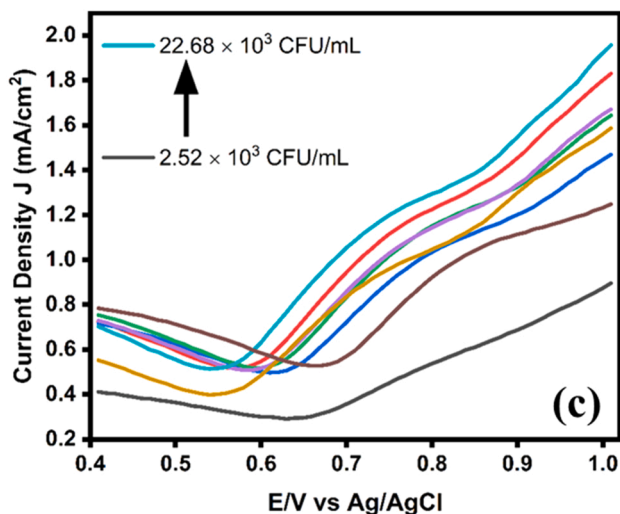
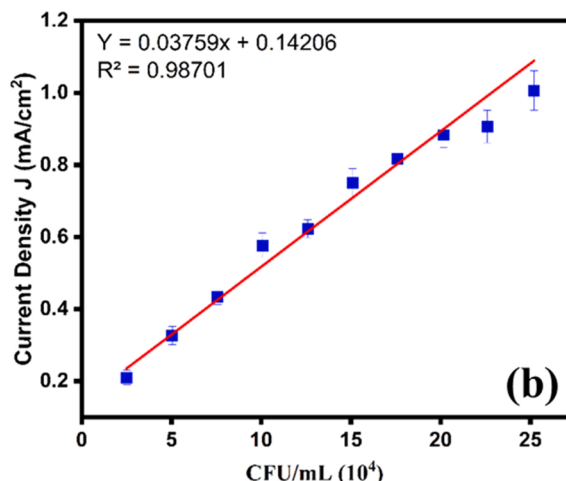
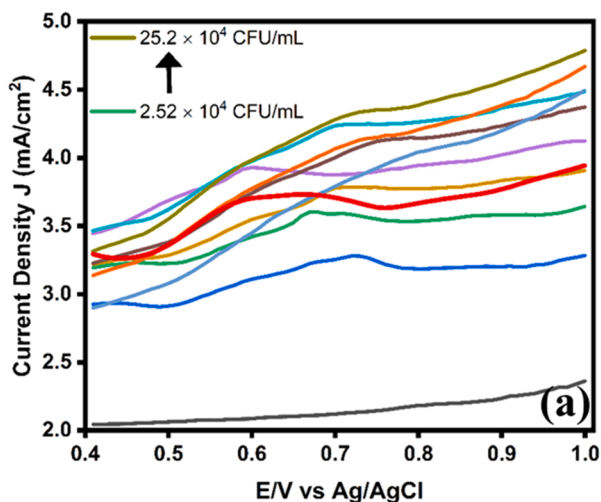


Fig. 3. (a) SWV response of the *E. coli* various concentrations  $(2.52\text{--}25.2) \times 10^4$  CFU/mL; (b) Corresponding linear graph for *E. coli* concentration  $(2.52\text{--}25.2) \times 10^4$  CFU/mL by subtracting the background current density; (c) SWV response of the *E. coli* corresponding to lower concentrations  $(2.52\text{--}22.68) \times 10^3$  CFU/mL; (d) Corresponding calibration graph for *E. coli* concentration  $(2.52\text{--}22.68) \times 10^3$  CFU/mL by subtracting the background current density. (e) Square wave voltammetry response of the *E. coli* concentration (252–2268) CFU/mL. (f) Corresponding calibration graph for *E. coli* concentration (252–2268) CFU/mL by subtracting the background current density.

and protons was approximately equal which characterizes the Nernstian reaction [22].

### 3.4. Effect of *E. coli* concentration

The concentrations of *E. coli* were varied from  $2.52 \times 10^4$  CFU/mL to  $25.2 \times 10^4$  CFU/mL and the SWV technique was used to characterize the peak current density within the optimized parameter ranges. These parameters were defined as, Initial potential ( $E_i$ ) = 0.4 V, Final potential ( $E_o$ ) = 1 V, Pulse height 25.0 mV, Pulses width = 50.0 ms, and Step height = 10.0 mV. Fig. 3(a) illustrates the SWV responses. Here, the peak current density gets increase because of an increase in the *E. coli* bacteria concentration. In Fig. 3(a), the lowest read line in red is GCE/GMC which is in only phosphate buffer solution, hence, a flat curve is obtained. Whereas, the next line, green, is GCE/GMC in LB media alone without bacteria, wherein, the slight bump is visible. Once the bacteria culture was added, the peak grew gradually upon increasing the bacterial concentration. Therefore, this signifies that the peak observed was due to the presence of bacteria. The lowest current density obtained from the green line, (blank) was subtracted from all the consecutive current densities of the concentration, and a baseline-corrected peak value was plotted for the linear graph.

Fig. 3(b) is the corresponding calibration plot. The given plot was made by subtracting the peak current density of LB media to each *E. coli* concentration. Fig. 3(b) showed good linearity of 96%. This signified the number of active sites present on the electrode [23].

*E. coli* concentration was further varied from  $2.52 \times 10^3$  CFU/mL to  $22.68 \times 10^3$  CFU/mL. The peak current density was calculated again using the SWV technique. As illustrated in Fig. 3(c), the current density gets increases, as the *E. coli* bacteria concentration increases. The actual current density of *E. coli* was drawn by subtracting the peak current density of the LB media. The corresponding base line corrected plot between the concentration of bacteria and peak current density is shown in Fig. 3(d) where linearity was observed with a slope of 0.02486 and an accuracy of 96.42%.

For the calculation of the LOD, *E. coli* was further diluted and concentration was lowered from the (252–2268) CFU/mL. This can be illustrated in Fig. 3(e), where the current density has been increased on increasing the *E. coli* concentration. The corresponding calibration graph has been plotted by subtracting the background current density as shown in Fig. 3(f). The slope of 0.0003462 and accuracy of 98.97% were

obtained.

The LOD was calculated by taking the peak average current density from the triplicated experiments. The slope was estimated by plotting the graph between the peak average current density and *E. coli* bacteria concentration. Standard deviation was calculated from the current density values derived from the triplicated experiments. Thus, theoretical LOD was obtained as 50.40 CFU/mL. Further, the presence of *E. coli* was detected for better selectivity and further used for real-time application.

### 3.5. Interference effect

Interference effect [24,25] from various bacteria, such as *Shewanella putrefaciens*, *Lactobacillus*, *Helicobacter pylori*, and *Bacillus anthracis* of  $1.008 \times 10^5$  CFU/mL concentration was performed to check the selectivity of the device in the presence of  $1.008 \times 10^5$  CFU/mL *E. coli*. A negligible amount of interference was observed from various other bacteria. As can be seen in Fig. 4(a) all the other bacteria gave responses at a slightly different potential than that of *E. coli* and *E. coli* gave predominant peak current value. Furthermore, as can be seen in Fig. 4(b), the bar graph depicts that simultaneous addition of other bacteria gave less reduction in peak current density < 5%.

### 3.6. Real sample analysis

The standard addition approach [26] was used to test the various real samples like tap water, pond water, and deionized water. Here, GCE/GMC was subjected to various spiked concentrations of *E. coli* and the approximate concentration of *E. coli* was calculated based on the average current density values obtained by triplicated experiments. Fig. 5a–c are the SWV responses of the standard addition method. The obtained results are illustrated in Table 2. The recovery values signify that the *E. coli* was selectively detected in the real sample of tap water, pond water, and deionized water [27].

Following that, real samples of water were taken in the 10 mL of the filtrate. This solution was analyzed with the developed method, and SWV was performed. The standard addition approach was used with  $1.008 \times 10^5$  CFU/mL,  $1.512 \times 10^5$  CFU/mL, and  $2.016 \times 10^5$  CFU/mL. *E. coli* and SWV were conducted once again. This procedure was performed three times in the linear region to notice an increase in peak current density values as *E. coli* concentration increased. At

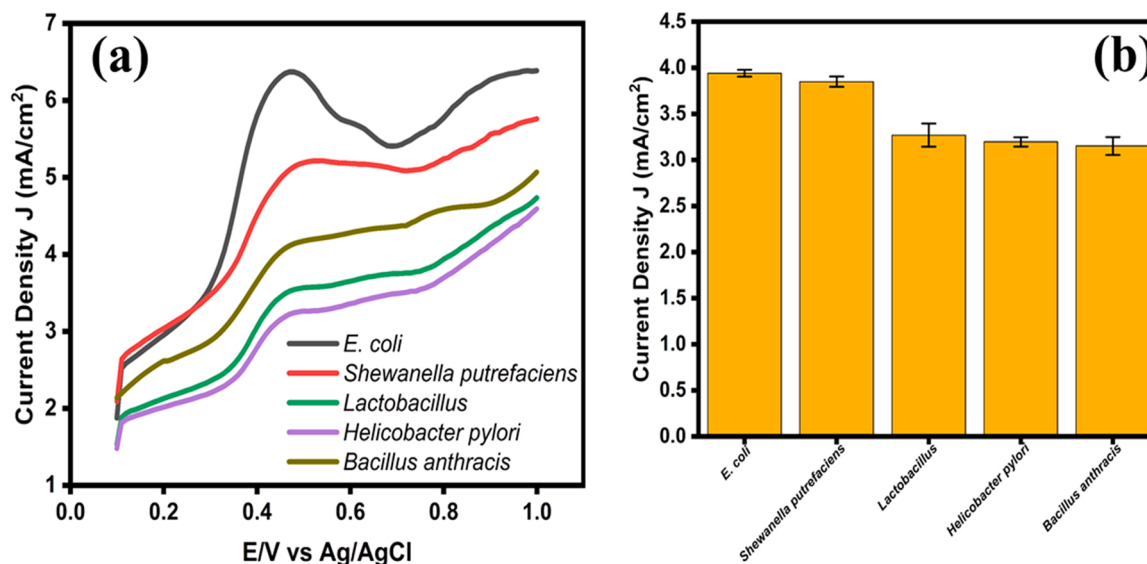


Fig. 4. (a) SWV response of various bacteria of  $1.008 \times 10^5$  CFU/mL concentration; (b) Bar graph representation for various bacteria of  $1.008 \times 10^5$  CFU/mL concentration.

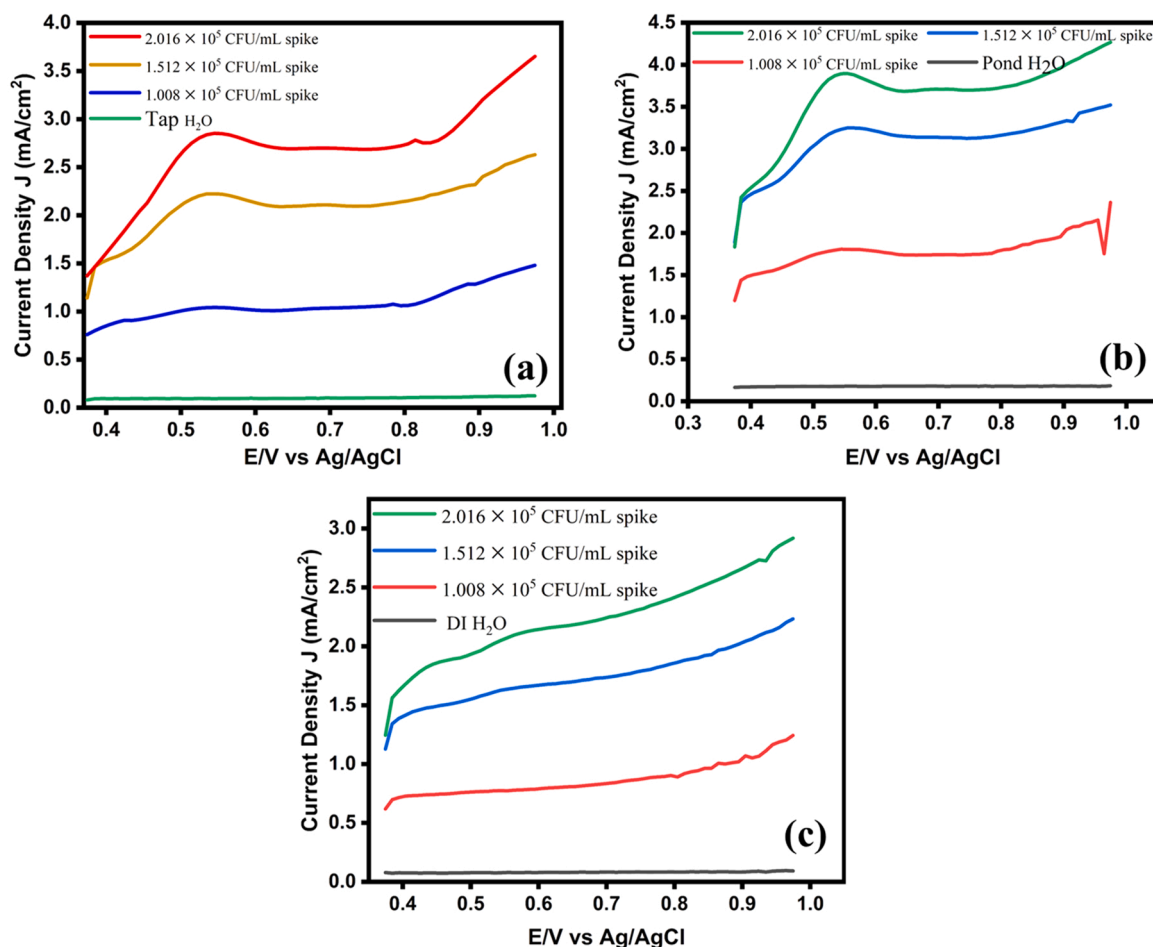


Fig. 5. (a) Square wave voltammetry response of *E. coli* in Tap water. (b) Square wave voltammetry response of *E. coli* in Pond water. (c) Square wave voltammetry response of *E. coli* in DI water.

Table 2

Real Sample Analysis of *E. coli* with Tap, Pond, and DI Water.

Sample	J1 (mA/cm <sup>2</sup> )	J2 (mA/cm <sup>2</sup> )	J3 (mA/cm <sup>2</sup> )	Average (mA/cm <sup>2</sup> )	SD	Added (A) ( $\times 10^5$ ) CFU/mL	Found (F) ( $\times 10^5$ ) CFU/mL	Recovery (A/F *100) %
Tap H <sub>2</sub> O	1.39	1.19	1.18	1.25	0.01	1.008	0.94	93.2
	1.85	1.47	1.33	1.55	0.022	1.512	1.54	101
	0.97	0.95	0.73	0.88	0.011	2.016	1.93	95.7
Pond H <sub>2</sub> O	1.44	1.46	1.43	1.44	0.001	1.008	1.03	102
	2.01	2.24	2.10	2.12	0.009	1.512	1.48	98
	2.36	3.02	2.77	2.72	0.027	2.016	1.99	99
DI H <sub>2</sub> O	0.73	0.94	0.98	0.88	0.011	1.008	0.99	98
	1.12	1.07	1.29	1.16	0.009	1.512	1.54	101
	1.52	1.51	1.59	1.54	0.004	2.016	2.036	100

$1.008 \times 10^5$ CFU/mL,  $1.512 \times 10^5$ CFU/mL, and  $2.016 \times 10^5$ CFU/mL *E. Coli* concentrations, significant recovery values were found for different kinds of water samples.

### 3.7. Microscopic characterization

SEM study was carried out to study the shape and size of various nanomaterials and also to calculate the value of components quantitatively [28]. Fig. 6(a)–(d) shows the particle shape and size for the Graphitized Mesoporous Carbon. The abundance amount of carbon can be seen on the glassy carbon electrode substrate. To carry out the SEM images,

## 4. Conclusion

The present work reports an *E. Coli* GCE/GMC modified electrode for *E. coli* detection. All the experiments were performed with a standard three-electrode system. It was observed that the electrochemical signals of *E. coli* bacteria increased with an increase in their concentration. A distinct oxidation peak found at 0.749 V, 0.742 V ( $E^0$  V v/s Ag/AgCl) using CV and SWV techniques respectively. A further effect of scan rate, pH, and concentration was also analyzed. The limit of detection was estimated as 50.4 CFU/mL using the SWV technique. An interference study was carried out to understand the interaction of *E. coli* with various bio-chemicals like Xanthine, Hypoxanthine, Uric acid, Dopamine, and Ascorbic acid. Finally, real sample analysis was carried out in tap, pond, and deionized water showing good reliability and recovery.

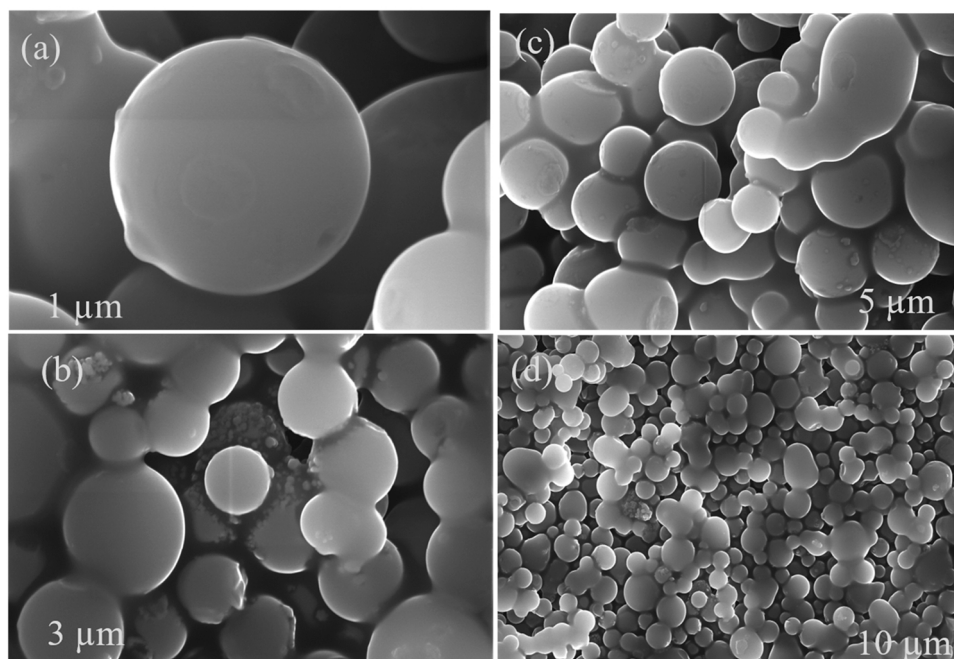


Fig. 6. SEM images of the GMC with different magnifications on the Glassy Carbon powder.

Because of the quick preparation time, simple modification process, and low preparation cost, the proposed sensor is easily scalable. Future research in this direction can pave way for the development of nanostructured electrochemical sensors for diagnostics and real-time monitoring of *E. coli* growth in diverse fields.

#### CRedit authorship contribution statement

**Manish Rishi:** Conceptualization, Data curation, Formal analysis, Writing – original draft. **Khairunnisa Amreen:** Data curation, Formal analysis, Writing – review & editing. **Jaligam Murali Mohan:** Data curation, Writing – review & editing. **Arshad Javed:** Project administration, Resources, Writing – review & editing. **Satish Kumar Dubey:** Project administration, Resources, Writing – review & editing. **Sanket Goel:** Funding acquisition, Supervision, Writing – review & editing.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# Emerging trends in miniaturized and microfluidic electrochemical sensing platforms

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Arshad Javed<sup>1,3</sup>, Satish Kumar Dubey<sup>1,3</sup> and Sanket Goel<sup>2,3</sup>

## Abstract

Electrochemical sensing has established a strong presence in diverse areas. The conventional electrochemical sensing approach consumes large sample volumes and reagents and requires bulky potentiostat, macro-electrodes, and other equipment. The synergistic integration of electrochemical sensing systems with miniaturized or microfluidic electrochemical devices and microelectrodes in a single platform provides rapid analysis with a disposable, reusable, and cost-effective platform for multiplexed point-of-care detections. Such microdevices have created scope for using several materials as electrodes and sensing platforms by using appropriate fabrication techniques. One of the most recent advancements in miniaturized devices includes the integration of automation and Internet of Things to realize fully automated and robust electrochemical microdevices. The review summarizes the emerging trends in fabrication methods of miniaturized and microfluidic devices, their multiple applications in real-time, integration of Internet of Things, automation, identifying research gaps with strategies for bridging these gaps, future outlook, and recent approaches to intelligent electrochemical sensing.

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Miniaturized devices, Microfluidic devices, Electrochemical sensing, Microfabrication, Nanotechnology.

## Introduction

The amalgamation of electrochemistry and microfluidics has allowed seamless integration of sensing and detection systems with automation, miniaturization, and simplification. These integrated systems, owing to their inherent advantages, are amenable to be used as point-of-care devices in fields such as biomedical diagnostics and environmental sensing. The evolution of newer technologies and upgradation of well-established methods of microfabrication has paved way toward the utilization of various substrates such as paper, glass, elastomer polymers, and so on for fabrication of these devices [1]. Depending on the substrates used, various fabrication techniques can be adopted. Generally, fabrication methods such as (a) screen printing, (b) three-dimensional printing, (c) laser-induced or laser-engraved, (d) photolithography, (e) cutting shaping, and (f) wax printing are used.

## Significance of miniaturized and microfluidic devices

Most present-day analytical methods primarily rely on laboratory-based analytical techniques that can be costly, cumbersome to use, have low sensitivity, and incur substantial testing expenses. Because point-of-care detection is crucial for several on-field applications, the focus has been shifted toward realizing smaller analytical instruments. Various miniaturized and microfluidic electrochemical (MMEC) sensors, with novel and smart materials, have gathered great demand in the domain of analytical science owing to their high sensitivity, mobility, operation, and rapid analysis [2]. The surface enhancement plays a vital role for detection of trace analytes by measuring electrochemical changes, such as voltage, current, and impedance potential, owing to the redox reaction (oxidation or reduction) signals of biological/chemical molecules with the use of electrodes and electrochemical units [3,4]. The ability of the sensor to detect various targets, such as tiny organic molecules, metal ions, and biomolecules, gives them enormous potential and advantages over conventional testing methodologies [5,6]. There have been numerous applications of MMEC sensors, particularly in the health-care industry, environmental monitoring, food safety, forensic analysis, military, defense, artificial intelligence, and so on. [7,8]. The present review focuses on the recent advancements in the fabrication of these

devices and their applicability. It also discusses the research gaps, scalability prospects, recent literature updates, and future outlook.

### Fabrication methods

A variety of fabrication techniques are generally selected depending on the specific requirements of a product. Although comparing the fabrication methods, diverse characteristics, such as the material, deposition thickness, uniformity of deposition, space, cost, size, and tolerance of the unit are considered. The established fabrication methods provide precise control over device dimensions, surface modification, and other related parameters and provide scope for the usage of diverse materials. The surface modification methods used in the production of MMEC devices include evaporation, sputtering, photolithography, and chemical vapor deposition. However, because the underlying expertise and experience required for traditional electrode manufacturing methods are enormous, the costs to use these techniques can be incredibly steep. Moreover, the associated equipment is costly and requires special facilities, such as cleanrooms for their proper working. In addition, a large amount of floor space is needed for many of these specialized equipment.

Several other manufacturing approaches such as ink-jet printing, screen printing, and microwire placement have emerged as alternative methods to overcome the aforementioned challenges. Numerous scientific research groups have demonstrated the use of electrode fabrication on different substrates including flexible and paper-based materials. Techniques such as photolithography, ink-jet, plasma treatment, wax printing, and screen printing have significantly contributed toward increasing the ease of fabrication. In addition, screen printing and ink-jet printing are two prominent methods for patterning of electrodes and incorporating hydrophobic barriers. Figure 1 represents the classification of general fabrication methods based on the type of substrate used.

#### Paper-based fabrication methods

Paper has been used in analytical chemistry for decades, but it was recently rediscovered as a valuable and affordable substrate to work as a sensor. Advantages such as biodegradability, biocompatibility, easy availability, facile fabrication method, simple surface modification, and flexibility make them a suitable candidate to work as a substrate for electrochemical sensing [6].

The commonly used paper is Whatman chromatography paper, which has a smooth surface uniformity on both sides, an average flow rate, and 0.18 mm thickness compatible with commercial office printers. These papers consist of 98%  $\alpha$ -cellulose, without any additives for strengthening or whitening agents, limiting the likelihood of any intervention. In addition, proper

tuning and property adjustment are required for paper-based electrodes based on the intended electrochemical experiment. Few fabrication methods that incorporate electrodes over the paper substrate are (a) screen printing, (b) ink-jetting, (c) pencil drawing, and (d) laser-induced printing. These methods are discussed in the next section.

#### Screen printing

Screen printed electrodes (SPEs) are the first type of electrodes to be integrated into paper analytical devices and become the most common electrodes [9–11]. Screen printing equipment is relatively low-priced, and several devices are reported so far [12–14]. This is popular in analytical instruments, making the manufacturing process more streamlined [15–17].

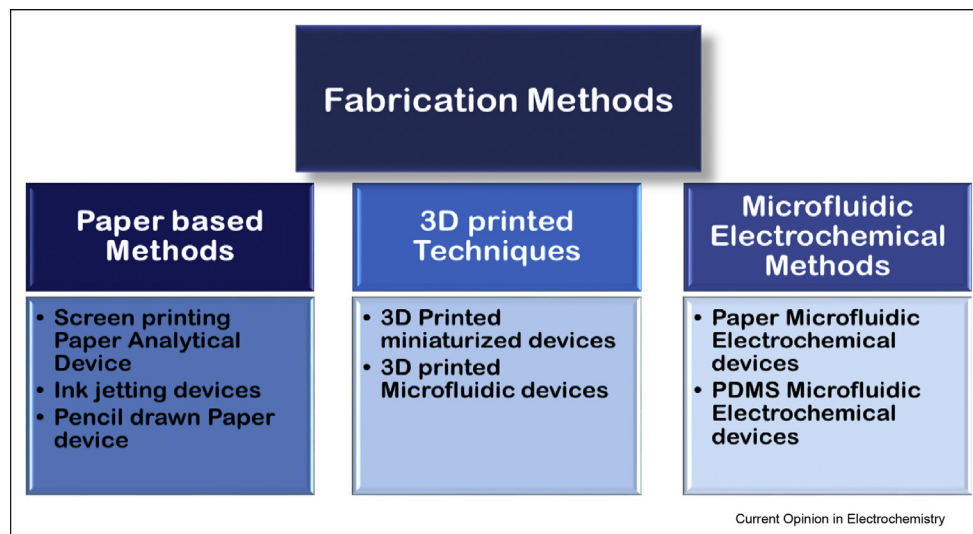
In a recent work where office paper was explored to develop screen printed paper strip for sensing of organophosphate pesticides as shown in Figure 2a. The strip was developed by both the wax printing and screen printing technologies. In the first step, a circular wax pattern was created to avoid solution diffusion toward the connector, and it might affect the readout of signal. In the second step, graphite ink and silver chloride ink were used to screen print the reference, counter, and working electrode. In the third step, the working electrode was modified with mixture of carbon black, Prussian blue, and butyrylthiocholine by drop-casting method. The developed portable strip can detect a lower limit of 1.3 ng/mL of organophosphate. To check its applicability, organophosphate was checked in the fruits, vegetables, and soil with the impressive recovery percentages in the range of 110%–90% [14].

Recently, Mohan et al. [18] reported a paper-based miniaturized screen-printed device for sensitive recognition of hydrazine on a paper substrate of dimensions 50 mm  $\times$  50 mm. As shown in Figure 2b, both reference and counter electrodes with dimensions of 40 mm  $\times$  5 mm were screen-printed on this substrate using carbon conductive ink and the Ag/AgCl ink. Furthermore, as a working electrode, a paper-based graphite sheet coated with the copper oxide nanoparticles was used. The working electrode was placed in between the counter and the reference electrode using double-sided tape. The wax barrier was produced to limit the 20  $\mu$ L of the sample volume in the vicinity of all three electrodes. Finally, the platform was examined with 20  $\mu$ M hydrazine. The actual sample analysis was also performed using tap and lake water for the device applicability, showing the appreciable recovery values.

#### Ink-jetting

Herein, ink-jet printing is used to deposit materials on the different substrates [19]. This technique aims to minimize the fixed costs of production while increasing

Figure 1



General classification of fabrication methods.

the number of materials used. The ink-jet printing technique overcomes disadvantages, wastage of the materials, lower production rate, and the nonuniformity of the conductive ink layer coatings [20]. The expensive materials can also be ink-jetted using ink-jet printing precisely with efficient resources [21].

A high level of accuracy and precision of required geometrical features of the electrodes is possible with ink-jet technology that satisfies specific industrial requirements [22]. The major ink-jet printing applications include light-emitting diodes and printed circuit board [23]. The ink properties, such as flow rate and viscosity values, must be adjusted as per the nozzle head for efficient application of this method. Various accessories and tools are required for the ink-jetting process, such as conductors, cartridges, paper, glass, or any flexible substrates and probe. Figure 4 gives a detailed representation of the ink-jet printing method. The process starts with filling the required conductive ink of specific viscosity in the cartridges using a fixed conductor. The substrates are fixed on the base plate of the ink-jet printing machine using substrate clamps. The final design is fed into the device with the compatible file format, as shown in Figure 3a. The distance between the substrate and nozzle is calibrated using the probe tool. The ink viscosity can be optimized with other parameters such as Z-axis distance, nozzle height, and line spacing [22].

Among various printing techniques, the ink-jet printing technique has a greater degree of flexibility and higher patterning capability and eliminates the need for predepositions or template/mask [24]. Multiple ink

cartridges may be used to print various materials at the same time. Several substrates such as paper, glass, and polyimide with high reproducibility can be used [25].

Bai *et al.* developed a biosensor system using ink-jetted printing technology as shown in Figure 3b. The developed biosensor system can able to detect phosphate in the saliva samples. From the fabrication point of view, a screen-printed electrode (SPE) was used to test the saliva samples. A bare SPE was cleaned with DI water and ethanol of 1:1 ratio. The cleaned SPE device was dried using nitrogen gas. Three different kinds of inks (Nafion, enzyme, and glutaraldehyde) were used to modify and to enhance the working electrode by ink-jet printing technology. These layers were formed layer by layer. The optimized parameters used for ink-jet printing were 6 kHz, 28 V [19].

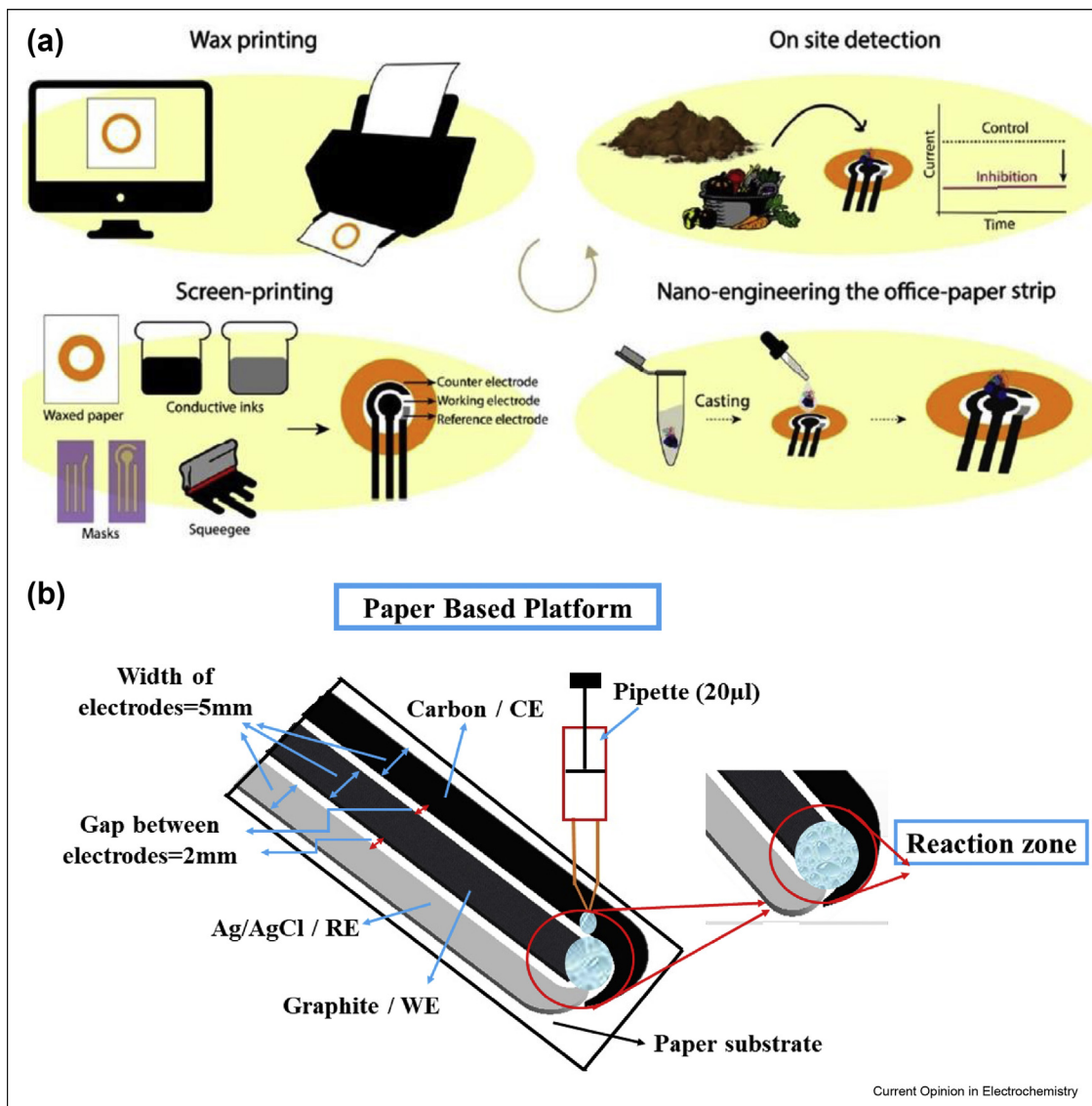
Srikanth *et al.* [26] reported a three-electrode device using the same ink-jet printer on the glass substrate, and one electrode at the tip is modified with Ag/AgCl paste using a microneedle. Then, the electrodes were kept in an oven to dry for 2 hours. The cured polydimethylsiloxane (PDMS) microchannel was prepared with a DLW system integrated on the glass ink-jetted electrodes using plasma bonding. Finally, the integrated device is tested with the three different concentrations of ascorbic acid. The oxidation peaks were observed at a flow rate of 1  $\mu\text{L}/\text{min}$ . Figure 3c is the reprint of the device with copyright permission.

#### *Pencil drawing*

Pencils can be used for fabrication of electrochemical devices as they are economic, available easily, and



Figure 2



(a): Representation of development of office paper strip for pesticide detection. Reprint with the copyright permission from [14]. (b): A paper-based screen-printed electrochemical device for hydrazine sensing. Reprinted with copyright permission from Mohan et al. [18].

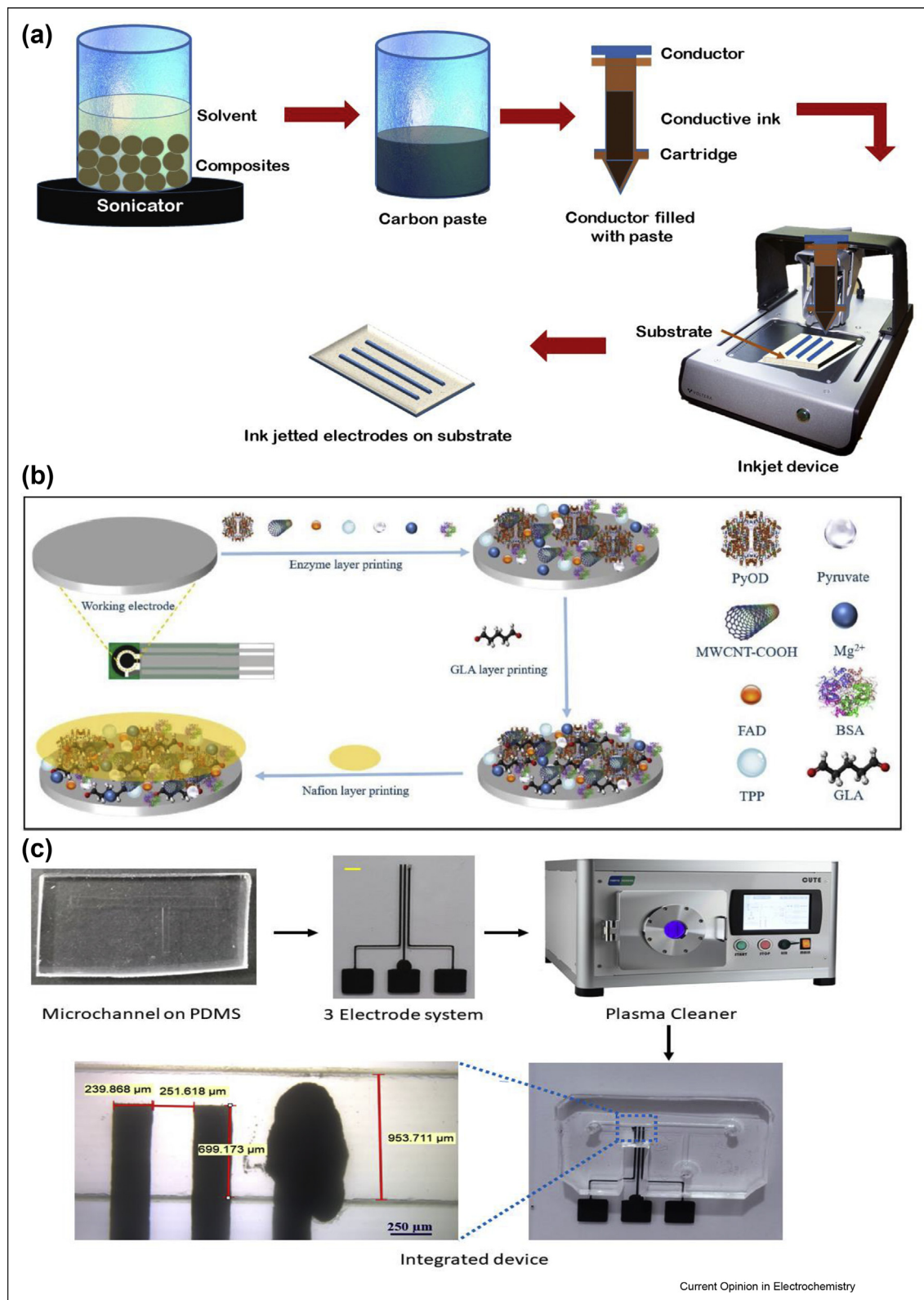
disposable. Some of the scientific reported works where pencil electrodes used are.

Franca et al. presented a simple and easily available inexpensive disposable electrochemical platform as shown in Figure 4a. In the fabrication, first, the tracing paper substrate was laminated with the laminating film using an office laminator. After that, the three electrodes were manually drawn on the tracing paper using a 6B-graded pencil. To draw the accurate shape and dimension of the electrodes, a layout of the design was produced on polyester film using the cutter and plotter. Later, the polyester film mold was placed over the tracing paper, and electrodes were drawn. Finally, to

delimit the sensing area, 14 mm diameter of adhesive tape was covered on the outside of three electrodes. Finally, the working electrode was modified with quantum dots to improve the sensitivity toward the dopamine. A lower limit of 96 nM/L was obtained on the developed platform [27].

Rocha et al. fabricated a pencil graphite on sand paper by friction process. Initially, the three-electrode layout was drawn using Corel draw software. Later, the layout design was printed on the sand paper using the ink-jet printing. Then, the working and counter electrodes were painted manually with a graphite pencil. The reference electrode was modified with Ag/AgCl ink. To

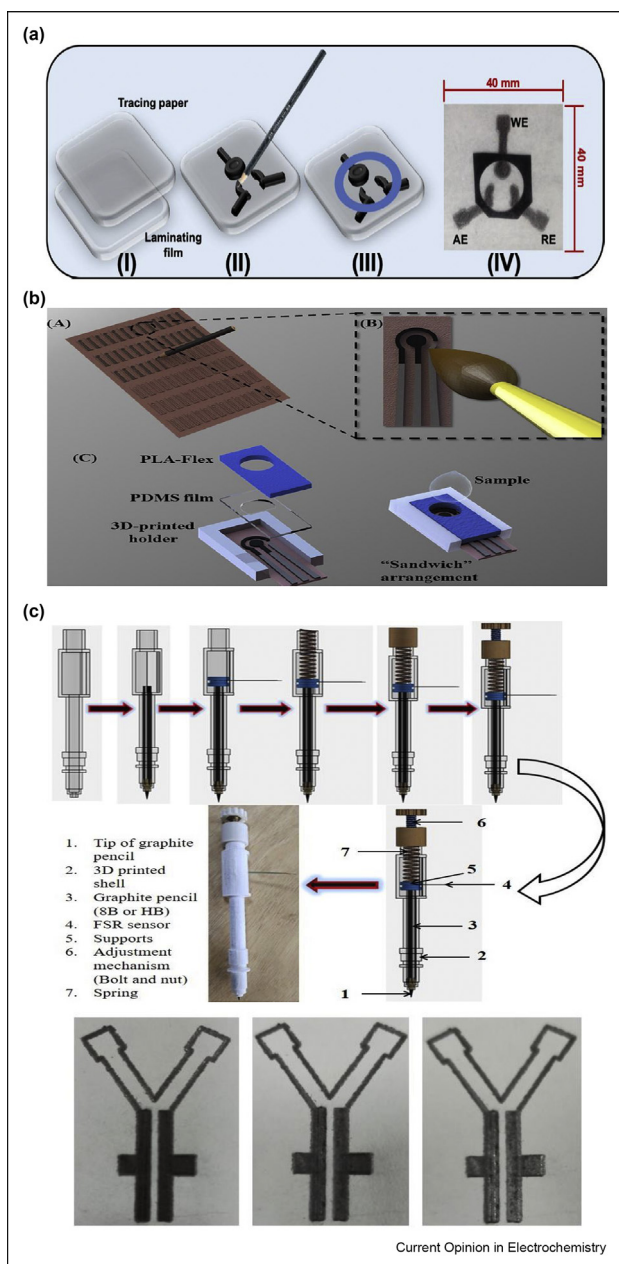
Figure 3



(a): Fabrication steps of ink-jet printing on substrates. (b): The functionalized procedure of the screen-printed electrodes is depicted in this diagram. Reprinted copyright permission from [19]. (c): An ink-jetted fabricated microfluidic electrochemical device for ascorbic acid detection. Reprinted with copyright permission from [26].

limit the geometric area, a three-dimensional (3D)-printed polylactic acid (PLA) was printed as shown in Figure 4b. To place the sand paper device, a 3D-printed holder was printed, in between the PLA and 3D-printed device holder, a PDMS film was sandwiched to avoid any leakages. The developed device was successfully tested with midazolam maleate which is also called as rape

Figure 4



(a): Fabrication steps for pencil-drawn electrodes for dopamine detection. Reprint copyright permission from [27]. (b): Step-wise fabrication with a pencil on sand paper substrate. Reprint copyright permission from [28]. (c): Different parts and an assembled automatic pencil stroke device for stroking. Reprinted with copyright permission from Srikanth et al. [29].

drug. To check the feasibility of the device, the device was also treated with vodka, beer, and liquor [28].

In another similar work, Rao et al., an automated graphite pencil stroke device, was developed to overcome human interaction at the manual electrode fabrication time, as shown in Figure 4c. The designed automatic pencil stroke device was used for paper-based microfluidic applications. This inexpensive automated pencil-based device is used in the fuel cell applications. Sulfuric acid is used as an electrolyte with formic acid, oxygen as fuel, and oxidizing agent. In this application, a power density, current density measured to be around  $135.5 \mu\text{Wcm}^{-2}$  and  $1305.5 \mu\text{Acm}^{-2}$ . Further fine-tuning with different pencil grades and various paper substrates, the fabricated devices can also be helpful in the electrochemical application for the various biological and chemical analyses [29].

#### Laser-induced and cut printing

Laser ablation over paper substrates is also versatile and low-cost in the manufacturing process [30]. This technique is often used for the production of paper electronics. Some reported works show platforms that were made using laser-induced on the different conductive polymers and mediator inks to produce conductive electrodes for electrochemical sensing applications [31–33].

Kothuru et al. had developed a graphene using  $\text{CO}_2$  laser ablation process on the polyimide sheet for various electronic applications as in Figure S1.a. One of application the laser engraved polyimide sheet was used for electrochemical sensing of  $\text{H}_2\text{O}_2$ . In the reported work, a rectangular-shaped laser-induced graphene (LIG) was used as the working electrode with  $50 \text{ mm} \times 3 \text{ mm}$  dimensions, platinum as the counter, and  $\text{Ag}/\text{AgCl}$  as the reference electrode. The bare LIG electrode was tested with the  $500 \mu\text{M}$  of  $\text{H}_2\text{O}_2$  in phosphate buffer solution. The bare LIG electrode was able to sense the  $\text{H}_2\text{O}_2$  up to a lower concentration of  $0.3 \mu\text{M}$  [34].

In recent work, Behrent et al. performed an excellent parametric study on the Kapton foil by  $\text{CO}_2$  laser engraving. To delimit the area of sensing, nail polish was used. To check the electron transfer rate using the laser engraving, various parameters were optimized as shown in figure S1.b. The electron transfer on the LIG electrode will depend on various inputs such as laser pulse density, laser scribing speed, and laser power. These parameters were varied with a laser power from 60% to 1% and the laser speed from 100% to 10%. The standard mediator, that is,  $5 \text{ mM K}_3\text{Fe}(\text{CN})_6$  and  $0.1 \text{ M KCl}$ , was used to check the proper electron transfer rate. Finally, it was found that lower levels of speed and power combined with high laser pulse density show the best electroanalytical performance. This optimized value was



used to detect the various biochemical analyses such as dopamine, uric acid, ascorbic acid, and paracetamol [35].

Zhu, Bicheng *et al.* fabricated a three-electrode system by laser scribing process using CO<sub>2</sub> laser on the polyimide substrate. The optimized parameters for the scribing graphene electrode were a laser speed of 0.45 cm/s, a laser power of 2.7 W, and 1000 pulse per inch. Furthermore, one of the electrodes was modified with a silver paste that acts as a reference electrode. The working electrode was modified with gold nanoparticles by electrodeposition process, as shown in [Figure S1.c](#). The fabricated device was analyzed and tested with glucose wherein, it showed good sensitivity toward glucose sensing. The lower limit of detection (LOD) was found to be 6.3 μM. The device also offered good sensitivity toward the other interferants such as dopamine, uric acid, and ascorbic acid [36].

### Three-dimensional-printed electroanalytical devices

Three-dimensional printing technology permits to making of three-dimensional solid objects from a designed file. The availability of 3D printing gives the ability to make complex shapes using less material than conventional manufacturing methods. Rapid fabrication, portability, and repeatability are unique features offered by 3D printing microfabrication. Although numerous 3D printing methods exist, only a handful have found mainstream use in production. They include stereo-lithography, fused deposition modeling, selective laser sintering, selective laser melting, and electronic beam melting. Among the aforementioned processes, fused deposition modeling, also mentioned as the fused filament fabrication technique, is the most widely used for desktop printers containing two standard filaments. This process extrudes filaments layer by layer onto a surface [37]. A nozzle-operated system uses filaments that melt and extrude filaments in a motion cycle. Then objects formed create 3D structures by extrusion layer by layer. Using fused deposition modeling technology, printable conductive filaments can be used in various sensors, antennas, metamaterials, filters, and various other applications [38]. The accessories which are used in the 3D printing fabrication are filaments and scrapers. Initially, the 3D model is designed using design software. The design is converted into the standard triangle language (STL), wavefront object (OBJ) file using the computer aided design (CAD) exchanger, Spin 3D software, and other related software. After the conversion of .STL file then again, the model is sliced into hundreds and thousands of layers by using the slicing software [39]. Finally, when the file is cut, it is ready for printing. Then the file is loaded on the 3D printer using USB, SD, or Wi-Fi. Then the sliced file is prepared to print the model layer by layer. [Figure S2.a](#) is the schematic representation of the 3D printing procedure [40].

An impressive number of improvements over conventional manufacturing techniques have been reported.

Katselli *et al.* reported that a dual extruder nozzle was used with filaments of nonconductive polylactic acid and conductive carbon-based filament to fabricate a three-electrode system, as shown in [Figure S2.b](#). The .STL file format was created using Tinkercard software printing conditions mentioned for 3D printing: PLA 200 °C, acrylonitrile butadiene styrene (ABS) 220 °C printing speed of 40 mm/s. The printed miniaturized electrochemical cell was used to test caffeine and paracetamol by using pulse voltammetry. The printed cell was susceptible toward both the analytes [41].

Katseli *et al.* fabricated a miniaturized 3D-printed electrochemical device. The device was printed using carbon conductive filament. The working electrode in the printed device was modified with gold particles by electroplating technique. The sensitivity of the integrated miniaturized cell was enhanced by gold film. The modified filament was successfully tested for the detection of Hg (II). The 3D-printed device was also successfully used to detect the Hg levels in fish oil and water. Anodic stripping voltammetry technique was used to detect Hg(II), as in [Figure S2.c](#) [42].

Rocha *et al.* had also used the similar electrodes and fabrication approach as shown in [Figure S2.d](#), PLA/carbon black composite was used as the working electrode for cadmium and lead determination. To increase the sensitivity of this, the composite electrode was chemically treated with 0.5M NaOH solution. The pretreated electrode shows better results toward the cadmium and lead in saliva and urine samples. The three electrodes, platinum (counter), Ag/AgCl (reference), and carbon black electrode (working), were assembled outer chamber. The outer chamber was printed with nonconductive ABS filament [43]. To limit the working electrode area and to drop the sample, O-ring was used to limit the working electrode area.

For instance, Katseli *et al.* [44] proposed a work where a file was saved in .STL format and transferred to the printer. The carbon-loaded PLA filament was used to print all three electrodes. One electrode was treated as the reference electrode. The other two electrodes serve as the working and the counter electrode. The 3D printer with the printed electrode is shown in [Figure S2.e](#). The printed electrodes do not require any posttreatment with solvents, unlike other 3D-printed carbon-based electrodes. For the proof of concept, the device was characterized in ammonia and acetate buffer solutions by scanning potential from 2.5 V to -2.5 V. The 3D-printed electrodes exhibited clear peaks for caffeine by using differential pulse voltammetry (DPV)



in the range of 0–90 mg/L<sup>-1</sup>. Later, the device was tested with Hg(II) different concentrations. The calculated LOD on this device was 1.9 µg/L<sup>-1</sup> with square wave voltammetry. The device was also tested for glucose by amperometric technique within the concentration ranges of 2–28 mM L<sup>-1</sup>.

Pal et al. designed and fabricated a single-step 3D-printed device for nitrite sensing. The three electrodes were printed with carbon conductive filament, and after that, the electrodes were treated with dimethylformamide as in Figure S2.f. Finally, the device was tested with 600 µL working volume of nitrite and oxidation peak observed at 0.82 V. Furthermore, the developed 3D printed device tested with other electrochemical studies such as scan rate and concentration analysis. The LOD found in this device was 1.96 µM [45].

### Maskless lithography

One of the processes used in the microfabrication of electrochemical devices is photolithography. In conventional photolithography, the mask pattern is transferred using light, and a geometric pattern is projected on the surface of the mask. Conventional photolithography is a relatively complex, sluggish, and inefficient approach to microdimensions and nanodimensions. For this reason, an improved process of the direct laser writer (DLW) approach is developed. DLW uses a precise ultraviolet radiation beam to form the desired geometric features on a substrate layered with photosensitive polymer. DLW technique is ideal for creating dimensions from the nanometer scale to micron dimensions [46]. DLW can be used for liquid photoresists and dry film photoresists of different thicknesses on the various substrates [47].

### Microfluidic electrochemical devices

Microfluidic devices provide the mixing of different reagents, regulate the flow of analytes, increase the flow rate of the analytes, reduce the volume of the analytes (microliter to nanoliter), increase the sensitivity of detection of the samples, and use a similar platform for sample preparation as well as for detection. Combining microfluidics and electrochemical technologies presents the ability to merge chemical and biological components into a single platform, which deals with new opportunities for future sensing applications, including real-time detection, portability, disposability, unparalleled accuracy, and simultaneous investigation of different analytes in a single device. The fabrication of these microfluidic devices is primarily achieved through the conventional soft lithography and laser writing process. The most common substrates that were used in the evolution and the fabrication of these microfluidic electrochemical devices are paper, PDMS, poly (methyl methacrylate), glass, and plastic, thread. Some of the reported articles where they had fabricated paper and

PDMS-based microfluidic electrochemical chemical devices are as follows.

Similarly, Silva et al. [48] reported a simple method to fabricate the linear microchannels in the PDMS substrates, replacing the soft lithography, which requires expensive materials and sophisticated instruments that require photoresists and clean room laboratory facilities. First, silicon mold was used to create microchannels. Then, the PDMS monomer was mixed with the curing agent at the ratio of 9:1. Then, this PDMS mixture was poured on the mold and cured at 70 °C for around 3 hours and then peeled off of PDMS to get microchannel. Furthermore, holes for the reservoirs were drilled using the biopsy punches. Later, a glass slide was cleaned with isopropyl alcohol and dried. Thereon, the pencil graphite was coated with epoxy glue and placed on the glass to cover the microchannel, and then the boundaries of the glass were sealed with the hot glue. Then, the pencil graphite was removed, carbon paste was added to the channel, and the glass slide was placed again cured. Finally, the developed device using electrochemical techniques successfully detected the iodide and ascorbate. Figure S3.a is the reprint of the procedure with copyright permission [48].

Regiart et al. has developed a portable microfluidic-based electrochemical sensor for the detection of sry box transcription factor 2 (SOX-2) cancer biomarker detection. The microfluidic channel geometry was prepared using CAD software. The mold was prepared using negative photoresist (SU-8) conventional lithography technique. The PDMS (elastomer and curing agent of 10:1 ratio) was poured on the SU-8 mold. Then the PDMS was peeled off and bonded on the glass substrate which was sputtered with the three-electrode system. All the three electrodes were prepared with gold using sputtering process. Later on, the PDMS microchannel and the gold-sputtered glass substrate were bonded using the plasma treatment as shown in Figure S3.b. For the detection of SOX-2 detection, the channels were treated with chemical to increase the hydrophilic nature. The SOX-2 antibodies and horseradish peroxidase were sandwiched in the channel. Finally, the quinone and the catechol solution along with H<sub>2</sub>O<sub>2</sub> were reduced on gold electrodes at +0.1 V [49].

Senel et al. fabricated a 3D-printed microfluidic chamber of a diameter of 10 mm and z height of 6 mm. The center of the chamber was manually drilled with a hole of 0.8 mm diameter. The pencil graphite electrodes were inserted into special cavities of the microfluidic chamber as shown in Figure S3.c. A tubing of an outside diameter of 0.762 mm was push-fit in the center of the 3D-printed chamber. Then, the electrochemical activation was performed by cyclic voltammetry for 50

cycles at  $100 \text{ mVs}^{-1}$ . Finally, the device was tested with clozapine and gave the oxidation peak at  $+0.2 \text{ V}$ . The fabricated device can able to detect very lower limits and up to  $24 \text{ nM}$  [50].

### Future outlook — Internet of Things

With the growing demand for the personal and integrated health-care system, there is a huge potential for Internet of Things (IoT) enabled point-of-care devices. IoT provides considerable ease for device interconnectivity, data analysis, and transmission. Miniaturized electrochemical devices, which are very sensitive, are promising for future IoT-enabled wearable biosensors and allied biomedical devices. Some reported works where the miniaturized electrochemical device integrated with a smartphone for various biological sample monitoring.

Alahi *et al.* developed an interdigitated electrode for nitrate sensing on the flame retardant (FR4) capacitance sensor. A smart IoT system was proposed as shown in Figure S4.a. Here, the nitrate sensor was connected to the impedance analyzer analog device (AD5933). The impedance analyzer was used to get the data from the sensor. The impedance analyzer collects the phase shift and impedance data. Later, the analyzed data were sent to IoT cloud server. The impedance analyzer was connected to the Arduino Uno wireless fidelity (WiFi) which acts as a microcontroller. Furthermore, a microcontroller unit was connected to the voltage regulator and power supply [51].

Nagabhooshanam *et al.* developed a micro electrochemical device for the detection of chlorpyrifos. Gold microelectrodes used a three-electrode system where the working electrode was modified with zinc metal-organic framework. The LOD found in the device was around  $6 \text{ ng/L}$ . To transfer data collected from the sensor, it is connected with k-stat (portable potentiostat) [52] as shown in Figure S4.b.

### Summary and conclusion

This review presents a wide range of fundamental aspects of electrode fabrications of miniaturized and microfluidic electrochemical devices, wherein many viable solutions to real-life applications were summarized. It is observed that researchers have attempted different fabrication techniques such as screen printing, ink-jet, 3D printing, laser engraving, and lithography for the development of the miniaturized electrochemical device. The screen printing technique is quite an old technique which is used for a long back in electrochemistry. However, its customized editions for digital printing are yet to come. However, ink-jet printing has emerged as a mature technology for digital and uniform printing of the miniaturized electrodes. Three-dimensional printing and laser engravings are the other promising and emerging technologies that pave the way for

cheaper and higher-quality electrochemical devices. Lithography technique can be used to cater to the need of empathetic submicron range electrode development. To summarize, miniaturized and microfluidics fabrication methods are an emerging multidisciplinary research subject with a wide range of biochemical sensing applications. These platforms are ideal for applications including wearable biosensors, point-of-care devices, forensic testing, drug delivery and screening platforms, and microreactors for *in situ* productions of different chemicals owing to their low cost, portability, and disposable nature. It is possible to meet a vast majority of market needs by mixing and adapting these surface modification materials with the appropriate production procedures.

Furthermore, MMEC devices can attain industrial-scale production through a sequence of improvements in interrelated research domains. MMEC has received a lot of attention from researchers worldwide, even though it is still in its infancy. As a result, this field is expected to rapidly extend its understanding and utility to diverse applications and thereby address some of current most serious issues. Integration of MMEC devices with IoT can also aid in transmitting analytical data to different users and stakeholders. The further integration of MMEC devices with smartphone technology may improve specificity and sensitivity in the detection system's performance. These MMEC platforms coupled with electrochemical detectors and IoT will be critical in growing scientific knowledge in bio/chemical sensing in the near future.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.coelec.2021.100930>.

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- \* of special interest
- \*\* of outstanding interest

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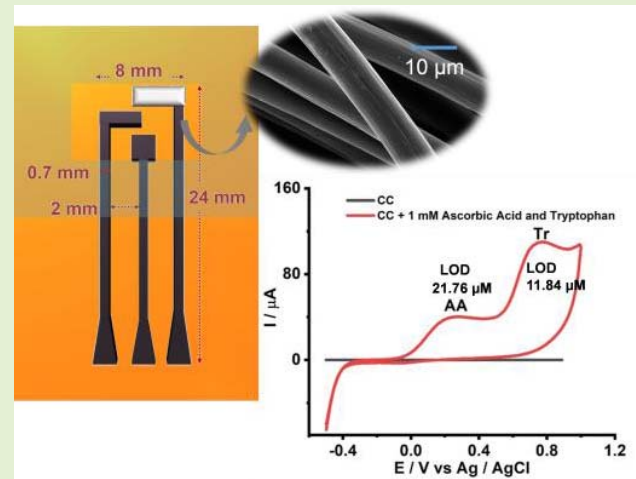


# Carbon Cloth-Based Electrochemical Device for Specific and Sensitive Detection of Ascorbic Acid and Tryptophan

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**Abstract**—Herein, a novel, flexible and inexpensive carbon-cloth based electrochemical device (CCED) is proposed with polyimide (PI) sheet as a base substrate. CC, embedded with numerous microfibers, was used as a sensing substrate. A CO<sub>2</sub> laser was employed for laser cutting three CC electrodes with desired dimensions, which were bonded on the PI sheet using a double-sided tape. Unmodified CC was used as working and counter electrodes, whereas Ag/AgCl ink modified CC was a reference electrode. For creating a hydrophobic region and insulating the electrodes, lamination process was used. Further ascorbic acid (AA) and tryptophan (Tr) were used as analytes for sensing with natural and unmodified CCED surface. The electrochemical response was analyzed using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). Under optimized conditions, the CCED was observed to be capable of determining AA and Tr in a neutral pH (phosphate buffer solution) at 0.4 V and 0.6 V in a linear range of 30–8000 μM and 20–6000 μM, with LOD of 21.76 μM, and 11.84 μM respectively at (S/N=3). Additionally, it was thoroughly investigated for common interfering species including Xanthine (Xn), Hypoxanthine (Hx), Uric acid (UA), Cysteine (Cy), Glucose (G) and Folic Acid (FA). The sensing platform was successfully applied for determination of AA and Tr in human real serum sample with appreciable recoveries.

**Index Terms**—Carbon cloth, carbon cloth-based electrochemical device (CCED), ascorbic acid, tryptophan.



## I. INTRODUCTION

MITIGATED and simultaneous detection of multiple analytes in a heterogeneous mixture is of great significance for the diagnosis of multiple ailments. Simultaneous detection offers advantages like rapid response, cost-effective as one more analyte can be determined together, for compound where peak to peak difference is very small by altering the experimental parameters [1]. For early-stage detection, selective and sensitive analytical methods are of great interest. In this regard, several research groups are working on developing sensors for simultaneous detection of biomolecules and biomarkers for health management. Vitamins and amino acids are biomarkers

that indicate the well-being of a physiological system. Ascorbic acid (AA) or vitamin C is one such essential vitamins in human nutrition which is responsible for the enzymatic production of a particular neurotransmitter in the mammalian brain [2] [3]. AA plays a significant role in synthesizing triple collagen helix and its deficiency or surplus may cause disease like scurvy nephrolithiasis [4]–[6]. AA has been used as an antioxidant in food, beverages, animal, and pharmaceutical formulation. Likewise, L-Tryptophan (Tr), one of the important amino acids, is involved in the metabolism of the human body, establishment, and maintenance of positive nitrogen balance, and acts as a precursor of neurotransmitter and hormones like serotonin, vitamin B<sub>3</sub>, melatonin, dopamine or epinephrine [7]. Due to its biological significance, Tr can be easily obtained from different food sources as it cannot be biologically prepared. Lack of Tr leads to stress, depression, and even schizophrenia [8]–[11]. Considering the significance of these molecules in physiological fluids, like human blood and urine, it is essential to determine an accurate and selective method for the simultaneous and quantitative sensing of AA and Tr. The minimum normal concentration of AA and Tr

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in human body is 0.6 - 2 mg/dl and 0.735 - 0.80 mg/dl respectively. However, during the food intake these values are 75 - 125 mg /day (AA) and 900 -1000 mg/day (Tr) [12], [13]. A major issue for simultaneous sensing of AA and Tr is that they undergo the same potential using conventional glassy carbon or gold electrode with a distinct fouling outcome, which results in poor reproducibility [14]–[18]. Till now, several analytical methods, like capillary electrophoresis, high-performance liquid chromatography, and fluorescence, have been reported to address this issue [19], [20]. Chromatography techniques are selective, precise, and accurate but they require complex sample preparation which needs a considerable amount of time. On the contrary, electrochemical analysis has received mix attention to detect electro-active analytes as it is cost-effective, easy operation, high sensitivity, and quite selective response [21].

In recent times, significant efforts are made for exploring the possibilities to develop miniaturized and portable devices that render better sensing and excellent sustainability comprising disposable screen-printed electrodes (SPEs), and wearable sensors [22], [23]. All these devices offer precise ability with outstanding adaptability, operability, miniaturized, low-volume consumption for analysis, and less time for processing. The working electrode surface in the miniaturized devices is usually modified using carbon, metal, or nanomaterial [24], [25]. Recently,  $\mu$ PADs have attracted the attention of the researchers for the development of unique platform due to the advantage like cost-effectiveness, disposability, biocompatibility, and portability [26], [27]. However, the mechanical and electrical stability of  $\mu$ PADs can be affected by the surrounding moisture due to the hydrophilic nature of the paper. Therefore, it is crucial to find a simpler and effective way for enhancing the stability and performance of the material used in the development of the sensor.

To overcome this issue, herein, a carbon fiber in the form of cloth (CC) has been utilized to advance electrochemistry with new sensing design. CC, embedded woven fibers, has a large active surface area of 3D carbon fiber matrix, outstanding electrical properties, good corrosion strength, high conductivity, current transmission connectivity, and porosity [28]. While comparing with other carbon-based materials, like graphene, carbon nanotube and activated carbon, the most appealing advantage of CC is outstanding mechanical strength with superior flexibility and mechanical integrated. As an inexpensive carbon material, carbon cloth (CC) is composed of plentiful uniform carbon microfiber of  $\sim 5$  to 10  $\mu$ m diameter. CNT or graphene based thin films are synthesized using vacuum filtration while still these films suffer from poor mechanical strength but in contrast CC can be directly employed as a freestanding band flexible electrode for electrochemical sensor and energy storage application and this can be easily integrated into a miniaturized device with a tiny size and variety of shapes. However, very few efforts have been made in using CC as a working electrode for electrochemical sensor. CC can be easily functionalized to detect specific analytes, flexible supercapacitor and various electronic devices [29], [30].

In the past few years, CC, modified with transition metal oxide/ hydroxide, has been extensively used in improving the sensing performance of electrochemical sensors and energy

storage [29], [30]. Lu et al used CC as a base substrate for fabricating asymmetric supercapacitor, zinc ion batteries, and metal-air battery [31]. Similarly, Chun et al used CC for wearable and a skin-adhesive electronic sensor for analyzing human activities [32]. Jayapiriya *et al.* have reported CC for mediator less enzymatic biofuel cell (EBFC) [33]. Furthermore, an inexpensive, natural abundance and required mechanical properties of CC can help in reducing the difficulty in developing an integrated device. Such attributes have made CC an appropriate sensing substrate.

Various flexible substrates that are widely used in developing miniaturized devices enabling fluid flow control in the microchannel are paper, cloth, plastic, and polymer [26], [34], [35]. Polyimide (PI) films are one of the most widely used flexible and robust substrates for the development of flexible electrochemical sensors or electronic devices due to their outstanding mechanical, electrical, chemical properties. Further, polyimide film has an inert and highly hydrophobic surface that can be used for the development of the flexible electrochemical sensor. The flexible electrochemical sensor can be extensively used for development of various wearable application for the selective and sensitive detection of target analytes with the bodily fluid. The non-invasive sensing of the target analytes is the main reason for development of flexible electrochemical sensing.

This work delves upon developing a flexible CC-based electrochemical device (CCED) using CC as a sensing substrate, without any pre-processing. CCED consists of CC as working and counter electrodes, Ag/AgCl ink modified CC as a reference electrode, and a PI sheet as a base substrate. The CCED was constructed using a CO<sub>2</sub> laser whereby the PI sheet and the three-electrode CC system of the desired shape were laser cut and were attached to the PI sheet using an adhesive tape. The developed flexible CCED provides a new way to fabricate various sensing platforms and has a wide range of applications for simultaneous detection of multiple analytes at a point-of-care testing (POCT) level. As a proof of concept, the developed CCED was applied for the detection of AA and Tr. The fabricated CCED provides exceptional, increased sensitivity, good selectivity, and excellent electrocatalytic activity towards these analytes, and by modifying the working electrode (WE) surface, the same platform can be used for diverse sensing applications.

## II. EXPERIMENTAL

### A. Chemical and Reagents

All the chemicals were of analytical grade and were used as received, and all the aqueous solutions were prepared using milli-Q grade ultrapure water with 18.2 M $\Omega$  cm resistivity. Ascorbic acid (AA), Tryptophan (Tr), Xanthine (Xn), Hypoxanthine (Hx), Uric acid (UA), Cysteine (Cy), Glucose (G) and Folic Acid (FA) were obtained from Sigma (St. Louis, MO, USA), sodium phosphate monobasic anhydrous (NaH<sub>2</sub>PO<sub>4</sub>), and sodium phosphate dibasic dihydrate (Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O) from SRL Chemicals (Mumbai, India). Commercially available hydrophilic CC (Fuel Cell Store, USA) was utilized without any further treatment. The polyimide film (Kapton  $\pm$  125  $\mu$ m) was purchased from Dali Electronics.

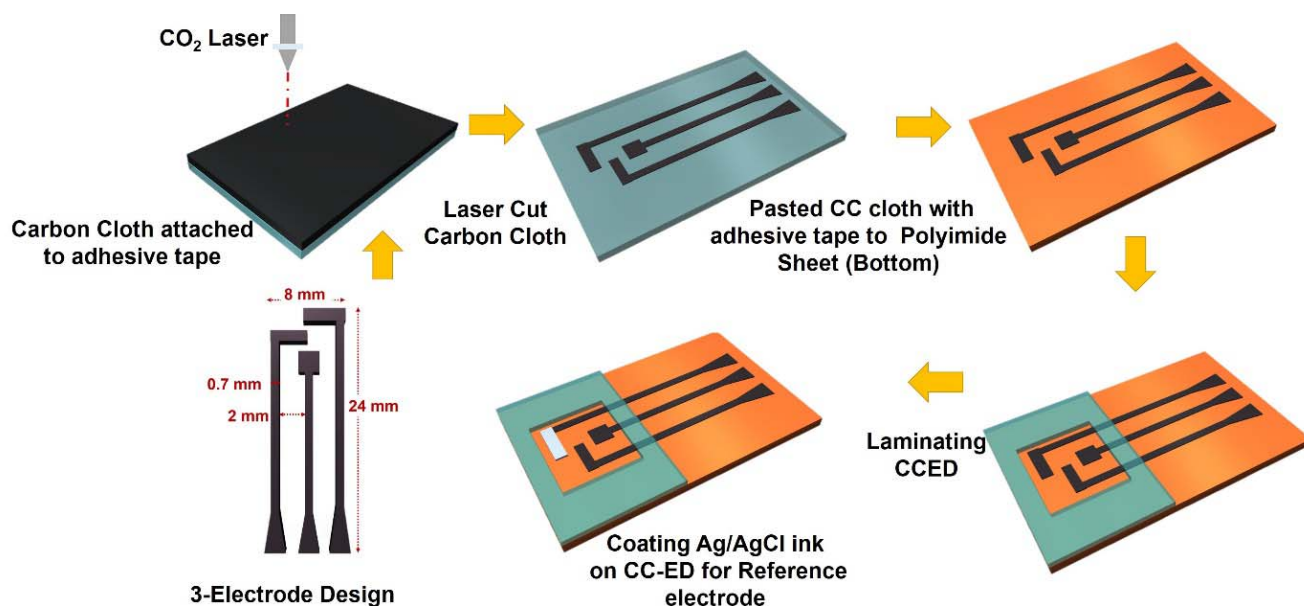


Fig. 1. Schematic representation of the process of fabricating carbon-cloth based electrochemical device (CCED).

### B. Apparatus

Electrochemical measurements of AA and Tr were performed using 0.1 mol/L phosphate buffer solution ( $\text{pH} = 7$ ) as a working electrolyte. Silver-Silver chloride (Ag/AgCl) ink was obtained from ALS Co. Ltd., Tokyo Japan. Electrochemical measurements were performed using a Electrochemical Workstation (SP-150 from Biologic, France) with a three-electrode system consisting of CC as a working and counter electrode and Ag/AgCl ink coated CC as a reference electrode.

A  $\text{CO}_2$  laser scriber (VLS 3.60 from Universal Laser System, Inc., AZ, USA), was used for laser cutting of PI sheets and CC. CC characterizations were performed using JSM-7610F field emission scanning electron microscope (FESEM) from JEOL Ltd (Tokyo, Japan). The pH meter (Oakton, Singapore) was used for measuring the accurate pH value of the required solution. For the standardization of the result, so that CC absorbed the sample volume ( $100 \mu\text{L}$ ) was established to be 60 s before starting the experiment.

### C. Design and Fabrication of the CCED

The designing and fabrication process of CCED is illustrated in Fig. 1. The overall size of the CCED was  $15 \text{ mm} \times 30 \text{ mm}$  with each electrode size was  $8 \text{ mm} \times 24 \text{ mm}$ . The parameters, like dimension and spacing between the electrode, were kept minimal so that the distinct peak with better sensitivity could be obtained. The working electrode (WE) was kept adjacent to the reference electrode (RE) to reduce the uncompensated resistance and the counter electrode (CE) was kept greater than the WE and RE so that unlimited current can transfer through the circuit.

The layout of the electrode design and the flexible substrate of the desired dimensions was designed in Corel Draw and then was transferred to the  $\text{CO}_2$  laser software. The CC was attached to an adhesive tape and then it was laser cut in the desired shape using a  $\text{CO}_2$  laser cutter. The PI sheet was

laser cut in rectangular-shaped ( $10 \text{ mm} \times 30 \text{ mm}$ ) and then the patterned CC 3 electrode system was attached to the PI sheet. Ag/AgCl ink was coated using a paintbrush on CC for preparation of reference electrode and was left for air drying for 15-30 mins at  $60^\circ\text{C}$ . A transparent polyester sheet, obtained from a local stationary shop, was used for a lamination process, to create a hydrophobic region on the developed CCED. To create the hydrophobic region, a rectangular shape was laser cut on a polyester sheet and then developed CCED was laminated using a laminator. The primary benefit of this fabrication process was that the design can be easily modified to form an electrode array to perform multiple electrochemical sensing.

### D. Electro-Catalysis of AA and Tr With CCED

The fabricated CCED was subjected to the electrocatalytic sensing of AA [ $1 \text{ mM}$ ] and Tr [ $1 \text{ mM}$ ] with  $100 \mu\text{L}$  of the target analytes. Cyclic voltammetry (CV) characterizations were performed in the voltage range between  $-0.5 \text{ V}$  to  $+1 \text{ V}$  at a scan rate of  $10 \text{ mV/s}$  for 2 cycles.

### E. Real Sample Preparation

Blood serum sample were collected from the Medical Centre at the campus and it was used without any further treatment. The dilution was 1:5 of serum in PBS for analyzing the trace of AA and Tr. The samples were single blind test. The chemical concentration of the analyte in the real sample was discretely measured. The obtained vitamin C was ground in a mortar, then the ground powder ( $1 \text{ mg}$ ) was dissolved in PBS ( $1 \text{ ml}$ ) and sonicated for 15 mins. The sample was then filtered and was used for analysis. The samples were analyzed blindly and concentrations were derived from the calibration curves.

## III. RESULT AND DISCUSSION

### A. Physico-Chemical Characterization

Fig. 2 a and b show the SEM images of CC at different magnifications revealing that CC has a microfiber structure of



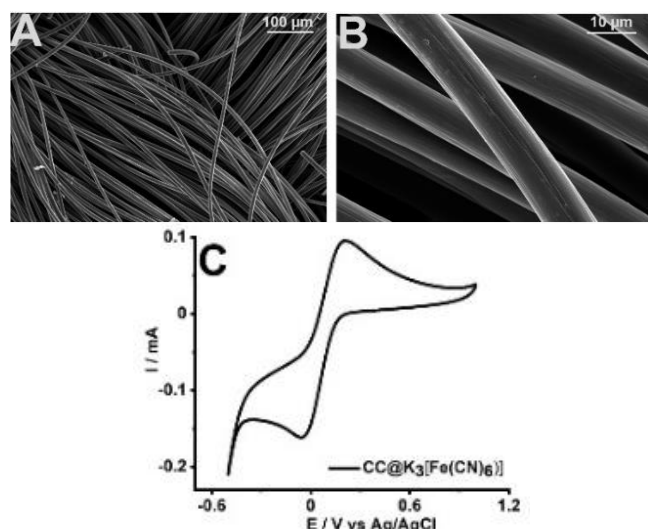


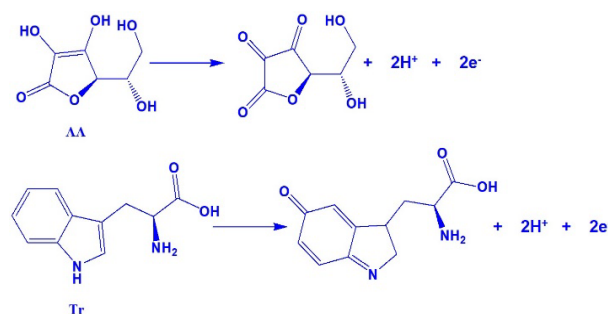
Fig. 2. SEM images of CC at different magnifications (A) 100  $\mu\text{m}$ . (B) 10  $\mu\text{m}$ . (C) Cyclic voltammetry response of CC in  $\text{K}_3[\text{Fe}(\text{CN})_6]$  [5 mM] KCl [1 mM] at 50 mV s<sup>-1</sup>, for 10 cycles.

an average diameter of  $8.5 \pm 0.5 \mu\text{m}$ . This type of woven textile structure (Fig. 2 b) gives a porous layout facilitating a greater surface area [33], [36].

Fig. 2 c shows the CV response for potassium ferricyanide  $\text{K}_3[\text{Fe}(\text{CN})_6]$  [5 mM] - potassium chloride (KCl) [1 mM] in a potential window of  $-0.5 \text{ V}$  to  $1 \text{ V}$  at 50 mV/s. CCED shows a reversible redox peak (I<sub>pa</sub>/I<sub>pc</sub>) for  $\text{K}_3[\text{Fe}(\text{CN})_6]$  as 0.5, with peak separation of 0.117 V as its E<sub>pa</sub> = +0.2 V and E<sub>pc</sub> =  $-0.035 \text{ V}$  [37]. This attributes to the redox couple electrochemicals which facilitates the mass transfer of ionic species towards the electrode surface [38]. The T value, that is surface excess value of electrode can be calculated using  $T = -nAF/Q$ . Where n = no of electrons, A is surface area of working electrode, F-Faraday Constant, Q = charge from CV. With positive charge value 0.337 mC  $T = 2.86 \times 10^{-8} \text{ mol/cm}$ .

### B. Electrocatalytic Determination of AA and Tr With CCED

AA and Tr were selected as target analytes for understanding the functionality of CCED. Cyclic voltammetry (CV) behavior of CCED was investigated by addition of AA [1 mM] and Tr [1 mM] in 0.1 mol L<sup>-1</sup> PBS (pH 7), respectively. In the absence of AA and Tr, there were no redox peak in the potential scan range. Fig. 3 a and b show the CV response with and without the presence of analytes respectively in the potential window of  $-0.5 \text{ V}$  to  $1 \text{ V}$ . A distinct oxidation peak was observed at  $\sim 0.4 \text{ V}$  for AA (1 mM) and  $\sim 0.6 \text{ V}$  for Tr (1 mM) at a scan rate of 10 mV/S signifying that the prepared sensor gave electrocatalytic oxidation of the analytes [39], [40]. Fig. 3 c shows the CV response for simultaneous sensing of AA and Tr with distinct oxidation peak at  $\sim 0.3 \text{ V} \pm 0.06$  (AA) and  $\sim 0.72 \text{ V} \pm 0.05$  (Tr). The plausible reason behind the increase current value of tryptophan may be that ascorbic acid also help in oxidation of tryptophan [41]. Further, the flexibility of the CCED was analyzed for the sensing analyte (AA and Tr). To evaluate the flexibility, AA and



SCHEME. 1. Electrochemical oxidation of ascorbic acid (AA) and tryptophan (Tr) [33], [34].

Tr were measured before and after continuously bending the CCED against a 10 mm cylinder for 100 times Fig. 3 d and e show the CV response for AA and Tr before and after bending and there was negligible reduction ( $\sim 5 - 7 \%$ ) in the sensitivity of the CCED, revealing that the CCED is a promising platform for development of flexible electrochemical sensor.

### C. Effect of pH on AA and Tr Using CCED

The electro-oxidation behavior of AA and Tr were studied in the pH range of 3 to 10 phosphate buffer solution [0.1 M]. It was observed that with the increase in pH, the oxidation peak shift to the less positive potential (Fig. 4 a -b). In case of AA, maximum current was obtained in pH 3 while in case of tryptophan no peaks were observed in pH 3 - 4 and max current was observed in pH 5. The relationship between the potential peak vs. pH (Fig. 4 c) appears linear with a slope value of  $-36 \text{ mV} \pm 3.4 \text{ mV pH}^{-1}$  (AA) and  $-25 \text{ mV} \pm 1.7 \text{ mV pH}^{-1}$  (Tr) [42].

This evidently illustrates the Nernstian behavior which shows that the complete process is dependent on proton and electron transfer is accompanied by equal number of electron and proton transfer involves in the reaction as shown in the Scheme 1 [43], [44]. Herewith, CCED was used for target analyte sensing in neutral pH = 7 for smooth simultaneous electrochemical sensing of AA and Tr applicable to real samples as well

### D. Effect of Variable Potential Scan-Rate on CCED

CV analysis was performed by applying an increasing potential scan rate for understanding the electrochemical electron transfer mechanism of AA and Tr oxidation with CCED. Fig. 5 a and c illustrate the increase in anodic peak current with an increase in the scan rate potential. Fig. 5 a and c show that the scan rate  $v^{1/2}$ , is directly proportional to the anodic peak current and Fig. 5 b and d show the baseline- corrected linear fit resembling surface-confined electrode transfer mechanism. Randles Sevcik's equations are usually utilized in this type of surface-confined electrons transfer reaction.

$$i_p = 2.69 \times 10^5 A_e D^{1/2} n^{3/2} v^{1/2} C \quad (1)$$

where,  $i_p$  = peak current,  $A_e$  = electrode surface area ( $\text{cm}^2$ ),  $n$  = number of electron transferred,  $v$  = scan rate ( $\text{V/S}$ ),  $C$  = concentration of electroactive species ( $\text{mol m}^{-3}$ ),  $D$  = diffusion coefficient equals to  $8.95 \times 10^{-15} \text{ cm}^2/\text{S}$  [AA] and  $7.13 \times 10^{-15} \text{ cm}^2/\text{S}$  [Tr]. As shown in Fig. 4 b and d,



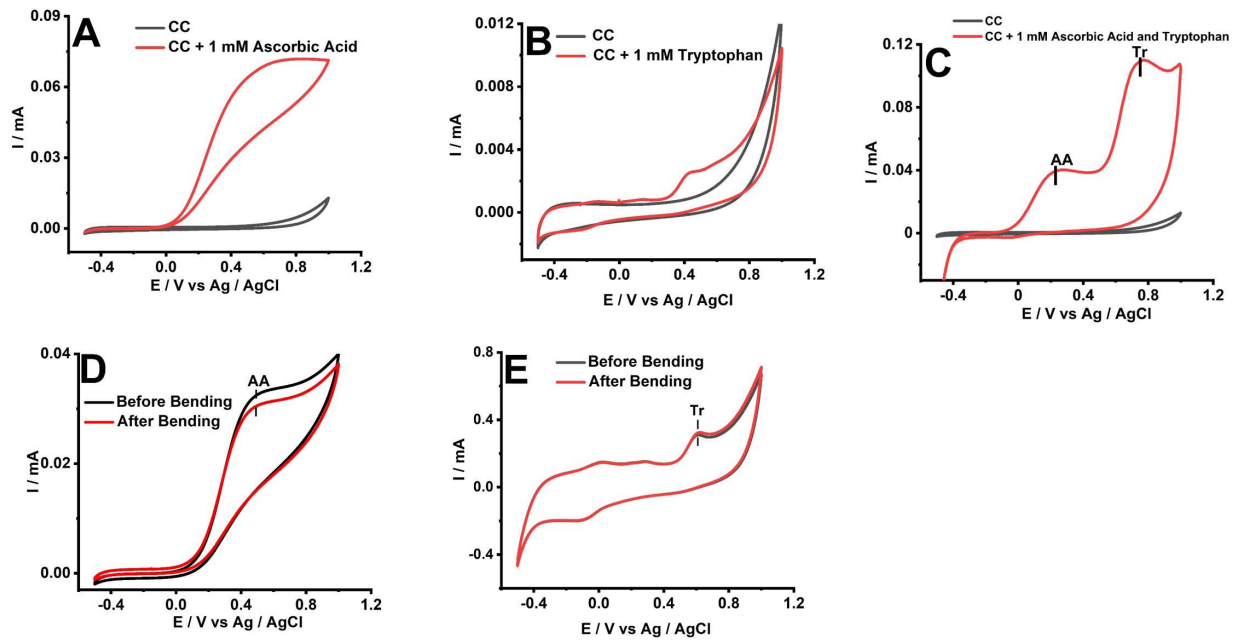


Fig. 3. CV response in 0.1 M PBS (pH = 7) solution (A) AA [1 mM], (B) Tr [1 mM], (C) AA + Tr (1 mM) at 10 mV/S and (D) AA [1 mM], and (E) Tr [1 mM] at 50 mV/S.

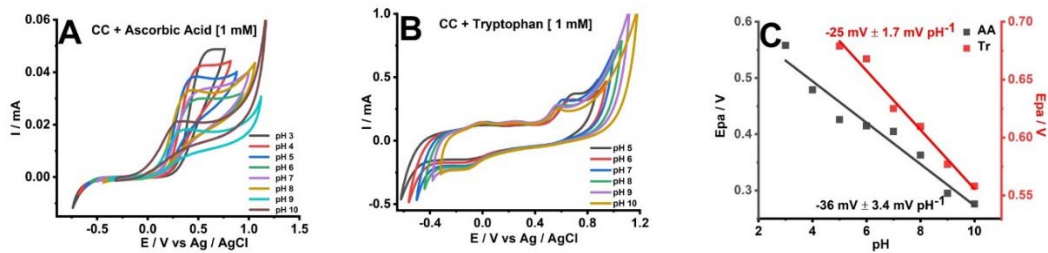


Fig. 4. CV response in 0.1 M PBS solution at 10 mV/S. (A) AA [1 mM] (pH=3–10). (B) Tr [1 mM] (pH = 5–10). (C) Linear fit response for Epa vs. pH for AA and Tr.

a calibration plot, giving slope value as 0.5, authenticates that the electro-oxidation of AA and Tr was a diffusion-controlled reaction. Using Laviron equation, for an irreversible electrode process  $E_p$  is calculated using the following equation (1) [45].

$$E_p = E^{\circ'} + \left[ \frac{2.300RT}{anF} \right] \log \left( \frac{RTk^{\circ}}{anF} \right) + \left( \frac{2.303RT}{anF} \right) \log v \quad (2)$$

where,  $\alpha$  = transfer coefficient,  $k^{\circ}$  = standard heterogeneous rate constant of the irreversible electrode reaction,  $n$  = number of electron transferred,  $v$  = the scan rate and  $E^{\circ'}$  = formal redox potential.

The  $an$  from slope and  $k^{\circ}$  from intercept were calculated using calibration plot of  $\log v$  vs.  $E_p$  and  $E^{\circ'}$  from intercept using calibration plot of  $v$  vs.  $E_p$ .  $\alpha$  was estimated using Bard and Faulkner equation (2) [46].

$$\alpha = \frac{47.7}{E_p - E_{p/2}} \text{ mV} \quad (3)$$

where  $E_{p/2}$  is the potential, when the current is half the peak value. Using Equation (2) and (3) for AA  $\alpha$  was calculated as 0.79 and  $n$  over the range of (1.75 – 2) and for Tr,  $\alpha$  was 0.9 and  $n$  was (1.9 - 2) [46].

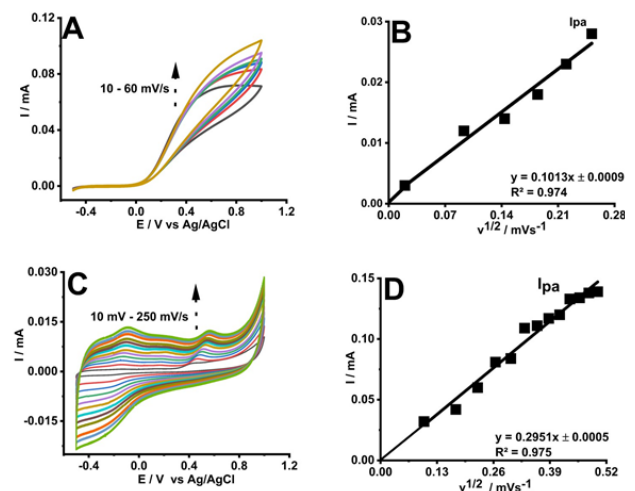


Fig. 5. (A) Comparative CV at variable scan rate for AA. (B) Corresponding  $I$  vs.  $v^{1/2}$ . (C) Comparative CV at variable scan rate for Tr. (D) Corresponding  $I$  vs.  $v^{1/2}$ .

### E. Individual Concentration Effect of AA and Tr

For analytical application, to understand the concentration effect, varying AA and Tr concentrations was used by investigating the upper and lower limit range. The specific sensing of analytes was performed using differential pulse

TABLE I  
COMPARISON TABLE WITH REPORTED LITERATURE

Electrode	Technique	Linear Range ( $\mu\text{M}$ )		LOD ( $\mu\text{M}$ )		Sample
		AA	Tr	AA	Tr	
Screen-printed graphene electrode [21]	DPV	4-4500	-	0.95	-	Human urine
Toray Paper [33]	SWV	100-1000	-	97.1	-	Human serum
(GNPs) on overoxidized-polyimidazole (PI <sub>ox</sub> ) film modified glassy carbon electrode (GCE)[15]	DPV	210-1010	3 - 464	0.136	0.770	Human urine, serum, Vitamin C tablets
carbon black nanoballs (CBNB) / carbon nanotubes (CNTs)/GCE [16]	SWV	20-400	0.025-4.8	5.71	0.011	Human urine, Vitamin C injections, milk
Carbon Cloth (this work)	DPV	30-8000	20-6000	16.85	7.69	Human serum

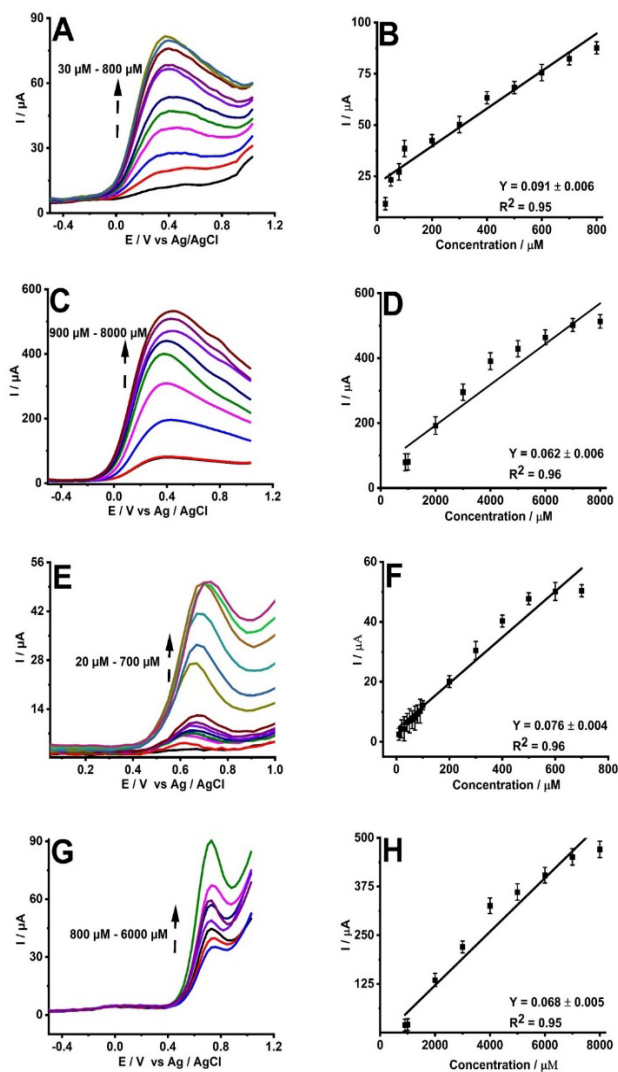


FIG. 6. Differential pulse voltammetry (DPV) for low (A) and high concentration (C) of AA. (B) and (D) corresponding calibration plots for AA. DPV for low (E) and high (G) concentration of Tr. (F) and (H) corresponding calibration plot.

voltammetry (DPV) under the optimized parameter as initial potential as  $-0.5$  V, final potential as  $1$  V, pulse height (PH) as  $50$  mV, pulse width as  $15$  ms, step height  $30$  mV and step time as  $30$  ms. Fig. 6 a, c, e, g depict that CC gives a good response with an increase in concentration range for AA and Tr respectively. Fig. 6 a and e show linear response for the concentration range of  $30$   $\mu\text{M}$  to  $800$   $\mu\text{M}$  for AA and

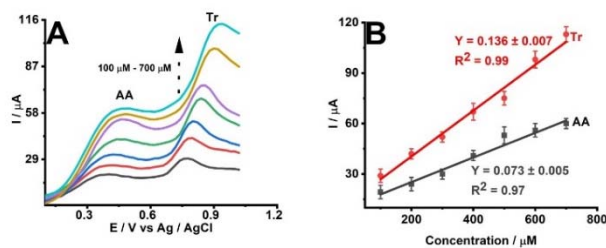


FIG. 7. (A) and (B) Differential pulse voltammetry (DPV) for simultaneous concentration effect of AA and Tr with its corresponding calibration plot.

$20$   $\mu\text{M}$  to  $700$   $\mu\text{M}$  for Tr, with a limit of detection to be around  $21.76$   $\mu\text{M}$  and  $11.84$   $\mu\text{M}$  for AA and Tr respectively calculated with the linear fit obtained from Fig. 6.b and f. Fig.6 c and g show the DPV response for higher concentration range with the corresponding linear fit (Fig. 6.d and h) in  $900$   $\mu\text{M}$  to  $800$   $\mu\text{M}$  for AA and  $800$   $\mu\text{M}$  to  $6000$   $\mu\text{M}$  for Tr.

Since, no reports are available on using unmodified CC for AA and Tr sensing, a couple of recent articles using a screen-printed electrode,  $\mu\text{Pad}$  were explored and summarized in Table I. Although these articles show better LoD, but they involve electrode surface modifications with various redox mediators and carbon materials. However, herein, the work demonstrates the unmodified CC for electrochemical analysis. Therefore, a skilled and time-consuming step of electrode surface modification is avoided. In addition, this also cuts down the cost of the sensor fabrication, hence, easy to commercialize.

### F. Simultaneous Electrocatalytic Oxidation of AA and Tr

The CCED was employed to study the simultaneous electrocatalytic oxidation of AA and Tr. DPV was carried out for within a potential window range of  $-0.5$  V to  $1$  V, pulse height as  $50$  mV, pulse width as  $30$  ms, step height as  $20$  mV, step time as  $40$  ms and step percentage as  $20$ . Fig. 7 a shows that the CCED gives well defined distinguished anodic peak at  $\sim 0.35 \pm 0.05$  V for AA and  $\sim 0.735 \pm 0.07$  V for Tr. An increase in the oxidation peaks were observed, when the concentration of the analytes were increase, which indicate that CCED helped the target analytes to oxidize at their corresponding potential. The achieved anodic peak current linearly increased with the concentration of the analytes in the range of  $100$   $\mu\text{M}$  to  $700$   $\mu\text{M}$  with lower detection limit of  $82.19$  (AA) and  $88.23$   $\mu\text{M}$  (Tr) (Fig. 7 b). Although there is a negligible shift observed in the peak potential due to presence

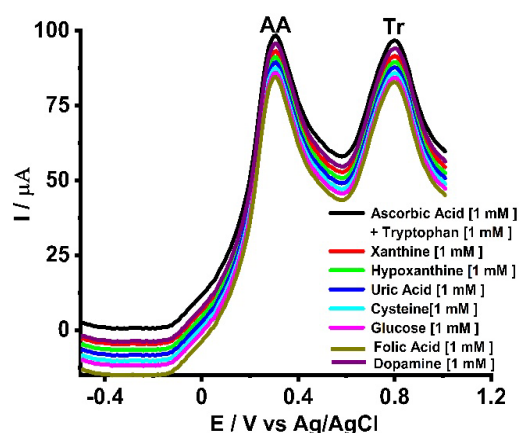


FIG. 8. Differential Pulse Voltammetry (DPV) response for effect of interference with AA [1 mM] and Tr [1 mM] with other biochemicals [1 mM].

TABLE II  
REAL SAMPLE ANALYSIS

Sample	Analytes	Detected (μM)	Added (μM)	Found (μM)	Recovery %
Human Serum	Ascorbic Acid	35	30	66.074	101.652
			40	74.153	98.870
	Tryptophan	15	50	85.123	100.144
			20	34.456	98.445
Vitamin C Tablets	Ascorbic Acid	50	30	46.175	102.611
			40	57.863	105.205
			50	81.241	101.551
	Ascorbic Acid		40	92.146	102.384
			50	98.183	98.183

of the high concentration of other analyte, yet distinct peaks can be seen manifesting no interference from each other.

### G. Reproducibility and Interference Effect

The reproducibility of the developed CCED, with disposable electrodes, was checked by measuring the response towards 1 mM AA and Tr using CCED under the same condition for successive 7 times. The CCED shows a similar DPV response with a relative standard deviation of 4.1% specifying good reproducibility. Further, evaluating the selectivity, the CC was tested with various biochemical to analyze the interference effect. Herein, biochemical like Xanthine (Xn), Hypoxanthine (Hx), Uric acid (UA), Cysteine (Cy), Glucose (G), and Folic Acid (FA) were analyzed in presence of 1 mM AA and Tr using DPV with optimized parameters. Fig. 8 shows the distinct peaks of AA and Tr at 0.3 and 0.8 V respectively and in the presence of interfering biochemicals reveal no distinct peak and negligible changes in current value. Thus, the developed CCED not only gives good stability and reproducibility towards AA and Tr sensing, but also selective response even in the presence of interfering biochemical that results in the wide practical applications.

### H. Real Sample Analysis

For determining the future applications and precision of the developed CCED, it was applied for AA and Tr sensing using a standard addition approach in a human serum sample and vitamin C tablets. For three spikes, the concentration was in

the range of (30 μM, 40 μM, 50 μM) for AA and (20 μM, 30 μM, 40 μM) for Tr for analysis. The recovery was found in the range of 98% to 105 % as shown in Table II. This proves that the proposed sensor gives accurate and reliable detection of AA and Tr in the serum sample.

## IV. CONCLUSION

In this work, a simple fabrication technique for CCED has been presented by direct transfer of CC electrode as a sensor on PI sheet as a supporting substrate for determination of AA and Tr. The CCED was successfully applied for determining AA and Tr with a corresponding distinct oxidation peak at 0.4 V and 0.6 V respectively. Effect of scan rate, solution pH, variable concentration, and interference from other biochemical were explored. Further CCED was applied for real time analysis in human serum samples validating its practicability. While comparing it with conventional techniques, fabrication of such flexible CCED avoids complicating techniques, tedious pretreatment process, and costly electrode material. CC is found to be a very promising material in electrochemical biosensor due to the characteristics such as easy and versatile surface modification, sensitive and amenability to facile integration with miniaturized and portable devices. This will be favorable for the future development and plays a key role in the advancements of flexible, wearable, and sensitive sensor for biological application in addition to the proposed prototype of CCED. Such flexible electrochemical sensor provides diverse opportunities for real time application in health diagnostic, continuous human body health monitoring with the help of conventional electrochemical methods like potentiometric or amperometric technique using various bodily fluids like blood, saliva, sweat and urine.

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# Artificial Intelligence In Training And Development For Employees With Reference To Selected It Companies

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## ABSTRACT

The scope of Artificial intelligence is expanding with today's technology. Organizations collaborate with holistic functions rather than isolated processes. As a result, AI can build teachable systems that can both evaluate content and take action. AI also make it easier for systems to pair and collaborate with one another. This is an excellent starting point for new and exciting opportunities. The present study focused on the adoption of AI and influencing factors in the effective implementation. The study has considered the convenient sampling method for the collection of primary data from the employees, who have gone through the training with the AI in the organization. The study has considered the Technology adoption model to understand the usability i.e. perceived ease of use and intention to adopt the AI with the support of statistical method of SEM. The study applied the machine learning statistical method of Neural network to identify the influencing factors for the effective implementation of AI in the IT companies for the training and development. The study result indicate that AI will make an employee adopt the AI and learn the usage, which will help them to take new decisions and plan for projects and will enrich the employee's development.

**Key words:** AI, Training and development, Machine learning, Neural networks.

## INTRODUCTION

Artificial intelligence (AI) is increasingly transforming every part of people's lives, including our workplaces and professional lives. In this paper, the study explains how AI will impact learning development and employee training. Today, almost every company and business is witnessing the spread of AI. AI-powered tools and applications are rapidly being available in the workplace. There is no question that Artificial Intelligence can play an important role in how we are recruited, on boarded, and inducted into an organization, as well as the professional testing and personal growth

services we obtain. This will eventually pave the foundations for us to pass on our knowledge and insights to the next generation.

Even now, AI-powered solutions silently crunch data in the context and include insights about how to improve efficiency. AI is changing employee recruiting and participation, especially in HR and training and development. With the US corporate training industry worth \$130 billion, companies stand to profit from Artificial Intelligence's potential for improved training

personalization and predictive analytics of learning patterns.

The majority of industry leaders and researchers believe that artificial intelligence (AI) is our future. However, if we consider it carefully, AI is not the future – it is the current! As an example, consider your email service. It employs artificial intelligence to filter emails so that you do not get spam. Amazon and other e-commerce platforms use AI to offer products to consumers based on the knowledge they have gathered. Siri, Alexa, and Google Assistant are turning to artificial intelligence to improve their users' experiences. Despite the fact that technology is still in its early stages of growth, several businesses have already invested large sums in AI, believing that AI-powered products have a bright future.

Why AI is Importance in Learning and Development: Artificial intelligence is the new normal, and it is here to change the way we have learned and developed for millennia. AI has evolved tremendously, paving the way for people to discover limitless ways of absorbing knowledge today. The role of AI in education and learning has revolutionized a learner's experience, and it has been one of the biggest tech trends since 2018. Artificial intelligence has infiltrated every aspect of human life, from social media to retail, coding to warfare. The relationship between humans and artificial intelligence is so interlinked that nothing is possible today without machine intervention. When it comes to learning and development, AI has brought about a lot of convenience and improvement. Today, the world's largest sources of learning are applications and websites that allow people to improve, upskill, and grow.

Learning (Training) and Development is one of the areas that AI has the potential to completely change in the immediate future.

Learning and development professionals must be mindful of constantly evolving technologies to utilize it to improve learning processes. In terms of AI, L&D professionals can investigate and incorporate AI advancements in order to establish innovative teaching methods and techniques. Gartner, a leading consulting and consultancy firm, estimated that by the end of 2020, approximately 85 percent of customer services will be provided by robots (bots) rather than humans. According to another study, AI will deliver approximately 20% of training materials. Furthermore, according to Bank of America, by 2025, AI would be the driving force behind \$14-33 trillion in annual industrial development. The present study focused on the role of AI in the employee training and development with reference to information technology companies. The study has considered the two major organizations, which are extensively utilizing the AI in large scale. The AI adoptability also viable in big size IT companies, as the tech companies always focus on the enhancement of skill set of employees to adopt the latest changes of the globe to serve the clients.

## **STATEMENT OF THE PROBLEM**

Artificial intelligence (AI) is transforming every area of people's lives, including our employment and professional life, at a breakneck pace. In this article, the study will explore how AI will change learning and development and employee training. Artificial intelligence is now present in practically every business and area. AI-powered products and solutions are becoming more common in the workplace. Artificial Intelligence will undoubtedly play a significant part in how we are employed, on boarded, and initiated into organizations, as well as in the skills training and personal development programs we get. This will progressively establish the foundation for passing on our knowledge to the next

generation of workers. (**Eric Premnath and Arun Antony Chully (2019)**)

Despite the fact that technology innovations have changed organizational Training and development over the last decade, Organizational leaders still face several common issues, one of which is the absence of individualized learning. When it comes to urgent skills training or staff training programs as a whole, L&D experts are often accused of providing generic and non-customized learning. The time factor connected with content generation is one element that may be blamed for this. Second, the majority of LMSs on the market have a complex user interface, making it difficult for employees to browse and adapt in the long term. As a consequence of the difficulty in looking for and discovering appropriate learning information, the User Experience suffers. **Richard D. Johnson, Hal G. Gueutal (2011), Sandeep Gandhi. (2017).**

## REVIEW OF LITERATURE

The review of literature collected various papers and articles, which have focused on the usage of artificial intelligence on training and development for employees in organizations.

The artificial intelligence (AI) progress has been made and continues to achieve long-term educational objectives. **Beverly Park Wool (2015)**. The article describes two educational problems that require AI: customizing education and mastering skills from the 21st century. First, this paper discusses AI and some of its history before arguing that AI is so important in the development of educational systems. Instructional programmes which utilize AI technologies, such as analytical resources to customize teaching, to improve student experience and to support new educational theories, are listed. Moreover, several intelligent tutors offer academics new ways to study and

understand large data sets for classroom activities. The teaching and learning environment has evolved unintendedly in the educational system, explained **Nitin Borge (2016)** about artificial intelligence management (AI). New technology can help students more efficiently achieve and maintain their educational goals. With AI, each student in a class that is either a bad student or is too lazy to understand the subjects the teacher has discussed will be better examined. The analysis would provide a good understanding of each subject by the student. If a pupil lags or cannot grasp certain topics in certain fields, this report will be presented to Professors, Tutors or Parents by the AI review to allow appropriate measures to be taken. The AI study can also propose topics for students with simple examples or in a way that is easily understood to develop their skills in the field where they are not sure. In addition to sparing resources, the author found that the ability of information systems to offer this degree of perspective could provide the details that teachers might not have a real face value to understand. **Stefan (2017)** explores the phenomenon of artificial intelligence development and use in the field of higher education and learning. It examines new technology's educational impact on how students learn and how organizations train and develop. In a new environment where artificial intelligence is woven into university fabrics, recent technological advances as well as the growing pace at which emerging innovations in higher education are adopted are being examined. In implementing these teaching, learning, student service and administration innovations and future directions, we identify several obstacles for higher educational institutions and student learning. The digital technology in education and related materials were discussed by **Maud Chassignol (2018)**. The aim of the present paper is to identify and learn about the potential impact of artificial

technology on the learning process. The current literature suggests that teaching approaches, technology assessment and student contact are all important. Artificial intelligence would reshape the educational environment, according to the conclusions.

The objective of this research is to create an innovative and accessible platform for adults to help scientist and researchers develop their own self-deployment or do-it-yourself DIY by means of the virtual reality learning system. By the year 2030, it was most likely that self-exploration was only possible for extremely valuable usage cases, which affected the value proposition against the provision of educational service, improved stakeholder and value focused proposals fostered through constant contacts with the Heutagogical strategy and stakeholders. **Silvia Pokrivcakova (2019)** focuses on the use in foreign language learning and teaching of artificial intelligence (AI) that covers a variety of tools and approaches such as computer learning, adaptive learning, natural language processing, data analysis, crowd-sourcing, neural networking and an algorithm. Firstly, the paper deals with the development of foreign language education resulting from the use of IA-powered technologies and addresses ICALL, as a subset of CALL. Second, it sums up eight forms of foreign language training IA-powered resources and limited relevant findings of the current study. Thirdly, the framework for effective teacher training in foreign languages is addressed, in order to make AI's resources more simple, time consuming and successful in their teaching. The author calls for the current qualification framework for CALL teachers to be rethought. **Paschal Jeannette (2019)** Clarify the technical phenomenon known as artificial intelligence and how can it assist with business-to-business knowledge-based marketing? In particular, this article examines the fundamental building blocks and inter-relationships of each

artificial intelligence framework. This paper discusses the consequences for business awareness in B2B marketing of the different building blocks and outlines the future directions for studies. The paper describes AI by the input-process-output prism and describes the six basic building blocks of every AI system. The interaction of components transforms data into facts and understanding is discussed even. In this article, the phenomenon of artificial intelligence, the way it works and how it relates to the knowledge marketing in B2bo companies are discussed, aimed at general marketing leaders rather than IP experts. The paper focuses on the examples of how B2B marketing functions can be affected by IA.

In the teaching of increased reality courses, **Alexandra Klimova (2020)** has conducted an examination of the present trend. The objective of the study is to assess students, scientists and policy makers to inform them on learning strategies, objectives, expertise and skills in the area of enhanced reality. Due to the rapid growth of Augmented Reality technology and the growing scope of its implementations, highly trained professionals in this industry are in strong demand. **Xieling Chen (2020)** has been investigating influential AIED studies in an exhausted and systematic way. The author has examined 45 publications, most commonly used terms and theories and innovations, in terms of annual circulation, top papers, organizations, countries/regions. We compare and explain the relationship between AIED, mining of information, computer-based education, and teaching analytics in general and narrow definitions of ITED. The findings indicate that AIED research has a greater interest in and impact; little has been done to incorporate deep learning technologies into educational contexts; traditional AI technology, like natural language processing, is commonly used in education



contexts, although advanced technologies are seldom used; and studies are lacking.

The study found that AI, especially by educational institutions, is widely used and widely adopted in education. In order to fulfil trainers' tasks and functions individually or in colliance with instructors, AI initiated computation and technology, went to web based and online intelligent learning, and subsequently to the use of integrate computer systems in conjunction with other technologies. Through the use of such platforms, educators have been able to perform a variety of administrative tasks with greater precision and reliability, for example, updating, evaluating and improving their teaching skills.

### **RESEARCH GAP**

The present study based on the above review of literature it is clear that Artificial Intelligence plays the vital role in every area including training and development. Many academicians and scholars have done extensive research in this aspect and few of them are in the following directions.

- Few studies have discussed on the usage of AI to test emerging design approaches and techniques that can be used to advance AI research, education, strategy, and practice in order to better the human condition.
- The study focuses on the AI in management, teaching, and learning Based on a narrative structure for evaluating AI defined from preliminary research.
- Papers discusses studies on the usage of AI to test emerging design approaches and techniques that can be used to advance AI research, education, strategy, and practice in order to better the human condition

Therefore, it is evident based on the above review of literature no study attempted by considering the Artificial Intelligence role in Training and development of employees. Thus, the present study attempted to fill the research gap with the proposed title “Artificial intelligence in training and development for employees with reference to select IT companies”. In this study adoption of AI for employee training and development and the factors which are influencing the AI implementation in training and development.

### **RESEARCH QUESTIONS**

Based on the research gap the following research questions are framed

1. Will the employees adopt the Artificial Intelligence for the training and development?
2. Does factors effecting the employee training and development in implementing the AI?

### **OBJECTIVES OF THE STUDY**

1. To understand the adopting of AI among the Employee for T& D of select IT companies
2. To know the factors effecting the employee training and development in implementing the AI by using the Neural Networking

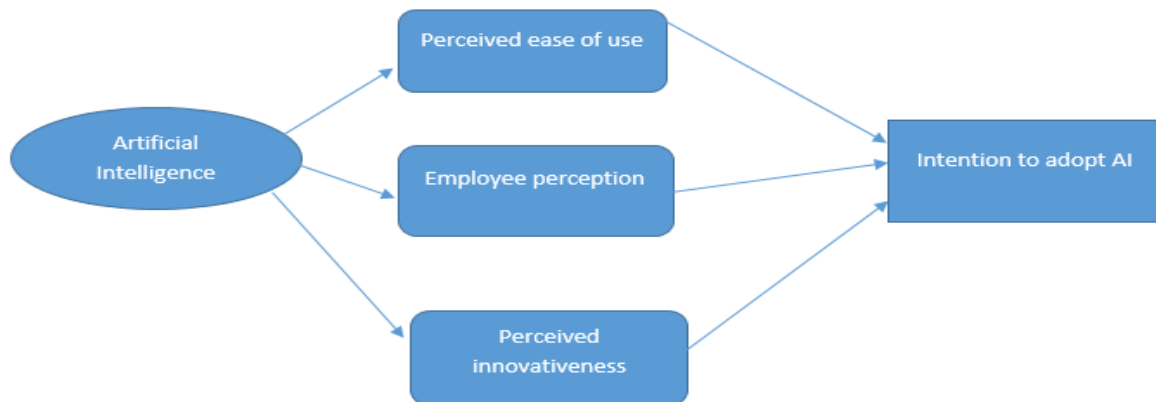
### **RESEARCH METHODOLOGY**

The study has adopted the descriptive oriented research to examine the AI role in the training and development of employees by the HR department in IT companies. The study has considered the IT companies located in Hyderabad region, which are effectively implementing for their employees training and development.

### **Parameters Support**

The present study has considered the Technology Adoption Model (TAM) developed by **Gurinder Singh et al, (2020)<sup>1</sup>**, to know the ease of use for the adoptability of AI in the IT companies. The authors have examined the Delhi-NCR region IT companies HR manager's opinion has been considered. The present study has focused on the employees who have undergone for the training in the organization with the AI support. The study has considered the TAM model variables to examine the employee intention to adopt the AI such as Perceived ease of use, Employee Perception, Perceived Innovativeness, and Intention to adopt AI.

### Research Framework



## HYPOTHESIS OF THE STUDY

### 1<sup>st</sup> Hypothesis

The study examines the influencing factors on the employees training and development with the implementation of Artificial intelligence. The study has considered the following factors, which were found to be influencing the effective implementation of AI in the organizations (**Bossam**)

**H0:** There is no significant difference between the factors influencing on the employees training and development with the effective implementation of AI

**H1:** There is a significant difference between the factors influencing on the employees training and development with the effective implementation of AI

### 2<sup>nd</sup> Hypothesis

The study has considered the TAM in adoption of AI in training and development of employees in the organizations. The study has considered the parameters based on the TAM model and examines the impact on the intention to adopt AI. The study has considered the TAM model variables to examine the employee intention to adopt the AI such as Perceived ease of use, Employee Perception, Perceived Innovativeness.

**H0:** There is no significant Impact of TAM parameters on the Intention to Adopt AI

**H1:** There is a significant Impact of TAM parameters on the Intention to Adopt AI

**Source of Data:** The study has considered the primary data for the examination of proposed objectives.

**Sampling method:** The study has taken primary data using Convenience Sampling method. Convenience sampling is a sampling method in

which the first available primary data source used without any additional study criteria.

**Sample Units:** The study has considered the respondents as those employees, who are undergone the training and development with the AI technology in recent past at their working place. The study has considered the Deloitte and Amazon India organizations. These two organizations are extensively utilizing the AI at various stages under the human resources management, such as, employees' selection process under the recruitment to training and development for the intake employees.

**Sample Size:** The questionnaire was distributed to 150 - 200 employees of Deloitte and Amazon.

**Questionnaire:** The study has framed the questionnaire relating to two objectives. The responses were collected in likert scale structured 1 to 5 points.

**Data Reliability:** The study applied the Cronbach's alpha for the primary data reliability for the questions. The calculated value observed to be 0.863, which greater than base value of 0.7. Therefore, the study can consider the primary data for the analysis.

### Statistical Tools

The statistical tools used in the study were using SPSS Statistics software. The study applied the following statistical methods for the examination of framed objectives:

**Structure Equation Model:** The Structural Equation Model (SEM) is a very general statistical modelling technique that is widely used in the behavioral sciences. It can be viewed as regression or path analysis. The interest in SEM is frequently focused on theoretical constructs, which are represented by latent factors. The relationships between the theoretical constructs are represented by

regression or path coefficients between the factors. Browne (1993).

**Neural Network:** The study has considered the factors, which are influencing the training and development of employees in effective

Case Processing Summary			
		N	Percent
Sample	Training	81	67.5%
	Testing	39	32.5%
Valid		120	100.0%
Excluded		0	
Total		120	

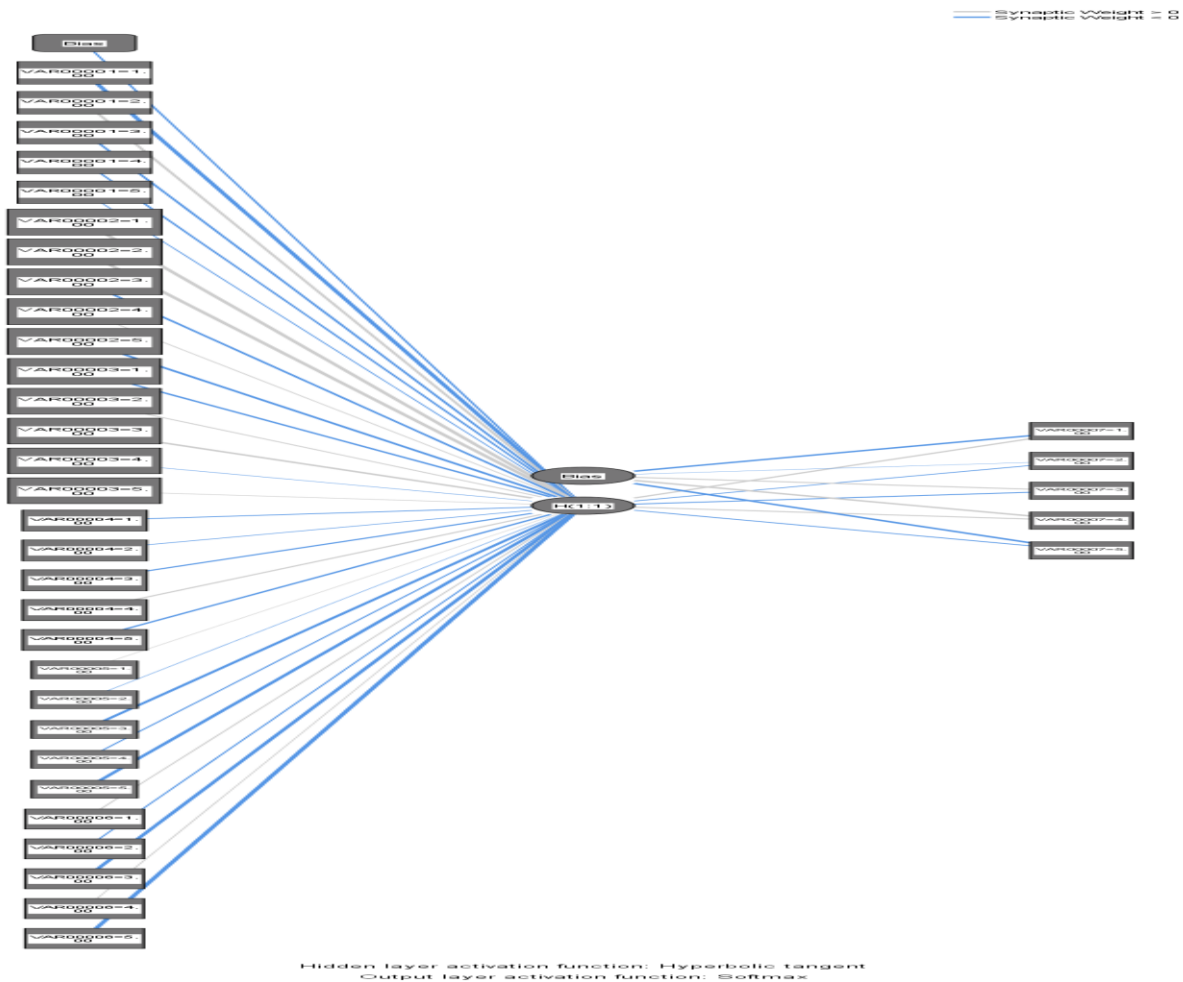
implementation. The study applied the neural network to identify the higher to lower influencing factors by prioritizing them with the importance level.

### TABULATION OF DATA ANALYSIS

**Objective 1: To know the factors effecting the employee training and development in implementing the AI by using the Neural Networking.**

The below study is witnessing regarding the Neural Network to know about the Adoption of the Artificial Intelligence in the training and development for the employees in IT sector.

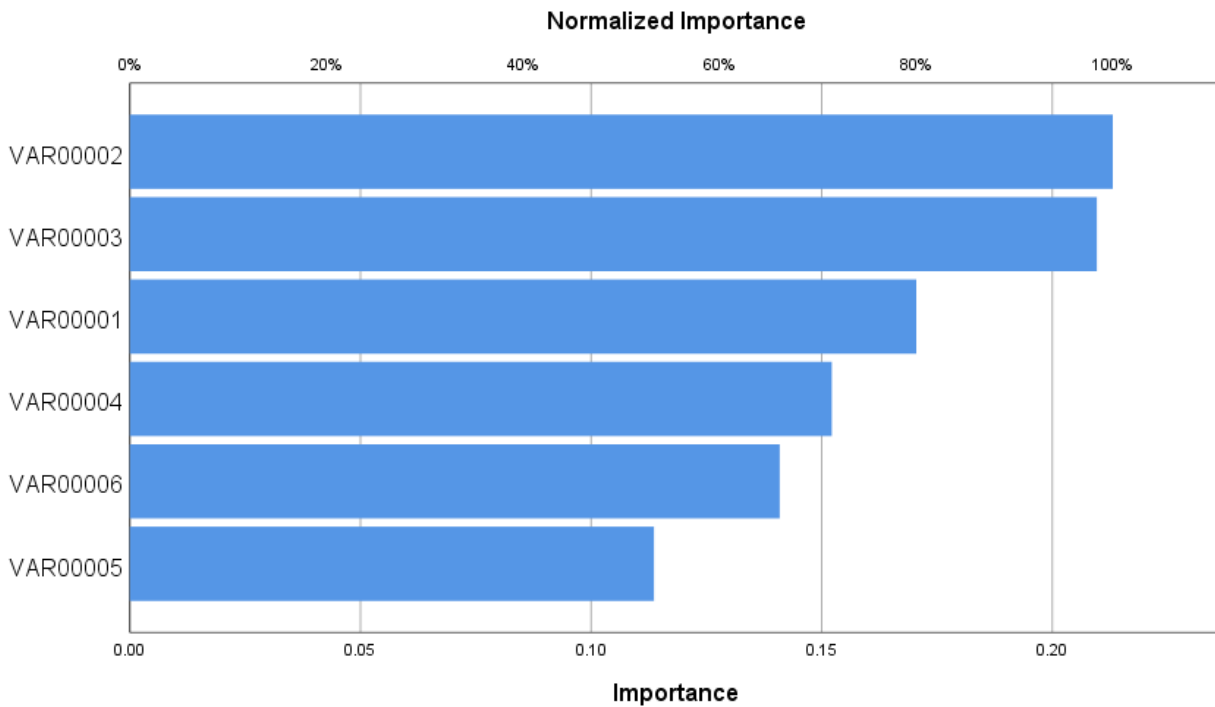
The study examines the case processing summary, to check the model fitness. Here, the training test has the 67.5% allocated and testing test have allocated 32.5%. Hence, this study results is to evaluate the Neural Networking





The study has considered one hidden layer in it with adoption of artificial intelligence to prioritize the influencing factors with the effective implementation in the organization from the perspective employees.

Independent Variable Importance		
	Importance	Normalized Importance
Machine learning through AI customized learning programs	.171	80.0%
Short and fast learning programs	.213	100.0%
Real time feedback available	.210	98.4%
Training cost is low	.152	71.4%
leads in time savings	.114	53.3%
uses statistical analytics in addressing employee's efficiency	.141	66.1%



The table represents the adoption of Artificial Intelligence in the training and development in

the IT sector. The highest importance level is seemed to be in “short and fastest learning

programs” with 100% meaning that these learning programs will increase the decision making capacity in the employees. The next importance level is “Real time feedback available”, seemed that this feedback will be able to improve the Artificial Intelligence adoption in the IT sector. “Machine learning through AI customized learning programs” is having the importance level as 80.0% through these programs by AI will be able to understand the employees easily. “Training cost is low” as the importance level as 71.4% meaning that this will reduce the cost of the organization expenses also. The factor “uses statistical analytics in addressing employee’s efficiency”, this factor will improve the employees’ efficiency through these AI adoption. The least factor which effects the adoption of AI is “leads in time savings” with 53.3%. Hence, by improving the short and fastest teach employees training will be certainly developed as well, organization and employees will improve the efficiency **Farah Zahidi (2020)<sup>2</sup>**. Therefore, null hypothesis has been rejected and accepted the alternative hypothesis. Thus, it signifies that influencing factors are having the significant difference between them in implementing AI.

### **Objective 2: To understand the adopting of AI among the Employee of select IT companies**

Structure Equation Modeling (SEM) is a set of statistical used to explain relationships between multiple variables. It aids in the analysis of interrelationships between multiple dependent and independent variables at the same time. To begin with, the reasons for selecting SEM for data analysis were that SEM has the ability to test causal relationships between constructs using multiple measuring objects. Second, it provides efficient and robust statistical

procedures for dealing with complex systems. The current study focuses on how Artificial Intelligence will improve Training and Development with specific reference to IT companies. In this study, the model attempts to consider the model-estimated goodness of fitness index first, followed by model consistency, indicating that the model is significant. Finally, the hypothesized model (SEM model) has been framed to test the estimated results, and the results are presented in detail below. The Goodness of Fit is implied by the table below, which includes Fit statistics, Recommended and Obtained values, and the result is explained below.

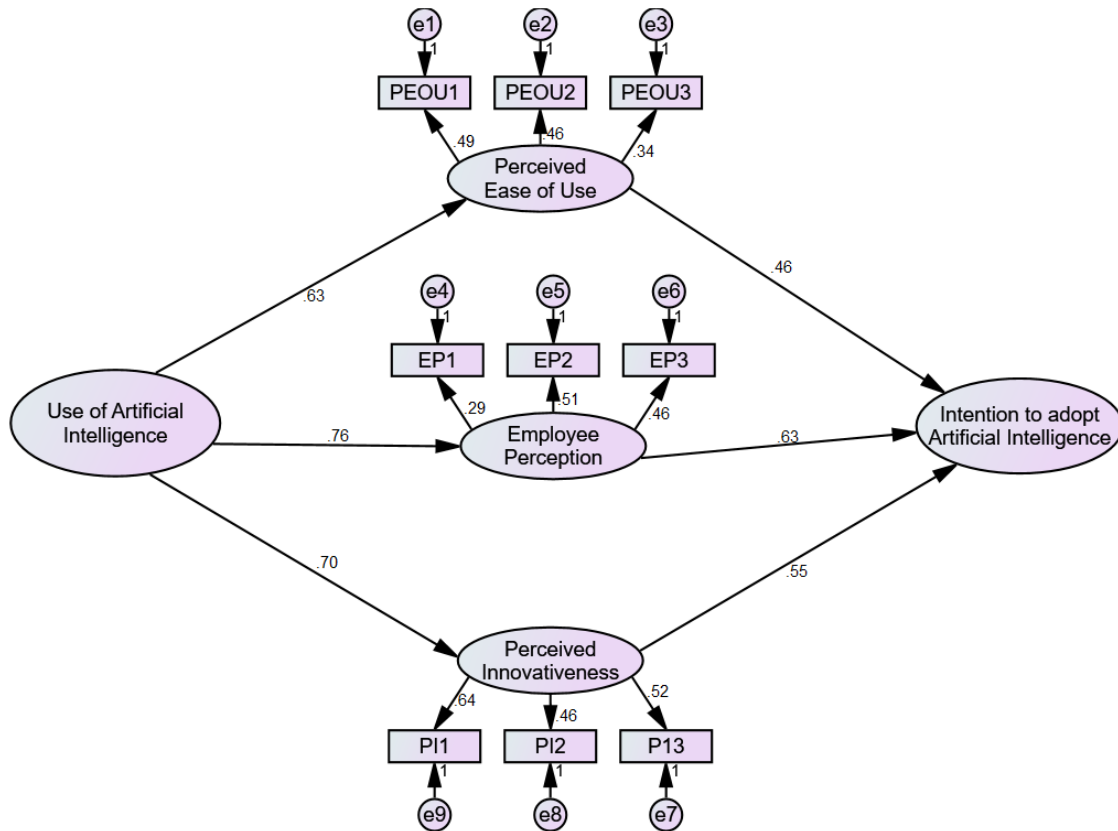
**Table-1: Goodness for Fit Index of SEM**

Fit statistic	Recommended Value	Obtained Value
Chi square		242.416
Df		4
Chi square significance	$p \leq 0.05$	0.000
Goodness Fit Index	>0.90	.985
Adj. Goodness Fit Index	>0.90	.992
Normed Fit indexes	>0.90	.741
Relative Fit Index	>0.90	.813
Comparative Fit Index	>0.90	.961
Tucker Lewis Index	>0.90	.900
RMSEA	<0.05	.008

Goodness of fit index indicates with respect to the fitness of hypothesized model. The result indicates usage of Artificial Intelligence in training and development for employees in IT sector. GFI (Goodness Fit Index) is 0.985 and Adjusted Goodness of fit Index is 0.992 that are observed to be above the recommended level. Normed fit Index seems to be greater than 0.741

and Relative fit index is 0.813. Goodness index like Comparative Fit index (0.961) and Tucker Lewis Index (0.900) are observe to be above the cut-off level. Root mean Square is 0.000, which implies that significant of the model. Therefore, goodness of fit index concludes that the model is satisfactory.

**Figure-1 SEM model with respect to identify the Artificial Intelligence in Training and Development with reference to TAM**



Source: Field data

Note: There are two basic requirements for the identification of any kind of SEM Model: (1) there must be at least as many observations as free model parameters ( $df \geq 0$ ), and (2) every unobserved (latent) variable must be assigned a

scale (metric).The below table examines regarding the Artificial Intelligence adoption in the IT sector by improving the employees training and development.

**Table- 2: Regression weights with respect to Use of Artificial intelligence**

			Estimate	S.E	C.R	P-value
Employee Perception	<---	Use of Artificial Intelligence	.760	.591	2.135	***
Perceived ease of use	<---	Use of Artificial Intelligence	.629	.903	3.147	***
Perceived innovativeness	<---	Use of Artificial Intelligence	.696	.421	3.113	***

The table attempts to describe how the use of artificial intelligence will affect employee

training and development in IT companies. According to the research results, the use of AI



has a significant positive impact on the Technological Acceptance Model scales. In which, with 0.760 units, the Employee Perception appears to be highly influenced, implying a favorable impact of employees on the adoption of Artificial Intelligence in their

Training & Development. It also reveals that AI in training and development programmes will increase employee innovativeness, and that understanding capability has increased with the help of Artificial Intelligence.

**Table- 2: Regression weights with respect to Measuring items of TAM model**

			Estimate	S.E	C.R	P-value
Using the AI product would be easy	<----	Perceived Ease of Use	0.491	0.162	3.030864	0.034
Interaction with the AI product would be clear and understandable	<----	Perceived Ease of Use	0.462	0.124	3.725806	0.026
I would find the AI product difficult to use	<----	Perceived Ease of Use	0.341	0.096	3.552083	0.016
Using the AI product would improve my work performance	<----	Employee Perception	0.292	0.089	3.280899	0.028
It would not be easy to operate the AI product	<----	Employee Perception	0.514	0.134	3.835821	***
It would not be easy to use the AI product technically	<----	Employee Perception	0.462	0.126	3.666667	0.022
I would like to try all kinds of new inventions or new ideas.	<----	Perceived Innovativeness	0.642	0.252	2.547619	***
I could often think of different ways to solve difficult problems	<----	Perceived Innovativeness	0.461	0.221	2.085973	0.032
I believe new technology devices can trigger my creativity	<----	Perceived Innovativeness	0.521	0.236	2.207627	***

Table illustrated that, majority of the respondents agreed that with the use of AI they are able to understand clear and easily, but few of them are states that AI product difficult to use. It reveals that use of AI has shown significant impact on their perception level towards the use of AI in their training and development program. The study also

suggesting Use of AI product will enhance the work performance of employee and it is more beneficial for cost reduction. Innovativeness is another step will lead to enhance the Employee productivity as well as Organizational Effectiveness. From the p-value, the study signifies that reject the Null hypothesis and accept the Alternative Hypothesis.

**Table – 3: Regression weights of Intention to adoption of AI**

			Estimate	S.E	C.R	P-value
Intention to adopt Artificial Intelligence	<---	Perceived ease of use	0.462	0.129	3.581395	***
Intention to adopt Artificial Intelligence	<---	Employee Perception	0.632	0.144	4.388889	***
Intention to adopt Artificial Intelligence	<---	Perceived innovativeness	0.552	0.162	3.407407	***

Table indicates that, three measuring items/scales are seeming to be significantly positively impact on the Intention to adopt of Artificial Intelligence. In which, Employee Perception is found to be highly impact with 0.632, followed by Perceived innovativeness and Perceived Ease of Use. The study suggests that implementing the AI in training and Development program in their organization will improve Employees in taking decision making, Quick work and eagerness to complete the work faster.

#### **FINDINGS OF THE STUDY:**

1. The study implies the Artificial intelligence usage through the training and development for the employees is through the Employee perception, they by giving their word of mouth or through any feedback will impact the AI to adopt in the IT sector.
2. The result witnessed the adoption of AI in IT sector is mostly due to the usage of technology, as the new generations are habituated to adopting new technologies. It will make an employee adopt the AI and learn the usage, which will help them to take new decisions and plan for projects, which will enrich the employees' development and enhance the adoption of AI. The study result validated with the author **Nitin Borge (2016)** findings that training on AI developed the skill set of employees that supported to meet the project timelines.
3. The study examined the factors, which are influencing for the adoption of AI in the IT organizations. The study applied the Neural network methodology and prioritized the factors, which reveals the factor of “short and fast learning programs” (0.213). Thus, it stated that AI would framed the content in less time as per the requirement of the employees.
4. The study found that in training and development for the employees' implementation of AI has given greater results. It indicates that “Real time Feedback available” (0.210). Therefore, it indicates that real time based feedback to each and every employee is possible without the support of AI.
5. The study observed that lower training cost factor (0.152) plays the vital role in adopting the AI in the organizations, where large pool of workforce involved for the training and development.
6. The study indicates the Artificial Intelligence adopted through the training and development for employees in IT sector by the shortest and the fast learning programs effectively, as these programs will create the awareness for the employees to know the AI and its usage. The study also found that through the feedback that has given genuinely is an added advantage for the improvement of the adoption of AI for the Employees in the IT sector.

7. Majority of the employee agreed that with the use of AI they are able to understand clear and easily, but few of them are states that AI product difficult to use.

### CONCLUSION OF THE STUDY

The present study has focused on the AI role in employee training and development in select IT companies located in Hyderabad region of Telangana state. The study has considered the Deloitte and Amazon India organizations, which are using the AI extensively in the organization including training and development. The study has considered the Technology Adoption Model and examined the perceived intention to adopt the AI in the organization for the employee training and development with the structural equation model and the result reveals that ease of use and employee perception significantly related to the adoption of AI in the organization. The study identified the influencing factors with the neural network of machine learning method and observed that AI will frame short and fast learning programmes for the employees and give the real time base feedback on the training and development to the employees. The study also stated that adoption of AI will reduce the operation cost and improves the operational efficiency in Training and development.

### FURTHER RESEARCH SCOPE

The present study has focused on the adoptability AI and usability in training and development in the select organizations located in Hyderabad. Therefore, the study suggests expand the study scope to focus on the non-IT sector organizations. The study also recommends focus on the AI usability for the education sector, so that students learning ability can be monitor and feedback to improve learning ability of the students.

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# मध्य भारती

मानविकी एवं समाजविज्ञान की द्विभाषी शोध-पत्रिका



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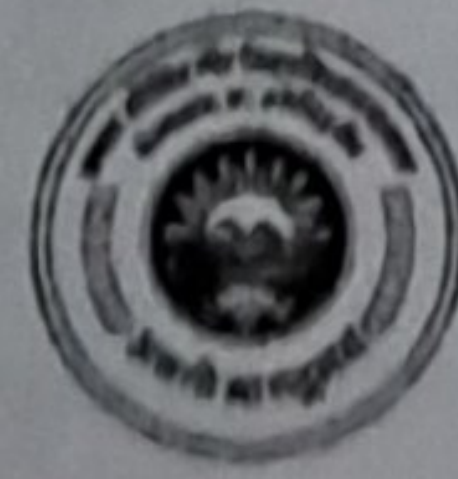
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**Introduction:**

Learning is the best path towards a successful and happy life, as it is conducive to a better understanding of life, and all acquired knowledge can be used towards fulfilling one's ambition. The magnitude of the importance of education in life is huge as well as multi-fold that helps to refine our character, strengthen morality, broaden the outlook and it prepares a nation for a better tomorrow. Well defined and futuristic education policy is essential for a country at school and college levels to be implemented by the governments in order to shape the future of the nation for its overall development.

A new chapter in education policy has begun after Indian independence. The implementation of previous policies on education has focused largely on issues of access and equity. The unfinished agenda of the National Policy on Education 1986, modified in 1992 (NPE 1986/92), is appropriately dealt with in this policy. A major development since the last policy of 1986/92 has been the Right of Children to Free and Compulsory Education Act-2009 which laid down legal underpinnings for achieving universal elementary education.

The National Education Policy (NEP) - 2020 replaces the National Policy on Education of 1986. In January 2015, a committee under former Cabinet Secretary T. S. R. Subramanian started the consultation process for the New Education Policy. Based on the committee report, in June 2017, the draft NEP was submitted in 2019 by a panel led by former Indian Space Research Organization (ISRO) chief Krishnaswamy Kasturirangan. The Draft New Education Policy - 2019 was later released by Ministry of Human Resource Development, followed by a number of public consultations. After a gap of 34 years, the Indian government consolidated feedback from 2.5 lakh village-level stakeholders to two national parliamentary level committees, over more than 50 months of consultations and workshops.

**National Education Policy-2020:**

National Education Policy 2020 is the first education policy of the 21st century and aims to address the many growing developmental imperatives of our country. This Policy proposes the revision and revamping of all aspects of the education structure, including its regulation and governance, to create a new system that is aligned with the aspirational goals of 21st century education, including Sustainable Development Goal, while building upon India's traditions and value systems. NEP lays particular emphasis on the development of the creative potential of each individual. It is based on the principle that education must develop not only cognitive capacities - both the 'foundational capacities' of literacy and numeracy and 'higher-order' cognitive capacities - such as critical thinking and problem solving - but also social, ethical, and emotional capacities and dispositions.

Built on the foundational pillars of Access, Equity, Quality, Affordability and Accountability, this policy is aligned to the 2030 Agenda for Sustainable Development and aims to transform India into a vibrant knowledge society and global knowledge superpower by making both school and college education more holistic, flexible, multidisciplinary, suited to 21st century needs and aimed at bringing out the unique capabilities of each student.

The Union Cabinet approved the National Education Policy (NEP) in July 2020 under the Prime Ministership of Narendra Modi, government of India. This policy will usher in sweeping changes to the education policy of the country, including a renaming of the Ministry of Human Resource Development as the Education Ministry. The Union Cabinet has approved the new National Education Policy 2020 with an aim to introduce several changes in the Indian education system - from the school to the college level.



- > Its aims at making "India a global knowledge superpower".
- > The Cabinet has also approved the renaming of the Ministry of Human Resource Development to the Ministry of Education.
- > The New Education Policy cleared by the Cabinet is only the third major revamp of the framework of education in India since independence.
- > The two earlier education policies were brought in 1968 and 1986.

**Background of NEP-2020:**

- The Ministry of Human Resource Development formed a committee chaired by Dr.K.Kasturirangan for preparing the National Education Policy. The Committee was constituted in June 2017. The Committee submitted its report on May 31, 2019. The National Policy on Education covers elementary and university education in urban as well as rural India.
- > The very first policy for education was promulgated in 1968 with the second one following in 1986.
  - > The first NEP was based on the recommendations of the Education Commission (1964-66). This policy sought to have a 'radical restructuring' of India's educational system and equalizing opportunities for education for all, to accomplish national integration and better economic and cultural development.
  - > The NEP also called for realizing compulsory education for every child until the age of fourteen, as mentioned in the Indian Constitution.
  - > It also aimed at providing enhanced training and improving teachers' qualifications

**Recommendations of National Education Policy-2020:**

- > **Early Childhood Care and Education:** The NEP-2020 recommended that early childhood care & education be developed in a two-part curriculum consisting of:
  - i. Guidelines for Parents & Teachers of students up to 3 years of age
  - ii. An educational framework for students between the ages of 3-8 years.

The NEP talks about the implementation of these recommendations by expanding and improving the quality of the Anganwadi system and co-locating them with primary school.

- > **Right to Education Act, 2009:** The NEP recommended extending the range of the Right to Education Act,2009 to include the following education levels:
  - i. Early Childhood &
  - ii. Secondary School

This will allow coverage of RTE to all children between the ages of 3-18 years. In addition, it suggested the elimination of detention of children until class eight.
- > **Curriculum Framework:** Reforms in the framework of the current curriculum of school education are based on the development needs of the students. The NEP recommends the 5-3-3-4 pattern explained in the table below:

Years	Stage	Curriculum
5	Foundational	3 years of pre-primary followed by class 1 and 2
3	Preparatory	Classes 3 to 5
3	Middle	Classes 6 to 8
4	Secondary	Classes 9-12

- > **School Exam Reforms:** Reforms in the school exam recommended by the NEP include tracking the progress of the students throughout their school experience.
  - i. It includes State Census Exams in class 3, 5 and 8.



- ii. Another important recommendation was the restructuring of the 10th board exam, which would mainly focus and test only the skills, core concepts and higher-order thinking & capacities.
- **Regulatory Structure and Accreditation of Higher Educational Institutions:** In terms of Accreditation and Regulatory structure, the NEP recommended the following changes:
  - i. Setting up NHERA (National Higher Education Regulatory Authority),
  - ii. Separating NAAC from UGC into an autonomous and independent body.
- **National Research Foundation:** In order to improve the quality of research in India, the NEP recommended:
  - i. Establishment of a National Research Foundation.
  - ii. It would be an autonomous body that would administer the mentoring, funding and capacity building for quality research in India.
- **Education Governance:** The NEP recommended establishing an apex body for education headed by the Prime Minister under the name Rashtriya Shiksha Aayog or National Education Commission. It also suggested changing the name of the Ministry of Human Resources & Development to the Ministry of Education.
- **Financing Education:** Doubling the public investment for education was one of the important recommendations of the NEP 2020.
  - i. NEP 2020 insisted on the expenditure of 6% of the GDP on education.
  - ii. Doubling the current 10% of total public expenditure to 20% in the next decade was recommended.
- **National Mission on Education through Information and Communication Technology:** The NEP suggested setting up an autonomous body that would facilitate decision making on the deployment, induction and use of technology. NEP said that this would be achieved by implementing the following measures:
  - i. Establishment of National Education Technology Forum. The recommended autonomous body would be administered under this mission.
  - ii. It will also include virtual laboratories in various disciplines providing remote access.
- **Vocational Courses:** Recommendations of NEP 2020 with respect to Vocational courses can be listed as follows:
  - i. Students in classes 9 to 12 must receive vocational education on at least one vocational.
  - ii. Schools should build expert curriculum delivery methods that are aligned with National Skills Qualifications Framework (NSQF) competency levels.
  - iii. Higher Education Institutes must also provide vocational courses that are integrated into undergraduate education programmes.
- **Three Language Formula:** The Policy recommended that the three-language formula be continued and flexibility in the implementation of the formula should be provided. The three-language formula states that state governments should adopt and implement the study of a modern Indian language, preferably one of the southern languages, apart from Hindi and English in the Hindi-speaking states, and of Hindi along with the regional language and English in the non-Hindi speaking states.

#### Challenges of NEP - 2020:

- **Opening universities every week is a herculean task:** Doubling the Gross Enrollment Ratio in higher education by 2035 which is one of the stated goals of the policy will mean that we must open one new university every week, for the next 15 years which is an undoubtedly massive challenge.
- **The numbers are no less daunting in reforms to our school system:** The National Education Policy 2020 intends to bring 2 crore children who are currently not in schools back into the school system. Whichever way you look at it, accomplishing this over 15 years requires the setting up of around 50 schools every week.



states even if the medium of education for children is not in mother tongue or regional language.

➤ **Funding is a big challenge in the Covid era:** The NEP-2020 envisages an increase in education spending from 4.6% to 6% of GDP, which amounts to around INR 2.5 lakh crores per year. What makes things tricky is that this policy comes into being at a time when the economy has been battered by Covid-19 related lockdowns, government tax collections are abysmally low, and the fiscal deficit was high even pre-Covid.

➤ **Current focus on healthcare and economic recovery to lower the execution speed:** Economists have been calling for large stimulus packages amounting to double-digit percentages of GDP, despite the strain on the exchequer.

➤ **Need to create a large pool of trained teachers:** In school education, the policy envisages a sweeping structural re-design of the curriculum a very welcome step. But in order to deliver this curriculum effectively, we need teachers who are trained in and understand the pedagogical needs. Many of the curricular changes require substantial mindset shifts on the part of teachers, as well as parents.

➤ **Inter-disciplinary higher education demands for a cultural shift:** In higher education, the National Education Policy 2020's focus on inter-disciplinary learning is a very welcome step. Universities, especially in India, have for decades been very silo-ed and departmentalized. The NEP-2020 has many initiatives to improve the quality and the broadness of the education system in India. The objectives of this study on National Education Policy 2020 are:

- a. To highlight and overview the policies of the newly accepted higher education system (NEP 2020).
- b. To compare National Education Policy 2020 with the currently adopted policy in India.
- c. To identify the innovations in new national higher education policy 2020.
- d. To predict the implications of NEP 2020 on the Indian higher education system.
- e. To discuss the merits of Higher Education Policies of NEP 2020.
- f. Suggestions for further improvements for the effective implementation of NEP 2020 to realize its goal.

#### Opportunities of NEP 2020:

New education Policy begins with the unfinished agenda of NEP-1986. NEP-1986 was rooted in a very different India. Over the years, remarkable strides have been made in terms of access and equity. Near universal levels of enrolment at primary levels, and subsequent increase in enrolment at higher education levels (GER: 26.3%) have been achieved. However, there has also been a drop in the quality of learning in public school systems, followed by an exodus of elite and middle classes. This also led to the weakening of accountability mechanisms. Despite poor returns on learning, the pay-structures in public systems have seen a gradual increase.

#### School Education:

- a. Revamping of 10+2 structure to 5+3+3+4. New pedagogical and curricular structure to include pre-primary years. It's a good departure as this was ignored in education policy documents, and referred to in informal sense.
- b. NCERT will focus on the development of new curricular and pedagogical structure for ECCE. Policy also delves deep into the development and training of Anganwadi trainers through short-term and long-term programs. A positive thrust towards formalisation, of ECCE structure and delivery.
- c. Focus on attaining foundational numeracy and literacy by grade 3. Ministry of Education will strengthen this, and run it in a mission mode through a separate national mission.



- d. A separate national book policy to develop libraries around the country and instill love of reading in children. Public libraries in India are scarce. If this could be strengthened through the public education policy, it's a plus.
- e. Mid-day meals to see an upgradation in nutrition component, wherever possible, local alternatives to be provided. Eggs are still a contentious policy issue; policy plays it safe by steering clear to avoid any unnecessary controversy.
- f. Design of programs and interventions to alleviate issue of dropouts in conjunction with the Ministry of Social Justice and Empowerment.
- g. Medium of instruction section for some reason has received lot of undue attention. However, the section remains fairly flexible to avoid all sort of controversy. Half-baked understanding and market push towards English and paternal perception of 'quality' could've led to this flexibility. Policy also doesn't thrust/force/prefer any particular language over the other and encourages learning multiple languages. It also recommends teaching foreign languages at secondary level, Korean, Japanese, Thai, French, German, Spanish, Portuguese, and Russian.
- h. Policy inserts a new term called SEDGs (socio-economically disadvantaged groups). This hitherto hasn't been used as a social category in technical documents. Though later sections highlight categories as caste, tribe, disability, transgender and have passing references to term minority. Technical criticism aside, policy envisages ample initiatives to be targeted at these groups to increase enrolment and retention.
- i. PARAKH, a new body to focus on assessments as NAS (National Achievement Survey) and SAS (State Achievement Survey). PARAKH could be an important instrument to look at learning gaps and support targeting of various ministry goals and programs.

#### Higher Education:

It's important to view the policy in context of what has been happening in public universities, and recent debacle of universities of eminence. There has been continuous erosion of university autonomy by the state. Though the document highlights regulatory autonomy, it would be worrisome if the document also meant financial autonomy.

- This 'imagined' autonomy is envisaged through replacement of UGC (University Grants Commission) and AICTE (All India Council for Technical Education). It would be body Higher Education Commission of India is based on the idea of division of functions and separation of activities.
- Policy also argues against commercialization of education. New breadth allows for foreign universities to come to India. However, in the same increase in number of private universities to come to India. There has been significant increase competition, it makes sense. However, insertion of the statement doesn't integrate technology in institutions is a necessary direction.
- National Research Foundation in institutions is another great idea. However, if these spaces get filled by individuals who are driven by ideological agendas, little could be expected.
- Indian Universities will be allowed to set up campuses elsewhere in the world there is a strong potential for this to develop in gulf-markets. There is a huge demand for quality education by Indian diaspora.

#### Suggestions for New Education Policy - 2020:

- Its implementation must contribute to improved realization of the RTE Act, lead to the strengthening of public education.
- State and national governments should push the envelope on ensuring educational equity and non-discrimination, address the needs of children below six and above 14, confront privatization and commercialization of education, tackle the rising commercialization of education, and stem the rising commercialization of education.



- tools.
- They must put in place a change management process for the upcoming governance reforms and strengthen the administrative backbone of the education system.
- Further, the government must ensure adequate resources for its implementation and amplifying mechanisms for citizen participation.
- Improve public schools for quality and social integration
- To improve the schooling system, budgetary allocations have to be increased.
- The next logical step for government should be to universalize secondary education.
- To implement NEP successfully at all levels the government will need to create stakeholder incentives so that the implementation is smooth and uniform.
- Formulate instruments in the form of legal, policy, regulatory and institutional mechanisms
- Build reliable information repositories
- Develop adaptability across Higher Education Institutions, regulatory bodies and government agencies
- Develop credibility through transparent actions and participation of all stakeholders;
- Develop sound principles of management

**Conclusion:**

The new National Education Policy – 2020 which has been approved by the central government to change the Indian education system to meet the needs of 21<sup>st</sup> century India, if it is implemented successfully, this new system will make India one of the world's leading countries. The aim of the NEP-2020 which came after 34 years, is to provide higher education to all students, which aims to universalize pre-primary education by 2025. NEP-2020 is marching towards achieving such objective by making innovative policies to improve the quality, attractiveness, affordability, and increasing the supply by opening up the higher education for the private sector and at the same time with strict controls to maintain quality in every higher education institution. By encouraging merit-based admissions with free-ships& scholarships, merit & research based continuous performers as faculty members, and merit based proven leaders in regulating bodies, and strict monitoring of quality through biennial accreditation based on self-declaration of progress through technology-based monitoring, NEP-2020 is expected to fulfil its objectives by 2030.

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ఎడిటోరియల్ విభాగం : పోస్ట్ బాగ్ అపార్ట్మెంట్, పోస్టల్ కాలనీ, 4వ లైను,  
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డి.టి.పి & ప్రింటింగ్ : తెనాలి ప్రబ్లమ్, జి.యల్.ఎస్. గ్రూప్స్, లేమల్లె,  
అమరావతి మండలం, గుంటూరు జిల్లా. ఫోన్ : 9494 660 509.



60. బోయి భీమన్న - సర్వోదయ భావనే - దీప సభ	- యం. ప్రదీప్	263
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## జానపదుని దర్శనం - సామాజిక జీవనం

- డా. ఉదాలి బిట్టపూరి, తెలుగు శాఖాధ్యక్షులు, సెంట్ ఆన్స్ మహిళా కళాశాల, మెహదీపట్నం, హైదరాబాదు.

గ్రామసీమలు దేశ సౌభాగ్యవికీ వట్టుగొమ్మలు.. అని గాంధీజీ చెప్పిన మాటలు ఆక్షర సత్యాలు. ప్రకృతి శోభను దర్శించాలంటే గ్రామసీమలకు పోవడానికి మించిన విహారయాత్ర ఉండదు. మానవులకు కావలసిన తిండి, బట్ట గ్రామాల నుంచే వస్తంది. వట్టణాభివృద్ధికి గ్రామాలు ఎంతగా తోడ్పడుతున్నాయో, అదే విధంగా గ్రామాభివృద్ధికి కూడా వట్టణమేమి చేస్తున్నదనే భీమన్న గారి ప్రశ్నలు 'జానపదుని జాబులు' లో ప్రశ్నలుగానే మిగిలిపోయాయి.

వ్యక్తుల మధ్య, సంస్థల మధ్య పరస్పర సందర్భాన్ని, సంబంధాన్ని బట్టి వ్రాసుకొనేవి లేఖలు. లేఖ రచనను సామాజిక చైతన్యానికి, మహిళాభ్యుదయనికి, సాంఘికాభివృద్ధికి, గ్రామాభివృద్ధికి చైతన్య వాహకంగా పలువురు రచయితలు స్వీకరించారు. ప్రముఖ వ్యక్తుల ఉత్తరాలను సాహిత్య రచనలుగా పరిగణించడం సంప్రదాయంగా వస్తున్నది. ఆ వ్యక్తుల అభిప్రయాలను, వివిధ వ్యక్తులతో వారికున్న పరిచయాలను, సంబంధాలను సమకాలీన సమాజ జీవన విధానాన్ని వాటి పరిణామాలను ఈ లేఖ రచన తెలియజేస్తున్నది. లేఖలను విమర్శకులు మూడు రకాలుగా విభజించారు. వ్యక్తిగతమైనవి, వ్యాపార పరమైనవి మరియు బహిరంగ లేఖలు. ఈ వరుసలో 'కారద లేఖలు' వ్రాసిన కనుపర్తి వరలక్ష్మమ్మ, 'జానపదుని జాబులు' వ్రాసిన డా. బోయి భీమన్న, 'ప్రేమ లేఖలు' వ్రాసిన గుడిపాటి వెంకటాచలం వంటివారు మనకు ప్రముఖంగా కనిపిస్తారు. సమాజానికి ప్రతిబింబాలే ఈ లేఖ సాహిత్యం! పైన పేర్కొన్న ఏ లేఖ చదివినా పలు సామాజిక అంశాలే ద్యోతకమవుతాయి.

'జనవాణి' పత్రికలోనూ, 1933లో 'జానపదుని జాబులు' అనే పేరుతో 'ప్రజామిత్ర'లోనూ ప్రచురింపబడ్డాయి. చదువుకొని పేదరికం వల్ల చదువు కొనసాగించలేక తన స్వగ్రామం పోయి పల్లెటూరి పనుల్లో మునిగిపోయిన జానపదుడు వట్టణంలోని శ్రీమంతుడైన మిత్రునికి తన అవస్థలను, గ్రామాల్లోని పరిస్థితులను లేఖల రూపంలో వివరిస్తాడు. ఈ లేఖలను డా. బోయి భీమన్నగారు రచించారు. ఈయన తూర్పు గోదావరి జిల్లా మామిడికుదురు గ్రామంలో 19 సెప్టెంబరు 1911లో పేద దళిత కుటుంబంలో జన్మించారు. 'నేనేమి చేస్తే అది కళాఖండం.. నేనేమి చెబితే అది వేదం' అని ఆత్మ విశ్వాసం ప్రకటిస్తూ దళితుల ఆత్మ గౌరవాన్ని పెంపొందించిన కవి శిరోమణి బోయి భీమన్న! 'గుడిసెలు కాలిపోతున్నై' రచనకు 1975లో సాహిత్య అకాడెమీ అవార్డు అందుకున్న ఈయనని భారత ప్రభుత్వం పద్మశ్రీ, పద్మభూషణ్ బిరుదులతో గౌరవించింది. విద్యావేత్తగా, సాహితీవేత్తగా, పాత్రికేయులుగా వివిధ రంగాల్లో రాణించి బహుముఖ ప్రజ్ఞను ప్రదర్శించి సమాజ శ్రేయస్సు కోసం పాటుపడిన మహామనీషి డా. బోయి భీమన్న! వీరి సాహిత్య ప్రక్రియలన్నీ కూడా సమాజంలోని అసమానతలను వెలికి తీసి ప్రజల్లో చైతన్య దీపికల్ని రగిలించాయి. ఈ క్రమంలో జానపదుని జాబులు సామాజిక పరిస్థితుల్ని తేటతెల్లం చేయడంలో ప్రముఖ పాత్ర వహించాయి.

జానపదుడు తన మిత్రునికి సర్వసమత్వం గురించి వ్రాసిన లేఖలో ఇలా ఉటంకించారు.. 'సర్వ సమత్వాన్ని సాధించాలంటే మారణ విప్లవం ఎందుకు? శాంతి విప్లవం చాలదా? అన్నారు. 'కాలేజీలో అసమత్వానికి చెందిన రాముడు, గర్వానికి చెందిన తేవిడు, నిరంకుశత్వానికి

ఎంత స్నేహంగా ఉండేవాళ్లమో.. అని ఆయన గుర్తు చేసుకుంటాడు. ఈ వాక్యంలో సర్వమత సామరస్యంతో కూడిన తన మిత్రబృందాన్ని ఆయన పరిచయం చేస్తారు. దీనిని కొనసాగిస్తూ ఆయన ఇంకా ఇలా అంటారు.. 'అ స్నేహం కాలేజీకి వెలుపల ఎందుకు ఉండదు? అది విశ్వవ్యాప్తం ఎందుకు కాదు?' అని వాపోతారాయన.

మిత్రబృందాన్ని తలచుకొని తన పాకలో గడ్డిపొచల కింద దాచిన వ్రాత పుస్తకం, పెన్సిలు తీసి, కిరసనాయిలు బుడ్డి వెలుగులో ఈ పద్యం వ్రాశారు..

"వాలుగింటికి కళాశాల మూసిన నాటి  
పైరు చేల్లట్ల విహారములను,  
సెలవులలో వేద్య చెలగు సంజల నాటి  
నైనతేయ తటోపయానములును,  
వెన పుస్తకములపై విసుగు పుట్టిన నాటి  
నెలవైన వెన్నెల పులినములును,  
పువ్వుకో కాయకో పుట్టునాడుల నాటి  
క్షణికములగు ప్రేమ కలహములును,  
మరపునకు రావు నెచ్చెలి, మరపురావు,  
బ్రతుకు తెర్యుల వెన్నంటి వచ్చు తుదికి.

...  
జీవిత పథాలు వేరయి, జీవయాత్ర  
వెదలి దూరమై పోయెడి వేళకూడ."

పై పద్య పాదాలలో ఆయన వెదజల్లిన స్నేహ సారబాలను నేటికీ మనం అభ్రాణించవచ్చు!

ఆయన దృష్టిలో పట్టణ వాసులకు వల్లెలోని నివాసాలైన కొబ్బరి చిగుళ్ల పాక తేలికగానూ, అసహ్యం గానూ కనిపిస్తాయి. కానీ అది గ్రామవాసులకు దివ్య భవనం, చర్చా సౌధం, సౌఖ్య వేదిక ఇంకా అదే విహారసీమ! తన జాబుల్లో జానపదుడు తమ జాతి జనులు ఎలాంటి శిథిలమయ్యారో నివాసాలలో బ్రతుకులీడుస్తారో ప్రస్ఫుటంగా వెల్లడిస్తాడు! ఆ మాటలు యథాకథంగా మీ ముందుంచుతాను..

**మద్రాసు విశ్వవిద్యాలయం - ప్రత్యేక సంచిక**

"జోగయ్య ఒక మామూలు రైతు. సుబ్బయ్య ఒక ఈనాందారు దగ్గర పాలేరు. ఈ ఇద్దరూ నా కు మిత్రులు. కుప్ప దగ్గర కుప్పకు అనుకొని ఒక గట్టు. ఆ గట్టు మీద కొబ్బరి అకుల చివుళ్లతో ఒక పాక. ఆ పాకలో మేము వదుకొన్నాం.. వట్టి గడ్డి మాకు పరుపు. కొబ్బరి తుంటలే తల దిందులు. కొబ్బరి దాపలే దుప్పట్లు. వెల్లకిలా వదుకొన్నాం ముగ్గురమూ! మా పాకలో నుంచి మిణుకు మిణుకు నక్షత్రాలు ఎంతో హాయిగా ఉన్నై.. ఆ నక్షత్రాలు తొంగి చూసే సందుల్లో నుంచి చల్లగా మంచు సొస దిగజారుతోంది. చలి ఉపహపహలా అని విస్తుంది. కట్టుకొన్న పంచెలు ఆ చలిని అపలేకపోయినై. పంచెలు విప్పి కప్పుకొన్నాం.. చలి అగలేదు. దగ్గర ఉన్న కొబ్బరి దాపలు మీదకు లాక్కొని, వాటి మీద వట్టి గడ్డి కప్పుకొన్నాం.."

ఈ మాటల్ని క్షుణ్ణంగా గమనిస్తే.. వల్లె వాసులు ఎంతటి దుర్భర జీవితాన్ని అనుభవిస్తున్నారో దానిని కూడా మెరిసే నక్షత్రాల సౌందర్యంలోనూ, కురిసే మంచు సొసల చల్లదనంలోనూ మరచిపోతూ కాలం వెళ్లడీయడం మనల్ని కలచివేస్తుంది!

జానపదుని జాబులు తన మిత్రుని కుకలములు విచారించడానికో.. తన పల్లె అందాలను పర్షించడానికో ఉద్దేశించినవి కావు. నివురు గొప్పిన నిప్పులా పల్లె గర్భంలో దాగిన చిచ్చును పట్టణ వాసులకు ఎరుక పరచడమే వాటి లక్ష్యం! అదే జానపదుని చేత మన గుండెల్లో ఈచెను దించే కరకు సత్యాల్ని బైట పెడుతోంది..

అకటి చిచ్చు చల్లారని బ్రతుకులకు సాక్షిగా ఆ పల్లెను తన జాబులో పేర్కొంటాడు జానపదుడు! పల్లె నుంచి తుక్కి కోసం దేశాంతరం పోయిన భర్తకై సంతోషంతో వైనతేయంలో దాక్కొన్న ఆ ముగ్గు.. తన భర్త మరణ వార్త విని అకటి చిచ్చుతో అలమటిస్తూ తన బిడ్డల్లో కలిసి చెరువులో పడి ఈ స్వార్థ సంఘానికి వీడ్కోలు తెలిపిందంటాడు! ఈ ఉదంతాలన్నీ కూడా నాటి సామాజిక పరిస్థితుల్ని కళ్లకు కడతాయి! వారి జీవితం నిరంతర



పోలాటమేనని జానపదుడు తన మిత్రునికి వ్రాసిన జాబుల్లో చెప్పకనే చెబుతాడు!

ఒకసారి పట్టణం నుంచి వచ్చిన మిత్రుని ఉత్తరం చూసి తిన్నుకయ్యాడు జానపదుడు. ఎందుకంటే అందులో పట్నం పేర్చాడు అనాటి దీపావళిని ఎంత విలాసంగా గడిపాడో వివరంగా వ్రాశాడు. కొత్త బట్టలకు, పిందులు వినోదాలకలకు, బాణసంచాలకు అకసెంకెంత అర్పు చేశాడో గొప్పగా వర్ణించాడు. కాని పల్లె జీవితం అలా లేదు కదా! అక్కడి జనం ఆ సందగ పూట ఎంత చైన్యంగా రోజు వెళ్లబుచ్చాలో చెప్పాలంటే దానికి ఈ జానపద మిత్రుడే సాక్షి! పేర్చాడుని జాబుకు జానపదుడు రాసిన జవాబులోనే మనకు పల్లె భ్రతుకులు ఎంతటి రయపీయమైనవో తెలుస్తాయి. “..కానీ ఆ వేళ మా పల్లెలో ఎంతమంది వస్తు ఉన్నారో! వంతుగ పూట వస్తు ఉండరాదని తప్పకుండా, సోలెడు తీయ్యం కోసం కొందరు ఎంత అవస్థ పడ్డారో తెలుసా..?” అంటూ పల్లె దృశ్యాన్ని వర్ణించాడు!

గ్రామంలో వంతుగ సందడి ఎలా ఉందో చూద్దామని వచ్చిన ఆ తూరి బడివంతులుకు ఆ తూళ్ల వృద్ధుడు తారస పడ్డాడు. ఆ ముసలాయన బడి వంతులుతో.. “ఏం వంతుగ బాబూ..! రోజులు మునుపటిలా లేవు. పూర్వం ఈ అరుగుల నిండా ప్రమిదలు వెలిగించేవాళ్లం..” అంటుంటే అతని గంతు బొంగురు పొయింది! ఎంతో మందికి సంతర్పణ జరిగేదని, ఎంతో మందు (బాణ సంచా) కాల్చి వంతుగను అనందంగా బరువుకానే వ్యామని పూర్వపు పల్లకులన్నీ నెమరు వేసుకొన్నాడు! జీవమున్న ఇందికి అనవారు వాకిలి ముందు ముగ్గు! కాని ఈనాటి వంతుగ పూట ఇంటి ముందు ముగ్గులు కూడా నల్లమొగం చేసుకొన్నాయి! ఎందుకంటే ముగ్గు ఏంటి కూడా కానే స్థామక లేక ఇదిక అవములలోని బూడిదను ప్రుగ్గులో కలుపుతారు. అందుకే వారి వాకిట్లో నల్లటి ముగ్గులే దర్శనమిస్తాయి!

ఆ పల్లెలో తీయ్యం నిల్వ చేసే వంతుమొకటి ఉంది. ఆ సంఘానికి సంబంధించిన కులాళ్లు దిన్న దిన్న వంతులతో ప్రతి ఇంటికి వెళ్లి తీయ్యం సేకరిస్తారు. ఆ తూరిలో రామాలయంలో వాటిని నిల్వ చేస్తారు. యువ ప్పాదయాలకు తట్టిన విశ్వజనన వర్తతి ఇది. ఆ గదిలో తీయ్యం సమ్మర్చిగా ఉన్నా అన్నాళ్లలో వాలుగు తీయ్యపు గింజలు కావాలని అదేగే అలాగులు ఈ పల్లెలో లెక్కకు మించి ఉన్నారని తీమన్న స్పష్టించిన జానపదుడు అనేదన చెందుతాడు. దేశంలో 90 వంతులు పల్లెటూర్లు అందకారంతో, అకటి చిచ్చుతో, అనేదనతో కుమిలి కృశిస్తూ ఉన్నాయి. అన్నాళ్లకు ఏముక్తి ఎప్పుడు లభిస్తుంది? పేద, దవిక, దిన్న పెళ్ల, కులమత భేదాలు మనుమల్లో ఎన్నుకు సాస్తాయో అన్నానే మానవ సమాజం అభివృద్ధి చెందుతుంది. మానవ సంఘాతివృద్ధికి నిరోధక మేమిటని ఆలోచిస్తే.. భయమే దానికి మూలకారణం! ఈ భయమే మన భారత జాతిని నిరేకియుల చేతులకు అప్పగించి నేనిన సెకాచమని తీమన్న అభోకిస్తాడు! భయం దావాలి. పరాక్రమం పుట్టాలి! మనం గౌరవించబడాలంటే, ముందు మనవాళ్లని మనం గౌరవించుకోవాలి. దేశానికి గౌరవం రావాలంటే ముందు దేశియులకు గౌరవం ఉండాలని తీమన్న గారు ఈ సంఘానికి చాటుతున్నారు!

గుజజాత గారి దేశదక్తి గేయంలోని వంక్తులను వ్యరిస్తూ, దేశం మట్టి కాదని, అది నింపిన మనుమల కూడలి అని ఎలుగెక్కి పాదతారు. పశం సంవదలలో దేశం కులకూగుకూ ఉండాలంటే ఆ దేశ పౌరులకు తగిన ప్రాణాన్యం ఉండాలని, మనుమలకు గౌరవం లేనివాడు దేశానికి గౌరవం ఉండదని ఉపదేశిస్తారు. ఇది తెలుగు వేలకూ వర్తిస్తుందని, తెలుగువాడికీ, తెలుగు వాడికీ ఈ దుస్థితి వట్టిందంటే అది తెలుగు లావకూ వట్టిన రొక్కాగ్యమేనని తన మిత్రునికి రాసిన జాబులో గర్విస్తాడు జానపదుడు!

వదునైన తన ఆలోచనలనూ, అభ్యుదయ భావాలనూ జానపదుని ప్రతిమను ముందు పెట్టి తన

గొంతును ఆతని ద్వారా వినిపింపజేసిన డా. బోయి భీమన్న, ఈ ఉత్తరాంతరంగాల్లో ఎన్నో కోణాల్ని స్పృశించి తన మనసులోని బావాల్ని మనకు చేరవేస్తారు! సంఘ పునర్నిర్మాణం గురించి కూడా బోయి భీమన్నగారు జానపదుని చేత జాబు రాయుస్తారు! ఆనాటి సామాజిక స్థితిలో స్వాతంత్ర్య సమరానికి ఏ గ్రామాలూ వనికి రావన్నారు. ఎందుకంటే ఇప్పుడున్న గ్రామాల పునర్నిర్మాణం జరిగితేనే అవి పోరాటానికి సంపూర్ణంగా సిద్ధమైనట్లని ఆయన అభిప్రాయ పడతారు. ప్రజల్లో మిలిటరీ ఉద్ధీపన, మిలిటరీ క్రమశిక్షణ అలవరచాలని,

అన్యాయాన్ని ప్రతిఘటించే దైర్యసాహసాలు పెంపొందించాలని జానపదుడు ఆవేశంగా చెబుతాడు. అప్పుడుగానీ ప్రజలు సైనికులు కాజాలరని మిత్రునికి వ్రాసిన లేఖలో నిష్కర్షగా వెల్లడిస్తాడు. వెరసి ఈ జాబుల ద్వారా వర్ణరంజితమైన పట్టణాలు భూలోక స్వర్గాలనీ, పల్లెలే దయనీయ స్థితిలో ఉన్నాయన్నది డా. బోయి భీమన్న గారి ముఖ్యోద్దేశం! "విజ్ఞాన నిదులకు గ్రామ వాటికలే ఆటపట్లు కావాలి. ఇది తప్పక సాధించాల్సిన లక్ష్యం" అన్నది ఈ జాబుల ద్వారా ఆనాడే బోయి భీమన్న గారు కన్న అపురూప స్వప్నం!!

**ఉపయుక్త గ్రంథ పట్టిక :**

1. జానపదుని జాబులు (పల్లెటూరి లేఖలు) - బోయి భీమన్న, దేశీ ప్రచురణలు, విజయవాడ.
2. భీమన్న సాహితీ సౌరభం, సంపాదకుడు: లక్ష్మవర్షుల రామమూర్తి, ఎమెస్కో బుక్స్, విజయవాడ.
3. డా. బోయి భీమన్న రచనలు - సామాజిక స్పృహ - కె.ఎస్.సుబ్రహ్మణ్యం, ఎమ్.పిల్ సిద్ధాంత గ్రంథం, కాకతీయ విశ్వవిద్యాలయం, వరంగల్.
4. భీమన్న సాహిత్యవలోకనం -డా. తాళ్లూరి అన్నపూర్ణ, విరించి పబ్లికేషన్స్, హైదరాబాద్.





RNI No. APTEL/2003/12253

# భావవీణ

కళలు, సాహిత్య సాంస్కృతిక భివృద్ధికి పాఠశాల



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మల్లెమూల సుందరరామిరెడ్డి



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## లలిత కళారాధనలో చలనచిత్ర గేయ సాహిత్యం

- డా॥ ఉడాలి విష్ణుప్రియ, తెలుగు విభాగాధిపతి, సెంట్ ఆన్స్ మహిళా కళాశాల, మెహదీపట్నం, హైదరాబాదు.

విజ్ఞానాన్ని అందించేది విద్య. ఆనందాన్ని కలిగించేది కళ. ఈ రెండూ కూడా అనునిత్యం మన జీవనంతో పెనవేసుకొనే ఉంటాయి. మానసిక ఉల్లసానికి ఒక మధుకలశం లాంటివే ఈ లలితకళలు. లలితమంటే మనోజ్ఞమని అర్థం! మనోజ్ఞమైన ఆ కళలే చిత్రలేఖనము, శిల్పము, సంగీతము, నృత్యము మరియు కవిత్వము. గీత గోవింద కర్త జయదేవుడు ఈ లలితకళలనే విలాస కళలని పేర్కొన్నాడు. కవిత్వంలోనూ, సంగీతంలోనూ సారస్వతం పాళ్లు కాసితం ఎక్కువ. కవిత్వాన్ని సంగీతంతో కలిపి వింటుంటే అందులోని రసాస్వాదనతో పాటు మన మనస్సులు కూడా ఆనందడోలికలలో తేలియాడుతాయి. అందుకే వీటిని చరకళలని, శ్రవ్య కళలని అంటారు. చిత్ర లేఖనానికి, శిల్పకళలకు స్థిరకళలని పేరు. చూడడం ద్వారా శిల్పాలను స్పృశించి, శిల్పి కళాత్మక వైశిష్ట్యాన్ని అనుభూతి చెందడం వలన మానసిక ఉల్లాసం చేకూరుతుంది. ఇవి కనులకు విందు చేకూర్చినందుకు అవి స్థిర కళలుగా, దృశ్య కళలుగా పేరుగాంచాయి. ఇక చివరిది నృత్య కళ. దీనిని అస్వతంత్ర కళ, సమాహార కళ అని విజ్ఞులు పేర్కొన్నారు. సంగీతమూ, కవిత్వముల సమాహారమే నృత్యం. ఇక ఈ వేదికపై లలిత కళలకు చలనచిత్ర గేయాలలో గల స్థానం గురించి నేనిప్పుడు వెల్లడించబోతున్నాను.

ప్రాచీన కాలంలో సాహితీ గ్రంథాలలోనూ, నృత్య, చిత్ర, శిల్ప కళల ప్రదర్శనల్లోనూ ఆస్వాదించే ఈ లలిత కళలు కాలక్రమేణ చలన చిత్ర రంగంలోనికి ప్రవేశించాయి. నాటక ప్రదర్శనలలో గోచరించే ఈ కళలన్నీ కూడా ఆధునిక వినోద సాధనమైన చలనచిత్రాలకు వ్యాపించాయి. మానవుని మానసిక ఉల్లాసానికి అప్పుడూ, ఇప్పుడూ అని కాకుండా ఎప్పటికీ ఈ లలిత కళలు

చెరగని ముద్ర వేస్తుంటాయి. తెలుగులో చలన చిత్ర నిర్మాణం మొదలైనప్పటి నుంచి ఈనాటి వరకూ కూడా లలిత కళల పాత్ర నిత్యనూతనం!

లలిత కళారాధనలో వెలిగే చిరుదివ్యను నేను

మధుర భారతి పద సన్నిధిలో ఒదిగే తొలి పువ్వును నేను.. అని 'కల్యాణి' చిత్రంలో వినిపించే ఈ గేయం తెలుగు సినిమాయే గొంతెత్తి పాడుతూ లలిత కళలకు తను పెద్ద పీట వేసిందన్న భావాన్ని కలిగిస్తుంది. ప్రముఖ రచయిత్రి మాదిరెడ్డి సులోచన రాసిన 'రాగమయి' నవల ఆధారంగా తెరకెక్కిన ఈ చిత్రంలో పల్లవించే ఈ పాటలోని ప్రతి పాదమూ మధుర స్వర సంపాతము!

'ఏ ఫలమాశించి మత్త కోకిల ఎలుగెత్తి పాడును

ఏ వెల ఆశించి పూచే పువ్వు తావిని విరజిమ్మును' అంటూ -

అవధి లేని ప్రతి అనుభూతికీ ఆత్మానందమే పరమార్థమని బోధించే ఈ గీతం కోకిల పాటల సార్థకతనూ, పూదావుల నిస్వార్థతనూ వెల్లడిస్తుంది.

ఏ సిరి కోరి పోతన్న భాగవత సుధలు చిలికించెను

ఏ నిధి కోరి త్యాగయ్య రాగ జలనిధులు పొంగించెను.. అనే పాదాల్లో రమణీయ కళావిష్కృతికి రసానందమే పరమార్థమని ఈ గేయంలో రచయిత డా. సి.నారాయణరెడ్డి లలిత కళలని ఆరాధించిన తీరు అనిర్వచనీయం! కళారాధనలో కోకిలగానం, పోతన భాగవత సుధామృతములు, మహా వాగ్గేయకారుడైన త్యాగరాజు రాగరసరంజితాల వరకు వివిధ రీతుల ఆత్మానంద రసానంద గుళికలను మనకు అందించడం విశేషం! సిని గేయాలలో ఏ కళలను ప్రస్తావించాలన్నా రెండు కళలు ప్రధాన పాత్ర పోషిస్తాయి. అవి ఒకటి సంగీతమైతే రెండవది సాహిత్యము!

శిల్పకళాచాతుర్యానికి నభూతో నభవిష్యతి అనదగిన రీతిలో ఆత్రేయ కలం నుంచి జాలువారిన 'మంచి మనసులు' చిత్రంలోని గీతం అపురూప తార్కాణంగా పేర్కొనాలి.

'రాజులే పోయినా.. రాజ్యాలు పోయినా  
కాలాలు మారినా.. గాడ్పులే వీచినా  
మనుజులే దనుజులై మట్టిపాల్చేసినా  
చెదరని కదలని శిల్పాల.. వైశిష్ట్యాన్ని ఆయన ఈ  
గీతంలో అక్షర శిల్పాలుగా చెక్కిన తీరు అద్భుతం!

'శిలలపై శిల్పాలు చెక్కినారు  
మనవాళ్లు సృష్టికే అందాలు తెచ్చినారు  
కనుచూపు కరువైన వారికైనా  
కనిపించి కనువిందు కలిగించు రీతిగా..' నాడు చెక్కిన  
శిల్పాల సౌందర్యాన్ని వాటి కంటే అందంగా తన పాటలో  
మలిచాడు కవి.

'ఏకశిల రథముపై లోకేశు ఒడిలోన  
ఓరచూపుల దేవి ఊరేగి రాగా  
రాతి స్తంభాలకే చేతనత్వము కలిగి  
సరిగమ పదనిస స్వరములు పాడా'యని ప్రాచీన  
శిల్పకళా ఔన్నత్యాన్ని కళ్లకు కడతాడు రచయిత.

హంపీ క్షేత్ర వైభవాన్నీ, ఆలయాలపై శిల్పులు చెక్కిన  
చారిత్రక సందర్భాలనీ ఈ గీతం చక్కగా ఆవిష్కరిస్తుంది.  
పేరోలగములో సభికులను ఉర్రూతలూగించే కవనాలు,  
మరో చోట ఉరికించే యుద్ధభేరీలు, ఒక చెంప శృంగార  
మొలుకు నాట్యాల వంటి నవరస భరితమైన దృశ్యాలను  
శిల్పీకరించిన తీరు అమోఘం.. అనన్య సామాన్యం! ఈ  
చిత్రంలోని కథానాయిక అంధురాలు. కథానాయకుడు  
ఆమెకు శిల్పకళా సౌందర్యాన్ని ఆస్వాదింపజేసే సందర్భం  
లోనిదీ గీతం. అందుకే కవి ఎంతో జాగరూకతతో, భావనా  
పటిమతో ఆయా శిల్పాల సౌబగులను ఎంతో హృద్యంగా  
రాశారు. ఈ పాట వింటున్నంత సేపూ శ్రోతలకు ఆ క్షేత్ర  
వైభవం మనోఫలకాలపై ప్రతిఫలించడం తథ్యం!

'అమరశిల్పి జక్కన' చిత్రంలో కథానాయకుడైన శిల్పి  
పాత్ర కోసం డా. సి. నారాయణరెడ్డి గారు మలిచిన గీతం  
వింటే జీవమున్న మనిషి కన్నా శిలలే నయమనిపించును..!  
ఈ గీతంలో ఒక శిల్పకారుడు శిల్పాన్ని మలచక ముందు  
ఆ శిలలతో అతనికున్న అనుబంధాన్ని ఎంతో ఉదాత్తంగా  
వర్ణిస్తాడు రచయిత! సంగీత దర్శకుడు సాలూరు  
రాజేశ్వరరావు గారు స్వరపరచిన ఈ గీతం ఆద్యంతం  
రసభరితం!

'ఈ నల్లని రాలలో ఏ కన్నులు దాగెనో

ఈ బండల మాటున ఏ గుండెలు మ్రోగెనో..' అని  
కదలని శిలలతో శిల్పి సంభాషించడం అపూర్వమని  
పిస్తుంది. పాపాలకు, తాపాలకు దూరంగా మునులవోలే  
కారడవుల మూలలందు అవి దాగున్నాయని కవి  
భావించడం విశేషం.

'కదలలేవు మెదలలేవు

పెదవి విప్పి పలుకలేవు.. కానీ -

ఉలి అలికిడి విన్నంతనే జలజలమని పొంగి  
పొరలు..'తాయంటూ ఉలకని బండరాయి కూడా ఉలి  
సవ్వడి చెవికి సోకగానే ఉలికిపడి లేస్తాయని అంటాడు  
కవి. పైకి కఠినంగా కనిపించినా లోపల వెన్నవంటి  
మనసుగల శిలలకు కవి చేసిన అక్షరార్చన అమోఘ  
మనిపిస్తుంది!

ఇక -

సంగీత సాహిత్యమే మేమే

నవ శృంగార లాలిత్యమే మేమే.. అంటూ

రాగానికి లాస్యం చేసి

భావానికి జీవం పోసి

నాట్యం లోకాలేలేము.. అని సంగీత, సాహిత్య,  
నృత్యాలకు జయభేరి మోగించిన చిత్రాలెన్నో తెలుగు  
తెరపై ఆవిష్కృతమయ్యాయి. మల్లాది రామకృష్ణశాస్త్రి,  
పెండ్యాల కలయికలో వచ్చిన 'రసికరాజ తగువారము  
కామా..' గీతం ఈనాటికీ సంగీత నేపథ్య గీతంగా  
వేదికలపై మన్ననలందుకొంటున్నది.



ఈ కోవలో 70వ దశకంలో వచ్చిన 'శంకరాభరణం' సంగీతానికి పెద్దపీట వేసి యావత్ ఆంధ్రదేశాన్ని సంగీత శిక్షణ కోసం స్వర గురువుల ముందు ఆసీనం గావించింది.

'ఓంకార నాదానుసంధానమౌ గానమే.. శంకరాభరణము' అని ప్రతి కంఠాన్ని ఎలుగెత్తి పాడించింది.

వేటూరి కలం నుంచి వెల్లువెత్తిన పదగతులు అద్భుత రసభావ విన్యాసాలతో ఈ చిత్రంలోని పాటలకు అజరామర కీర్తిని తెచ్చి పెట్టాయి.

రాగం తానం పల్లవి

నా మదిలోనే కదలాడి కడతేరమన్నవి

నాదవర్తులై వేదమూర్తులై

రాగకీర్తులై త్రిమూర్తులై

రాగం తానం పల్లవి

నా మదిలోనే కదలాడి కడతేరమన్నవి.. అని గానమే తన ప్రాణమని నమ్మిన సంగీతజ్ఞుని జీవితం ఈ చిత్రంలో అసామాన్యంగా తెరకెక్కించబడింది.

'శాంతి నివాసం' చిత్రంలో కథానాయిక తన ప్రాణ సఖుని రాక కోసం తుషార శీతల సరోవరాన అనంత నీరవ నిశీధిలోన వీణ మీటుతూ చేసే నిరీక్షణ అమేయ గానమై మన హృదయాలను ఆహ్లాదపరుస్తుంది.

'కలనైనా నీ వలపే

కలవరమందైనా నీ తలపే..'నంటూ నాయిక మది కథానాయకుడి పద సన్నిధికై పరితపిస్తుంది.

'కలవని తారకు కమ్మని కలలు

కళలూ కాంతులు నీ కొరకే'నని పల్లవించే ఈ గీతం లలిత రసాల పల్లవమై మన మనోవీధులలో సంగీత గంగా తరంగాలనుప్పొంగేలా చేస్తుంది! మనోల్లాసాన్ని కలిగించే గీతం అది భావ ప్రధానమైనా, రాగ ప్రధానమైనా దాని పరమార్థం మనలను ఆనందడోలికలలో తేలియాడించడమే!

హిందోళ రాగంలో పల్లవించే..

'మదిలో వీణలు మ్రోగె

ఆశలెన్నో చెలరేగె

కలనైన కనని ఆనందం

ఇలలోన విరిసె ఈనాడే..' అని సాగే దాశరథి గీతం వీణ పాటలలో అగ్రగామి! సాలూరు రాజేశ్వరరావు స్వరపరచిన వీణామృత రసధార ఈ గీతం! వెండితెర వీణ పాటలకు ఆయనది ప్రత్యేక ముద్ర!

చలన చిత్ర గేయాలలో సంగీతంతో సమానంగా నవరస నాట్య సమారోహమూ అంతే స్థాయిని సంపాదించుకుంది. పలు చిత్రాలలో నృత్యమే ప్రధానంగా సాగిన ఇతివృత్తాలను మనం మెండుగా చూడవచ్చు.

'నెమలికి నేర్పిన నడకలివి

మురళికి అందని పలుకులివి

శృంగార సంగీత నృత్యాభినయ వేళ

చూడాలి నా నాట్య లీల..' అంటూ కథానాయిక ఎంతో ఆత్మబలంతో పాడుతూ చేసే నృత్యం అమందానందకరం!

'సప్తపది' చిత్రంలో రసప్లావితం చేసే ఈ గీతం ఆద్యంతం రసానందభరితం!

'కలహంసలకిచ్చిన పదగతులు

ఎలకోయిల మెచ్చిన స్వరజతులు

ఎన్నెన్నో వన్నెల వెన్నెలలు

ఏవేవో కన్నుల కిన్నెరలు..' ఈ పాటలో అభినయించే కథానాయిక నాట్యంలో చూసి ఆనందించవచ్చు.

ఇదే గీతంలోని ఇతర పాదాలలో..

'కురులు విరిసి

మరులు కురిసి మురిసి

రవివర్మ చిత్రలేఖనా లేఖ్య

సరస సౌందర్య రేఖలను..' సైతం కవి తన కలం కుంచెతో చిత్రించిన తీరు అనితరసాధ్యం!

ఈ గేయంలో ప్రతి పదమొక కూచిపూడి ముద్రగా, అభినయ వర్ణ రంజితంగా అలరించడం కథానాయిక

నాట్య ప్రదర్శనకు మరింత వన్నె చేకూర్చింది. అందుకే ఈ గీతం తదనంతర తరాలకు కూడా చిరస్మరణీయ నృత్య గీతమై భాసిల్లుతున్నది.

‘స్వర్ణకమలం’ చిత్రం నాట్యకళ ప్రాభవాన్ని ఆధునిక జీవన శైలి ఎలా అడుగంటేలా చేస్తోందో చెబుతూ కథానాయిక పాత్ర ద్వారా దానికి తిరిగి జీవం పోసే ఇతివృత్తంతో అందరినీ ఆకట్టుకుంది. ప్రాచీన నృత్య కళ పాట్లకూటికి ఏమాత్రం పనికిరాదన్న అపోహను తొలగిస్తూ అవధులు లేని కళలకెప్పుడూ ఎలాంటి అవరోధాలూ ఉండవని నిరూపిస్తుంది చిత్రం!

‘అందెల రవమిది పదములదా  
అంబరమంటిన హృదయముదా  
అమృతగానమిది పెదవులదా

అమితానందపు ఎద సడిదా..?’ అంటూ తెప్పరిల్లిన హృదయంతో కథానాయిక చేసే ఎద సడి పదములపై నాట్య ప్రభలుగా ఎగజిమ్మడం నయనానందకరంగా అనిపిస్తుంది. సిరివెన్నెల పాళీ విన్యాసం, ఇళయరాజు స్వరోల్లాసాల మేళనంగా సాగే ఈ గీతం మరో అపురూప నృత్యగీతం!

అంగవైకల్యం కలిగినా తన కళాత్పష్ట తీరదంటూ నాట్యకళలో రాణించిన కథానాయిక జీవితాన్ని నృత్య నేపథ్యంలో జనరంజకంగా ఆవిష్కరించిన చిత్రం ‘మయూరి!’ ప్రజలలో నృత్యంపై ఒక నూతనోత్సాహాన్ని కలిగించిన ఈ చిత్రం కూడా నాట్య ప్రధాన చిత్రాల్లో మేటిగా నిలవడం గమనార్హం!

‘ఈ పాదం ఇలలోన నాట్య వేదం

ఈ పాదం నటరాజుకే ప్రమోదం..’ అంటూ తన పదఘట్టనలతో నాయిక అలరించడం విశేషం.

ఈ పాదమే మిన్నాగు తలకు అందం

ఈ పాదమే ఆనాటి బలికి అంతం

తనలోని గంగమ్మ ఉప్పొంగగా

శిలలోని ఆ గౌతమే పొంగగా..’ ఆ పాదం చేసే తాండవం ప్రతి అక్షరంలో కొత్త వుంతలు తొక్కుతుంది.

మణిరత్నం దర్శకత్వంలో వచ్చిన ‘ఇద్దరు’ చిత్రంలోని మరో నృత్య గీతం లలిత కళాభిమానులకు కన్నుల పండువగా పేర్కొనవచ్చు.

‘శశివదనే.. శశివదనే

స్వర నీలాంబరి నీవా..

అందెల వన్నెల వైఖరితో

నీ మది తెలుపగ రావా..!’ అనే పల్లవి ఎత్తుగడలోనే అనువాద సాహిత్యమైనా అద్భుతమైన పదచిత్రాలతో పాటని అలంకరించిన తీరు అపూర్వమని చెప్పవచ్చు. వేటూరి కలంకారీతనం ప్రతి పాదంలో అద్భుతంగా ప్రతిబింబిస్తుంది.

‘అచ్చొచ్చేటి వెన్నెలలో

విచ్చందాలు నవ్వగనే

గుచ్చెత్తేటి కులుకు సిరి నీదా..?’ అంటూ అసమాన మైన ప్రతీకలతో సాగే ఈ గీతంలో నాయిక నృత్యాభి నయం అభినందించదగినది.

సంగీత సాహిత్య నృత్య అభినయాలకు నెలవైన పలు రూపకల్పనలు ఈ చలనచిత్ర గేయాలలో మనం నిత్యం దర్శించవచ్చు. లలిత కళలకు పురాణ, ఇతిహాసాలలోనే గాక చలనచిత్రాలలో సైతం వాటికి నిండైన స్థానం ఉన్నదనడంలో ఎలాంటి సందేహమూ లేదు. స్వల్ప వ్యవధిలో శ్రోతలకు మానసిక ఉల్లాసాన్ని కలిగించడంలో సినిమాలే ఈనాడు అగ్రస్థానాన్ని ఆక్రమించాయని చెప్ప వచ్చు. అదే క్రమంలో పలు కళలకు సైతం ఇతోధికంగా చలనచిత్రాలు వేదికగా నిలవడం కళారాధకులందరూ ఆనందించదగిన అంశం!



ఉపయుక్త గ్రంథసూచిక :

1. పాటలో ఏముంది? నా మాటలో ఏముంది?  
మొదటి భాగం - రచన: డా.సి.నారాయణరెడ్డి
2. జయభేరి చిత్రం
3. శంకరాభరణం చిత్రం
4. ఆత్మేయ సాహితీ సంపాదకుడు: డా.పైడిపాల
5. స్వర్ణకమలం చిత్రం
6. సప్తపది చిత్రం
7. పగలే వెన్నెల - డా. సినారె
8. మయూరి చిత్రం
9. ఇద్దరు
10. యూ ట్యూబ్

★★★

# Comparative Analysis of Apriori and FP-Growth Algorithms For Frequent Item Sets

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## ABSTRACT

In Data Mining, the frequent item sets play an essential role in many tasks that try to find interesting patterns from databases, such as association rules, correlations, sequences, classifiers and clusters. The mining of association rules is one of the most popular problems of all these. Association rule mining is a standard method and research technique for discovering interesting relations between variables in huge databases. The identification of sets of items, products, symptoms and characteristics, which often occur together in the given data base is the most basic tasks in Data Mining. Association rule mining primarily focuses on finding frequent co-related associations among a collection of items. The goal is to find associations of items that occur together frequently from a sampling of all possibilities. We propose the most essential algorithms in mining such as Apriori and FP\_Growth algorithms, which are used to mine the frequent patterns. The Apriori algorithm generates candidate set in each pass, it reduces the data set by discarding the infrequent item sets that do not meet the minimum threshold from the candidate sets. To avoid the generation of candidate set, the FP\_Growth algorithm is used to mine the database. The aim of this paper is to determine the comparison of Apriori & Fp-Growth algorithms for frequent item sets on sample data.

Keywords : Frequent Item Sets, Association rules, Classifier, Apriori, FP\_Growth, Support, Confidence.

## I. INTRODUCTION

Data mining is an exploration and analysis of large quantities of data in order to discover meaningful patterns. It is also non-trivial extraction of implicit, previously unknown and potentially useful information from data. Some of the origins of data mining are machine learning, artificial intelligence, statistics, etc. Data mining contains the tasks like classification, clustering, association rule discovery, sequential pattern discovery, regression, etc.

In Data Mining, the frequent item sets play an essential role in many tasks that try to find interesting patterns from databases, such as association rules, correlations, sequences, classifiers and clusters. The mining of association rules is one of the most popular problems of all these. The identification of sets of items, products, symptoms and characteristics, which often occur together in the given data base is the most basic tasks in Data Mining.

Frequent pattern mining is an important area of data mining research. The frequent patterns are patterns such as item sets, sub sequences or sub structures that appear in a data set frequently.



For example, a set of items, such as milk and bread that appears frequently together in a transaction data set is a frequent item set. Another example a set of items mobile and sim card that appear frequently as well as together in a transaction set is a frequent item set. Sequence means if a customer buys a mobile he must also buy a sim card and then head phone etc. From the history of the database these transactions are happening sequentially is called sequential patterns. Frequent item sets have many application areas like customer relationship management, fraud detection, product assortment decisions (BRIJSet.al., 1999), episode mining (MANNILAet.al., 1995), functional dependency discovery (HUHTALAet.al., 1999)etc.

## II. LITERATURE (EXISTING SYSTEM)

In the literature on the frequent item sets the algorithms are usually studied from a practical view point. Traditional system based on the manual calculations and dynamic counting method. But it is very difficult to find frequent patterns from the large transaction using manual calculation. So it is difficult to implement DIC (Dynamic Item set Counting) algorithm [7][8]. DIC algorithm divides the database into partitions and uses a dynamic counting strategy. It determines some stop points for item set counting . Any appropriate points during the database scan, stopping counting, then starts to count with another item sets.

### Disadvantages:

- It require manual calculation.
- Very difficult to process the large transaction.
- Time requirement is high.
- Difficult to implement.

To achieve an optimal running time, specific implementation methods are applied. In this paper, frequent item sets are discussed from an algorithmic view point. Given the vast literature, mining frequent item sets has become a topic as classical as sorting arrays or finding shortest paths in a network.

Market Basket dataset mainly used in large databases. The dataset is frequently update at the time transaction, and also maintains frequent item sets. Association rule mining is one of the major tasks of the Data mining. Association rules were presented by R. Agarwal and others in 1993, It finds the better correlation relationship among a large set of data items.

Association rules is composed of the following two steps.

1. Find the large data sets that have transaction support above minimum support by using frequent item set mining algorithms.
2. From the discovered large item sets, generate the desired association rules based on some standard measures.

The motivation for searching association rules arose from the need to analyze so called Market Basket transaction data, that is to examine customer behavior in terms of the purchasing products. For example, “ bread=>butter (80%)” states that 80 % of the customers that bought bread also bought butter. Such rules can be useful for decisions concerning product pricing, store layout, and many other things.

Since its introduction in 1993, the task of association rule mining has received a great deal of attention. Today the mining of such rules is still one of the most popular pattern discovery methods in knowledge discovery methods. Frequent item set mining leads to the discovery of associations and correlation among items in large relational datasets. The algorithms most frequently discussed in text books on Data mining are Apriori (AGARWAL AND SRIKANTH, 1994, AGARWAL et.al.,1996), FP-Growth (HAN et.al.,2000,20004). Here a rough comparison between the best known algorithms is made, in order to get a better understanding. And these are used to mine the frequent patterns.

### III ASSOCIATION RULE MINING

Association Rule Mining aims to extract interesting correlations, frequent patterns, associations among sets of items in the transaction databases. The major purpose of it is to find the set of all subsets of items that frequently occur in many transactions and also to extract rules on how a subset of items influences the presence of another subset.

In general, the association rule is an expression of the form  $X \Rightarrow Y$ , where  $X$  is antecedent and  $Y$  is consequent. Association rule shows how many times  $Y$  has occurred if  $X$  has already occurred depending on the support and confidence value.

**Support:** It is the probability of item or item sets in the given transactional database.

$$\text{support}(X) = n(X)/n$$

where  $n$  is the total number of transactions in the database and  $n(X)$  is the number of transactions that contains the item set  $X$ .

so,  $\text{support}(X \Rightarrow Y) = \text{support}(XUY)$ .

**Confidence:** It is conditional probability, for an association rule  $X \Rightarrow Y$  and defined as

$$\text{confidence}(X \Rightarrow Y) = \text{support}(XUY) / \text{support}(X)$$

**Frequent Item Set :** Let  $A$  be a set of items,  $T$  be the transaction database and  $\sigma$  be the user specified min-support . An item set  $X$  in  $A$  is said to be a frequent item set in  $T$  with respect to  $\sigma$  if  $\text{support}(X)_T \geq \sigma$ .

This rule has two sub problems,

1. Find all set of items whose support is greater than the user specified min-support,  $\sigma$ , such item sets are called frequent item sets.
2. Use the frequent item sets to generate the desired rules.

### IV PROPOSED SYSTEM (ALGORITHMS)

We propose the efficient algorithms used to mine the frequent patterns.

**1. Apriori:** The first algorithm for mining all frequent item sets and strong association rule was Apriori[6]. This algorithm is proposed by R. Agarwal and R. Srikanth in 1994 for mining frequent item sets for Boolean association rules. The name of the algorithm is based on the fact that the algorithm uses prior knowledge of frequent item set properties. It is designed to operate on databases containing transactions. Apriori is a classical algorithm of association rules mining, adopts an iterative method to discover frequent item sets. The algorithm terminates when no further successful extensions are found. It consists of two steps.

1. The join step
2. The prune step



In the join step, a candidate  $k$ - item sets is generated by joining two frequent  $(k-1)$  item sets. In the prune step, all item sets whose  $(k-1)$  subset is not a frequent  $k$ - item sets , are removed from the candidate  $k$ - item sets. Then the database is scanned to compute the support of the candidate  $k$ - item sets. This process is repeated until no new candidate  $k$ -item sets is generated. A huge number of scans of the database and a complicated transaction process are required during the algorithm. It is an influential algorithm for mining frequent item sets for Boolean association rules.

The pseudo code of Apriori algorithm for discovering frequent item sets for mining is shown below.

Join Step :  $C_k$  is generated by joining  $L_{k-1}$  with itself.

Prune Step : Any  $(k-1)$  item set that is not frequent cannot be a subset of a frequent  $k$ - item set.

Code:

```

 $C_k$ : Candidate item set of size  $k$ 
 $L_k$ : Frequent item set of size  $k$ 
 $L_1 = \{\text{frequent items}\};$ 
for ( $k=1; L_k \neq \emptyset; k++$ ) do begin
 $C_{k+1} =$  candidates generated from  $L_k$ ;
  for each transaction  $t$  in database do
    increment the count of all candidates in  $C_{k+1}$ 
    that are contained in  $t$ 
   $L_{k+1} =$  candidates in  $C_{k+1}$  with  $\text{min\_support}$ 
end
return  $C_k, L_k$ ;

```

### Example :

Consider a database,  $D$ , consisting of 10 transactions.

Suppose minimum support count requires is 2 (i.e.  $\text{min\_sup} = 2/10 = 20\%$ )

TID	List of items
T10	I1, I2, I4
T20	I2, I4
T30	I2, I3
T40	I2, I4, I5
T50	I1, I2, I5
T60	I2, I3, I4
T70	I1, I5
T80	I1, I4, I5
T90	I2, I3, I4, I5
T100	I2, I5

Table 1: database consisting 10 transactions

**Step 1:** Count the number of transactions in which each item occurs (Table 2.a).

**Step 2:** In this step we remove all the items that are bought less than 2 from the Table 1 (Table 2.b)

C1

Item set	Sup-count
I1	4
I2	8
I3	3
I4	6
I5	6

(a)

L1

Item set	Sup-count
I1	4
I2	8
I3	3
I4	6
I5	6

(b)

Compare and Prune

Table 2: first scan of Apriori.

**Step 3:** Make all the pairs of items by using property JOIN L1 with L1 and count how many times each pair is bought together (Table 3.a)

**Step 4 :** Remove all the item pairs with number of transactions less than 2 (Table 3.b)

C2

Item set	Sup-count
I1,I2	2
I1,I3	0
I1,I4	2
I1,I5	3
I2,I3	3
I2,I4	5
I2,I5	3
I3,I4	2
I3,I5	1
I4,I5	3

(a)

L2

Item set	Sup-count
I1,I2	2
I1,I4	2
I1,I5	3
I2,I3	3
I2,I4	5
I2,I5	3
I3,I4	2
I4,I5	3

(b)

Compare and Prune

Table 3 : second scan of Apriori.

**Step 5:** To make the set of three items we need one more rule – self join

It simply means , from the item pairs in the above table we find two pairs with the same first item.

C3

Item set	Sup-count
I1,I2,I4	1
I1,I2,I5	1

(a)



Table 4: third scan of Apriori

According to the rule the statements I1,I2,I4 and I1,I2,I5 are generated whose minimum support is less than 2. So this not frequent.

Thus, the set of two items that are bought together most frequently are I2,I4.

#### Advantages:

- Easy and simple to implement.
- Uses large item set property.
- Very fast compare than existing system (DIC algorithm).

#### Disadvantages:\

- Requires many database scans.
- For candidate generation process it takes more memory, space and time.

**2.FP- Growth:** This algorithm was proposed byGrahneand is based on the usage of CPU time to compute frequent item sets using FP-Growth.It is a well known algorithm that uses the FP tree data structure to achieve a condensed representation of the database transactions and employs divide and conquer approach to decompose the mining problem by reducing the passes, shrinking number of candidates and facilitating support counting of candidates.It isan efficient and scalable methodto complete set of frequent patterns. It allows frequent item set discovery without candidate item set generation. It has two steps.

1. Build a compact data structure called FP-tree.
2. Extracts frequent item sets directly from the FP-tree.

Input : A transaction database D and a minimum support threshold

Output : FP-tree , the frequent pattern tree of D.

The FP- tree is constructed as follows.

- Scan the transaction database D once. Collect F, the set of frequent items, and the support of each frequent item. Sort F in support – descending order as F- list, the list of frequent items.
- Create the root of an FP-tree t, and label it as null . For each transaction Trans do the following.
  - Select the frequent items in trans and sort them according to the order of F-list. Let the sorted frequent item list in Trans be [p|P] where p is the first element and P is the remaining list. Call insert tree([p|P],T).
  - The function insert tree ([p|P],T ) is performed as follows. If T has a child N such that N. item- name= p. item- name, increment N's count by 1, else create a new node N, with its count initialized to 1, its parent link linked to T , and its node link linked to the nodes with the same item- name via the node-link structure. If P is non empty, call insert tree(P,N) recursively.

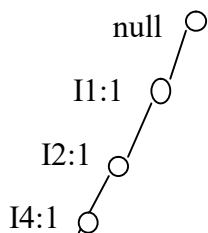
**Example :**

Let us create the FP-tree for the example Table 1:

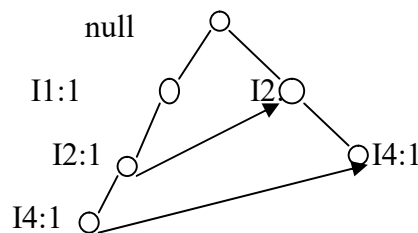
- First we scan the database and determine the set of frequent items (1- item sets) and their support counts (frequencies):  
 $L = \{\{I2,8\}, \{I4,6\}, \{I5,6\}, \{I1,4\}, I3,3\}$
- Then we create the root of the FP-tree and label it with “null”.
- We take each transaction, sort the items according to descending support count, and create a branch for it. For example the scan of the first transaction “ T10: I1,I2,I4 ”, which contain tree items: I2, I4, and I1 in sorted descending, leads to the construction of the first branch of the tree: (I2:1), (I4:1),(I1:1).

The second transaction T20 contains the items I2 and I4. This would result a branch where I2 is linked to the root and I4 is linked to I2. However, this branch share a common prefix I2, with the existing path for T10. Therefore, we instead increment the count of the I2 node by 1 and create a new node (I4:1), which is linked as a child of (I2:2).

TID	List of items
T10	I1, I2, I4
T20	I2, I4
T30	I2, I3
T40	I2, I4, I5
T50	I1, I2, I5
T60	I2, I3, ,I4
T70	I1, I5
T80	I1,I4,I5
T90	I2,I3,I4,I5
T100	I2,15

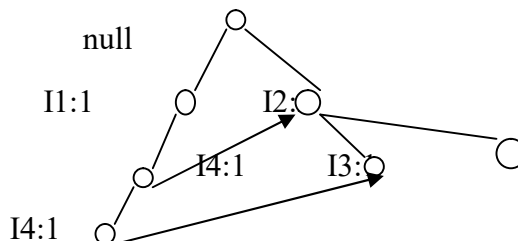


(i) After reading TID= T10



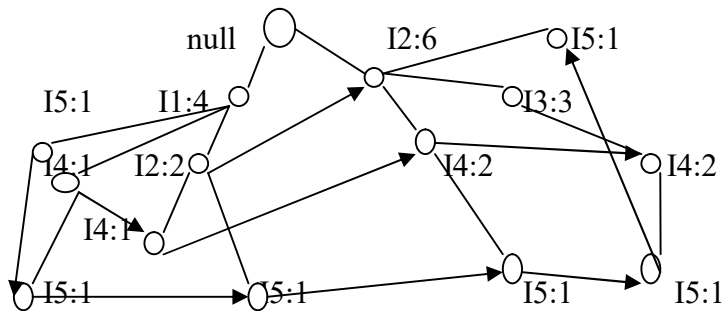
(ii)After reading TID-T20

I2:1



(iii) After reading TID=T30





(iv) After reading TID – T100

Item	Frequency
I1	4
I2	8
I3	3
I4	6
I5:	6

Now the FP- tree is mined as follows.

Start from each frequent length – 1 pattern, as an initial suffix pattern, construct its conditional pattern base, a sub database, which consists of the set of prefix paths in the FP-tree co-occurring with the suffix pattern, then construct its conditional FP-tree and perform mining recursively on such a tree. The pattern growth is achieved by the concatenation of the suffix pattern with the frequent patterns generated from a conditional FP-tree.

- Let us consider I3, which is the last item in L. I3 occurs only in one branch of the FP-tree.
  - (I2,I3:1)
- I3 is a suffix, so its corresponding prefix path is
  - (I2:1)
- The single path generates the combinations of frequent patterns:
  - (I2,I3:1)
- The frequent pattern (I2:1)

#### Advantages:

- Much faster than Apriori.
- No candidate generation.
- Compresses data set.

#### Disadvantages:

- FP tree may not fit in memory.
- Fp-tree is expensive to build.

## V COMPARISON

Both Apriori and Fp-Growth algorithms are used to find frequent item sets from database. Each will have its individual technique to discover or identify frequent patterns. The comparison between both algorithms based on capacity, number of scans, technique, use of memory, type of method and time consumed are discussed below.

**Capacity** : Apriori algorithm works well with large database, where as FP- Growth does not work with large database.

**Number of Scans** :Apriori algorithm takes multiple scans to generate candidate key. FP-Growth algorithm scans database only twice.

**Technique**: Apriori algorithm uses join and prune property for mining frequent patterns. FP-Growth algorithm constructs conditional pattern free and conditional pattern base from the database which satisfies the minimum support.

**Use of Memory** :Apriori algorithm requires large memory space because it works with large databases. FP- Growth algorithm requires less memory space due to its compact structure they discover frequent item sets without candidate item set generation.

**Method Type** : Apriori uses breadth- first search method and FP- Growth uses divide and conquer method.

**Time**: In Apriori algorithm execution time will be wasted in producing candidates for every scan. FP-Growth algorithm's execution time is less than Apriori.

## VI CONCLUSION

Frequent item set is an important area in data mining research Frequent item set mining is an important task in association rule mining. It has been found useful in many applications like customer relationship management, fraud detection, product assortment decisions, episode mining, functional dependency discovery, market basket analysis etc. In this paper, we have discussed about two classical algorithms, Apriori and FP-Growth with their different techniques. In Apriori we found that for all candidate item set, each level has to be discovered, so the length of the frequent item set becomes longer and also the number of candidate generation is more. To overcome the above problem we used FP-Growth algorithm, in which it uses tree method, is efficient in terms of speed but utilized more memory space. This can be overcome by using other techniques like partitioning etc. In this paper, comparison is also made between them.



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## **Innovative IoT Solutions For The Disabled**

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### **Abstract**

Currently over 15% of world's population are projected to be living in disability. Living with a disability may be difficult, but with the help of Internet of Things (IOT), it is possible to make their lives easier, productive and fruitful. Different disabilities affect people in different ways like Mobility & Physical impairments, Vision Disability, Hearing Disability, Learning Disabilities, Psychological Disorders, etc. IOT is a concept where each thing is uniquely identifiable through its embedded computer system and is able to interoperate with the Internet infrastructure. The IoT is enabled by smart devices, communication technologies, Internet protocols etc. The Internet of Things can offer people with disabilities, the assistance they require to lead a good quality of life and allows them to participate in all kind of activities. This paper presents an overview of IoT and how it can be used to help the people with disabilities and describes relevant IoT solutions.

*Key Words: Internet of things, Disabilities, Smart devices, Mobile Internet, Solutions.*

### **Introduction**

A disability is often used to refer to individual functioning, including physical impairment, sensory impairment, cognitive impairment, intellectual impairment, mental illness, and various types of chronic diseases. 3rd December is the International Day of Persons with Disabilities. Having disability is the vulnerable situation in the human life. The Disabled persons have to face many challenges to lead their everyday life. The *World report on disability (WRD)* [1] is the first document to give an extensive global picture of the situation of people with



disabilities, their needs, and the difficulties they face in participating fully in their societies. The aim of the report is to support the implementation of the Convention on the Rights of Persons with Disabilities (CRPD). Published in 2011 by the World Health Organization (WHO) and the World Bank, the report assembles scientific information on disability, with relevance to the fields of public health, human rights and development.

### Key findings of the WRD

- More than 1 billion people in the world have some form of disability. This corresponds to about 15% of the world's population.
- People with disabilities are more likely to be unemployed than non-disabled people. The employment rate of people with disabilities (44%) is slightly over half that for people without disabilities (75%).
- People with disabilities often do not receive needed health care. Half of disabled people cannot afford health care, compared to a third of non-disabled people.
- People with disabilities experience increased dependency and restricted participation in their societies. Even in high-income countries, 20-40% of people with disabilities lack the help they require to engage in everyday activities.

### IoT

The “Internet of things” (IoT) is becoming an increasingly growing topic of conversation both in the workplace and outside of it. It is a concept that not only has the potential to impact how we live but also how we work. As per Wikipedia Internet of things can be defined as *“the network of physical objects, devices, vehicles, buildings which are embedded with electronics, software, sensors, and network connectivity, which enable these objects to collect and exchange data”*. It is a joint and complex discipline that requires synergistic efforts from several communities such as telecommunication industry, device manufacturers, semantic Web, informatics and engineering [2].

The Internet of Things allows objects to be sensed and controlled remotely across existing network infrastructure, creating opportunities for more-direct integration between the physical world and computer-based systems, and resulting in improved efficiency, accuracy and economic benefit.

When IoT is augmented with sensors and actuators, the technology becomes an instance of the

more general class of cyber-physical systems, which also encompasses technologies such as smart grids, smart homes, intelligent transportation and smart cities. Each thing is uniquely identifiable through its embedded computing system but is able to interoperate within the existing Internet infrastructure. For technology to disappear from the consciousness of the user, the Internet of Things demands, a shared understanding of the situation of its users and their appliances, software architectures and persistent communication networks to process and convey the related information to where it is relevant, and the analytic tools that aim for independent and smart behavior. With these three fundamental grounds in place, smart connectivity and context-aware computation can be accomplished [3]. Experts estimate that IoT will consist of almost 50 billion objects by 2020. The Internet of Things is a prominent technology, which helps the persons with disabilities to improve their life and quality. The Internet of Things is made possible by what Alain Louchez, managing director of Georgia Tech's Center for the development and Application of Internet of Things Technologies (CDAIT), calls DNA [4]: **Devices** with miniaturization of sensors and captors, **Network** through radio signal, wireless Internet, etc. and **Applications**.

## Devices

Over the past few decades, researchers and industry players have developed Micro-Electro-Mechanical Systems (MEMS). These are miniaturized structures, sensors, actuators and microelectronics, the sizes of which range from microns to several millimeters. These miniature devices called nodes interconnect to form a wireless sensor networks (WSN) and find wideranging applications in environmental monitoring, infrastructure monitoring, traffic monitoring, retail, etc [5]. Micro sensors and micro actuators are categorized as "transducers," which are defined as devices that convert energy from one form to another. In the case of micro sensors, the device typically converts a measured mechanical signal into an electrical signal. These new microelements are very powerful: for example, researchers have placed small micro-actuators on the leading edge of airfoils of an aircraft and have been able to steer the aircraft using only these devices, as the industry organization MEM Exchange points out. As the technology evolves, the merger of MEMS with the integrated circuits that power computing will become more common, and will make more complex applications available to the end user. Ultimately, miniaturization



will allow any object to be connected, which could have substantial positive impacts for persons with disabilities as more and more products can be controlled from accessible computing platforms. The number of connected devices is set to explode from 4.9 billion this year, according to the Gartner Group, to 25 billion or even 50 billion by 2020.

## **Network**

As important as these advancements in microelectronics are, the Internet of Things refers not just to devices, but also to the connections between them. For IoT applications to work, the sensors and the actuators must be able to communicate with the devices that inform their action, whether it is a smartphone or something as simple as a remote thermometer. Communication between devices can be established by a variety of different modes, including radio signals, Bluetooth (for devices in close proximity) or an Internet connection (Wi-Fi and wireless broadband for a wider range of communication and for video). And, as our wired and wireless networks reach more and more people, the potential applications for the Internet of Things will continue to expand.

## **Applications**

New IoT applications are being introduced to the market every day. While IoT applications are expected to penetrate into many activities both consumer and industrial smart home applications seem to be the fastest growing segment. Connected home devices are expected to comprise 25% of all Internet of Things devices shipped this year, according to Business Intelligence, a market currently valued at \$61 billion and expected to jump to \$490 billion by 2019. Many of these, as the next section discusses, have potential to improve quality of life for persons with disabilities. Home automation applications and security systems are examples of this. Other examples include self-driving cars that identify traffic and obstacles and remote medical monitoring that makes access to care available to more people in more places.

### **Potential for Persons with Disabilities**

Many new IoT applications are comfort and security oriented. These applications enable a user to change his or her thermostat remotely, control door locks, activate alarm systems, etc. While these applications certainly add a level of fun and convenience for all users, the

applications take on a whole new level of importance when used by persons with disabilities and older adults. Smart phones can now be used by more persons with disabilities than ever before. Many manufacturers have also developed smartphones with easier and more intuitive interfaces. These interfaces facilitate use by older adults whose usage of smartphones may otherwise be impacted by vision or hearing loss or reduced dexterity and mobility. These accessible

smartphones are already becoming the remote controls for smart homes, smart transportation and smart cities, giving persons with disabilities a new and flexible instrument to navigate daily life and involve more fully in the economic and social environment. Industry experts agree that the Internet of Things has tremendous potential to bring benefits to persons with disabilities.

<b>Type of Disability</b>	<b>Examples of Useful Functionalities Enabled by IoT</b>
Physical and Dexterity	Remote support and services at home, Speech activated devices, Automated accessibility functions in public spaces.
Visual	Automation Speech, activated devices which communicate with speech output.
Hearing	Captioning glasses delivered by beacons, Visual cues about status of home devices on mobile device.
Cognitive	Localization and orientation, Automated reminders, Programmable safety processes.

### **Home Automation Technologies for disabled**

Of all the Internet of Things applications that have the potential to improve life for persons with disabilities, home automation technologies are among the most promising. Home automation technologies utilize a variety of connected cameras and sensors to relay information to the user's smart phone, allowing for remote monitoring and control of lights, thermostats, appliances, security systems and more.

Smart home technologies hold huge promise for persons with disabilities, improving quality of life and facilitating independent living. The examples include:



- For people who are blind or have low vision, home automation applications allow for easy control of appliances and the home thermostat, all with the touch of a button on a smart phone. It is crucial, however, that these applications be compatible with the smartphone's screen reader or other accessibility features.
- For people with mobility-related disabilities, smart home technology holds the promise of allowing the user to control things in his or her home that may be physically difficult to reach, such as lights, doorlocks and other security systems.
- For people who are deaf or hard of hearing, one benefit of home automation technology is improved security. A deaf person might not be able to hear a break-in, but a smart security system can help compensate for that by providing alerts through the user's phone in the event of suspicious movement picked up by a sensor outside the home.

Additionally, smart home technology allows the children and caregivers of older adults to monitor their daily routines, promptly alerting children and caregivers in the event that routine tasks are not performed. Several providers already have smart home platforms on the market today. One such platform is AT&T's Digital Life, which offers the "classic" features including cameras inside and outside the home, remote temperature monitoring and control, remote home light control, remote water detection and control, coded doorlock setc.

### **Conclusion**

Disabled people need extra help in carrying out their daily tasks and to lead better quality lives. Using IoT, people with disabilities can also participate in all walks of life at par with normal people. IoT has opened up previously unavailable avenues for the disabled in terms of opportunities for education, employment, healthcare, housing etc. IoT enabled devices are providing immense benefit to disabled people in making their lives better. The Internet of Things is still in its infancy, and it will take years before it becomes a part of our daily lives. Significant challenges still remain before the IoT can fulfill its promise to develop the lives of its users, particularly people with disabilities. Finally, we conclude that the Internet community can provide the resolution to increase and enhance the use of the Internet by persons with disabilities.

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## Mobile Application Development for Bank Loan Approval Prediction System using Machine Learning

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**Abstract**— Taking out a loan from a financial organization has become a widespread practice in today's environment. The number of people applying for loans is increasing every day, and they do so for a variety of different reasons. The banking system, which has a diverse range of products to provide, relies heavily on the credit system as its principal source of income. Because the interest earned on the loans, source of money. A big number of people, on the other hand, are asking for loans. It is not necessary that all application has been trustworthy, and the applicants can able to pay back the loan. Every year, we learn a batch of instances in which borrowers fail to repay their loans in full, resulting in considerable losses for the lending institutions. It's difficult to find a faithful borrower, he or she would repay the loan. When a procedure is carried out manually, there are plenty of possibilities for human mistakes. Predicting which customers are true is one of the most difficult tasks for any financial institution. This is very much important for further investigation of the loan approval prediction. To automate the process of granting bank loans, banking systems require an accurate modelling system to be implemented. To address this issue, a Machine Learning (ML) algorithm such as Logistic Regression (LR), Random Forest (RF), and C4.5 was applied on collected Kaggle data. The metrics of LR, RF, and C4.5 are used in a comparison to determine which ML algorithm is the most appropriate for the task at hand. The RF model was selected because of its high accuracy rate of 90.86%. A smartphone application has been developed to assist clients in saving both time and travel. Customers can use the app to determine whether or not they are eligible for a loan.

**Keywords**— Bank, Loan, Attributes, Categorical data, Metrics, Mobile application

### I. INTRODUCTION

People all around the world rely on banks to supply them with loans to get out of debt and achieve their objectives for a variety of reasons [1]. Because the economy is always

changing and the financial industry is becoming more competitive, borrowing money has become an unavoidable aspect of everyday life for many people. Small and large banks alike rely on loan lending to generate revenue and keep operations running smoothly during periods of financial duress. The banking industry is primarily reliant on loans, which serve as both a significant source of revenue and a significant source of risk. One of the most important components of a bank's total assets is the interest that it earns on the loans it makes. Due to intense competition, banks must now work incredibly hard to obtain a competitive advantage to grow their overall company. Banks have recognized that customer retention and fraud prevention are key components of a successful approach for healthy competition. The availability of a significant amount of data, the growth of a knowledge base, and the efficient use of that data have all helped banks establish efficient delivery channels [2]. Even while lending money is a win-win situation for both parties, there are major risks involved. 'Credit Risk' [3] is the risk that a borrower will not be able to repay a loan when it is due, as agreed upon by the lender and the borrower. That is why, before approving a loan, it is necessary to do a complete credit assessment. In the traditional lending procedure, banks frequently employ the '5C idea' to assess a borrower. For a long time, banks hired highly qualified individuals whose primary task was to review loan applications, make a final judgment on the applicant's appropriateness, and inform the applicant of the evaluation results. To establish whether or not a person was eligible for a loan, a numerical score known as the 'Credit Score' was utilized. Lenders and government agencies use this score to predict whether a borrower will be able to make timely debt payments based on their credit or payment history and other considerations [4].

Bank employees manually analyse the applicant's information and make loans to those who qualify. Going through all of the candidates and verifying their information takes a long time. Going through all of the applicants' information takes a long time and a lot of effort. Because every information is manually verified, there is a chance of human error. An ineligible applicant may be assigned the loan. As a solution, applied ML approaches to generate autonomous loan predictions. To train the system, we'll use the prior dataset. As a result, the machine can analyze and comprehend the process. The system will then do a background check and return the findings to us. The proposed project has benefits. As a result of this adjustment, loan approval times will be reduced. There will be no opportunity for human error because the entire process will be computerized. Loans will be made available to approved candidates as soon as possible.

The journal paper is partitioned into 8 parts. The necessity of the research is detailed in part I, the old works are analysed in part II, the steps of the research are overview in part III, part IV discuss the data collection, information, and processing, the working of ML algorithms are debated in part V, the creation of the mobile app and its language are summarized in part VI, part VII suggest the best algorithm and working of the designed app.

## II. LITERATURE SURVEY

The previous work done on this area is detailed in this part. Research [5,] presents a method for predicting whether a loan request will be approved or rejected based on its trustworthiness. This will substantially benefit the banking industry by opening up new avenues for effective delivery. The Decision Tree (DT) Algorithm is used to make the prediction. Other methodologies that outperform standard data mining algorithms in the domain must be developed and validated. For the sake of saving time and money, the author [6] tries to minimize the danger by selecting the safest person. Big Data from previous loans is mined and used to train the machine using an ML model, which then uses the most accurate



information from those prior loans to train the machine. Predicting whether a certain individual will be safe to lend money to is the primary goal of this research. In the last few years, a slew of machine-learning-based approaches to the problem of loan approval has been proposed to address the issue. However, throughout the processing phase, these algorithms failed to take into consideration the Real-time paradigm. The author proposes Real-Time classification to deal with loan approval [7], which he believes will be more efficient. The proposed approach, which makes use of a Deep Neural Network (DNN), is capable of classifying loan applicants as either good or bad risks. A DNN-based Real-Time model, such as the one proposed by us, outperforms typical classifiers in terms of accuracy, and precision, demonstrated by our experiments.

Utilizing standard ML techniques [8] it is possible to profit from the financial information of customers. The author investigated the role of DNN in this research because of its high success rate in a variety of fields such as picture identification, audio recognition, and natural language processing. Contrasted old approaches such as Naive Bayes, DTs, and K-Nearest Neighbour to more recent learning methods such as reinforcement learning. When put through a set of tests, DNN outperforms its traditional competitors. DNN has an accuracy of 0.73 and a recall of 0.42 on the accuracy and recall tests. When compared to traditional approaches, it has an F1-score that is 25 percent higher than the best in the industry. To construct the loan prediction project, ML algorithms such as LR are being applied [9]. To deploy the website, the Python code developed in Colab is used to implement the code, and the HTML pages are created in Visual Studio code. The proposed system has the potential to produce high accuracy results with only low data loss when used for training and validation data. Finally, but certainly not least, the findings demonstrate that the model was effectively deployed and is exceptionally accurate in its predictions. It is possible to reduce the scope of this work to improve its accuracy even further, although this is not recommended. To develop better predictions about how much a consumer will be able to borrow based on demographic data from several sources, first understand how loan systems work [10]. Then must use ML techniques to this model. The primary purpose of the project will be to predict which clients will be able to pay back their loans and which will not. Popular algorithms such as DT, LR, and RF will be employed to accomplish this goal. Reach an accuracy of 86% with the least number of mistakes by analysing the LR Confusion Matrix, which is comparable to the procedures used in DT and RF.

### III. RESEARCH FLOW

The steps are taken to automate bank loan approval based on the customer information detailed in this part. Figure 1 depicts the research flow in pictorial format. The flow of the research are 1) Bank loan data collection from Kaggle, 2) Identify and cleaning the missing values present in bank loan dataset, 3) Conversion of categorical elements in the dataset to numerical values by encoding method, 4) To reduce the training time and overfitting problem important features are identified using heatmap, 5) Train the ML model using 80% of data, 6) Identify the best ML model by employing 20% of data, 7) Develop user-friendly mobile app by deploying the best ML model.

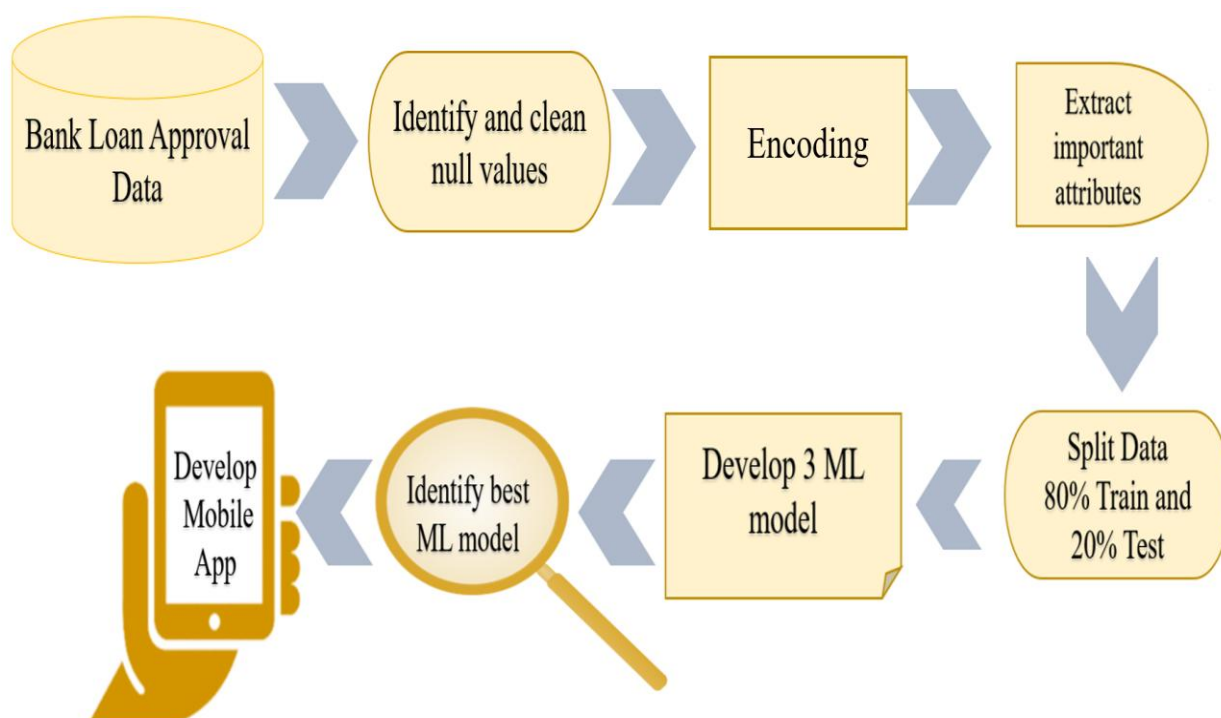


Fig. 1 Research Flow of the proposed work

#### IV. DATA ACQUISITION AND PROCESSING

The classification learning methods utilized are heavily influenced by the quality and quantity of data needed to train a classification model. This section uses exploratory data analysis to increase prediction accuracy by pre-processing the Kaggle loan approval dataset. Approximately 30% of the data will be used for testing, with the remainder being used for training (70%). A training set is a collection of data that acts as the program's starting point as it learns and applies increasingly complex technology. Furthermore, the labelled example is used to calculate the weights and biases of training models. The ML method builds models for the training dataset that look at multiple examples in an attempt to find the model with the least degree of loss. It does, however, have the same probability distribution as the training data set and is used to quantify performance independently of it. The training data will be used to fit the model, and the test data will be used to evaluate which model best fits the data. The obtained data is not directly fed into the ML algorithm. Need to employ some pre-processing procedures to get the data ready. The following section goes over the pre-processing methods used in this project in further depth.

##### A. Cleaning

Inconsistencies in the data may develop as a result of the presence of missing values. Pre-processing of data is required to obtain better results from the algorithm. Getting rid of the outliers and doing the things that need to be done are priorities right now. By using the simple python function “`isnull()`”, the total null values present in the data are identified. Figure 2 shows the number of null values present in the data. The attributes where null values are present are gender, marital status, number of dependents, nature of employment, loan details, and past credit details. For eliminating the null values, the random, mean, and median methods are used.



```

#      Column      Non-Null Count  Dtype
---  -
0      Loan_ID      614 non-null    object
1      Gender        601 non-null    object
2      Married        611 non-null    object
3      Dependents      599 non-null    object
4      Education       614 non-null    object
5      Self_Employed   582 non-null    object
6      ApplicantIncome  614 non-null    int64
7      CoapplicantIncome 614 non-null    float64
8      LoanAmount       592 non-null    float64
9      Loan_Amount_Term  600 non-null    float64
10     Credit_History    564 non-null    float64
11     Property_Area     614 non-null    object
12     Loan_Status       614 non-null    object
dtypes: float64(4), int64(1), object(8)
memory usage: 62.5+ KB

```

Fig. 2. Data information

### B. Data Transformation

When an artificial neural network is used to handle category data, the data will likely be converted to a numerical variable or a vector whose members are of numerical data type. Based on Figure 2, it appears that the first six characteristics are categorical. There are three basic ways for transforming the datatypes of the properties in question. In this study, the term "encoding" is used to describe how information is stored. When dealing with categorical data, the Label encoding (LE) method allocates each label to the integer number that is most suited for that label. Even though this strategy is basic, it may be difficult to identify the most appropriate assignment for a given problem. The Python Scikit-learn library [11] contains a Category Encoders module, which includes an Ordinal Encoder (OE) as well as other encoding algorithms. Ordinal Encoding is covered in detail in the OE documentation provided by Scikit-learn. Either the LE or the OE assigns a single integer value to each of a categorical variable's different values, regardless of which encoder is used. The data before and after LE are depicted in figure 3.

	Loan_ID	Gender	Married	Dependents	Education	Self_Employed	ApplicantIncome	CoapplicantIncome	LoanAmount	Loan_Amount_Term	Credit_History	Property_Area	Loan_Status
0	LP001002	Male	No	0	Graduate	No	5849	0.0	NaN	360.0	1.0	Urban	Y
1	LP001003	Male	Yes	1	Graduate	No	4583	1508.0	128.0	360.0	1.0	Rural	N
2	LP001005	Male	Yes	0	Graduate	Yes	3000	0.0	66.0	360.0	1.0	Urban	Y
3	LP001006	Male	Yes	0	Not Graduate	No	2583	2358.0	120.0	360.0	1.0	Urban	Y
4	LP001008	Male	No	0	Graduate	No	6000	0.0	141.0	360.0	1.0	Urban	Y

↓

	Gender	Married	Dependents	Education	Self_Employed	ApplicantIncome	CoapplicantIncome	LoanAmount	Loan_Amount_Term	Credit_History	Property_Area	Loan_Status
1	1	1	1	1	0	4583	1508.0	128.0	360.0	1.0	0	0
2	1	1	0	1	1	3000	0.0	66.0	360.0	1.0	2	1
3	1	1	0	0	0	2583	2358.0	120.0	360.0	1.0	2	1
4	1	0	0	1	0	6000	0.0	141.0	360.0	1.0	2	1
5	1	1	2	1	1	5417	4196.0	267.0	360.0	1.0	2	1

Fig. 3. Data before and after encoding

C. Identifying important attributes

Feature selection is the technique used for reducing training and testing time, simplifying models, and enhancing the likelihood of simplification to avoid overfitting issues [12].

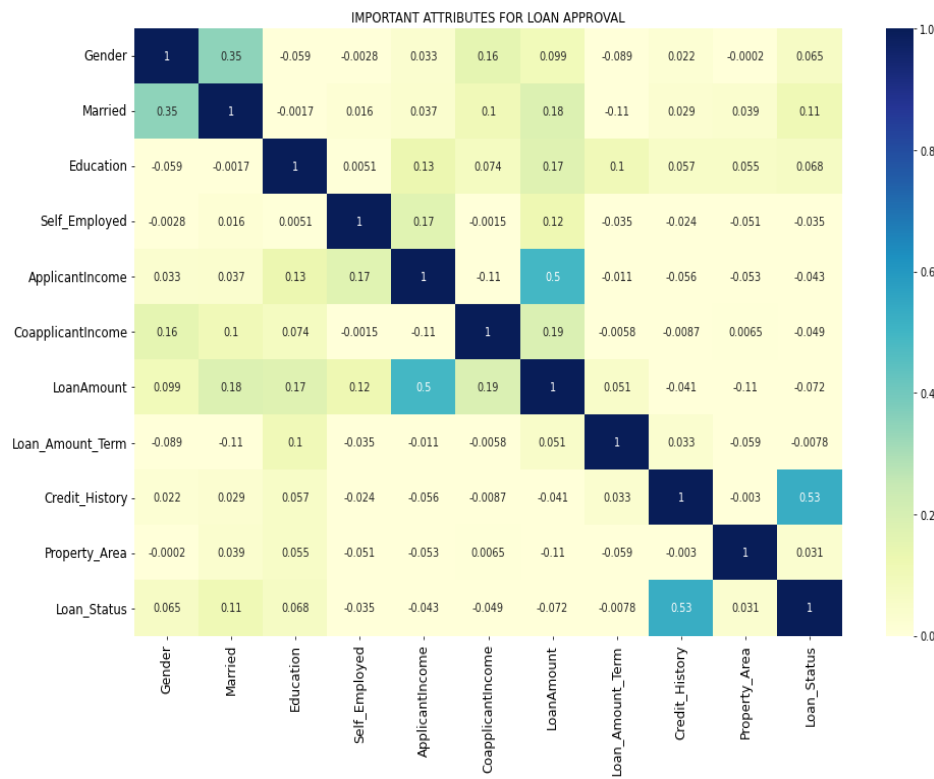


Fig. 4. Bank loan approval data correlation plot

A correlation plot is used in this study to assist narrow down the list of key features. Correlation analysis is a helpful statistical method that may be applied to a wide range of data in various fields of study. A single correlation matrix can represent many variables, making it simple to see the relationships between them. The bank loan data approval correlation plot is depicted in Figure 4.

Table 1 shows the data count in each category. The raw data downloaded from Kaggle contains 981 samples, after pre-processing the data is reduced to 926 samples. The pre-processed data divides into 740 for the train and 186 for the test. Figure 5 represents the number of data used in each stage by bar graph.

TABLE I. DATA COUNT IN EACH CATEGORY

Data	Raw data	Pre-processed data	Train	Test
Bank Loan Approval	981	926	740	186



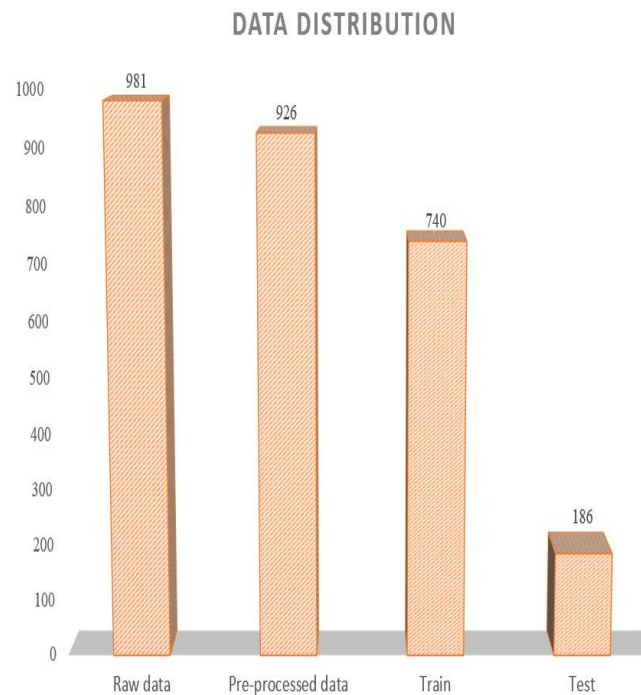


Fig. 5. Data distribution

## V. CLASSIFICATION METHODS

Classification algorithms make educated guesses about which groups a particular data item based on the information from previously categorized data. It makes use of a range of input features to aid in the learning process. For example, when it comes to ML, classification algorithms are extensively used to determine whether or not someone is eligible for a bank loan, among other things. The algorithms that were used in this investigation are summarised in the following section:

### A. LR

LR has been added to "regular" linear regression. This sort of analysis is applicable when the dependent variable, Y, is categorical. The author [13] introduces a "yes/no" type of variable in binary LR. The Y variable is generally referred to as "1," and the two Y categories are numerically represented. Binary LR can investigate how well a group of variables anticipates a categorical dependent variable in addition to determining the model's "goodness-of-fit." Binary LR was initially used in epidemiological research, but it has subsequently been embraced by a variety of industries, including marketing and general business. Its popularity has skyrocketed in the previous two decades. If the LR is applied to the bank's approval prediction, "1" represents "loan acceptance," while "0" represents "loan rejection." LR, one of the most often used classification algorithms, employs sigmoid functions to divide its output into two discrete categories, 0 and 1. As a result, an optimal classification is achievable. Categorizing data points with estimated values of more than one or fewer than 0 might be problematic if linear regression is used. Thus, LR can be considered an enhancement over the linear regression model when it comes to classifying data. More information on the logistic function can be found in the sections that follow.

$$\text{logistic}(y) = \frac{1}{1 + \exp(-(y))} [1]$$

As move from linear regression to logistic regression, the  $y$  can be thought of as the equation from the linear regression model. Afterward, the logistical role is transferred as

$$P(y(i) = 1) = \frac{1}{1 + \exp\left(-(\beta_0 + \beta_1 x_1^{(i)} + \dots + \beta_p x_p^{(i)})\right)} [2]$$

### B. RF

The term "RF" refers to an ensemble ML approach that uses categorization and decision regression trees to learn from data. It is an attempt to construct a statistical model to forecast the values of response variables based on prior knowledge of the projected values of predictor variables. To build a DT, the space of predictor variables is divided recursively and the values of the response variables are assigned to each division. In paper [14], Breiman suggested an RF learning approach that was based on the well-known classification tree ensemble learning methods of boosting and bagging. The element of chance was then included in the game of bagging. In this sense, the two strategies differ from one another: if a previous forecast was incorrect, subsequent trees will weight it more highly in the boosting process, but if an earlier prediction was correct, the following trees would weight it less heavily. At the end of the day, a weighted vote is utilized to determine whether or not a loan is accepted. Bagging instead uses a bootstrap sample of the dataset to build each successive tree sequentially. When it comes down to it, the approval or denial of a loan is determined by a simple majority vote.

### C. C4.5

Data mining algorithms, such as the C4.5 algorithm, can be used as part of the categorization groups. Ross Quinlan first introduced the C4.5 Algorithm as an improvement on the ID3 Algorithm in 1993 as a version of the ID3 Algorithm [15]. To utilize the C4.5 technique to design a DT, the entropy of attribute values, as well as the information gain and gain ratio, must be calculated. C4.5 algorithms are used to construct a DT. The approach C4.5 generates a DT that may represent and model the results of critical data explorations, making it easier to identify the knowledge or information gained from these results. In approach C4.5, Entropy ( $S$ ) and Gain ( $S, A$ ) are employed to locate early roots. An attribute is obtained from an old source, and the most essential one is the value of Gain. If a case has multiple values, establish a new branch for each one. Repeat the process for each branch until all of the branches have the same class C4.5 algorithm formula in the form of a DT.

$$\text{Gain}(S, A) = \text{Entropy}(S) - \sum_{i=1}^n \frac{|S_i|}{|S|} * \text{Entropy}(S_i) [3]$$

$$\text{Entropy}(S) = \sum_{i=1}^n (-p_i * \log_2 p_i) [4]$$

Here,

$S \rightarrow$  Set

$N \rightarrow$  number of features

$|S_i| \rightarrow$  Set case in partition  $i$

$A \rightarrow$  Features

$p_i \rightarrow$  proportion of  $S_i$  to  $S$



## VI. MOBILE APPLICATION

It is the process of developing software for mobile devices such as cell phones that is primarily intended for this purpose. As a result, it is now an essential component of any business that can think of. Mobile penetration has reached a significant level in today's banking sector and has risen dramatically over the last decade. Because of the mobile app, customer service has taken on a whole new meaning [16]. The increased use of these applications has piqued the curiosity of academics, who have discovered a variety of development-related issues. This study's purpose is to develop a client mobile application for virtual bank loan consulting. The practice of developing an app exclusively for an operating system, such as Android or iOS, is referred to as "native app development." It has all of the functionality of the operating system as well as the ability to fully utilize the capabilities of a mobile device. It's far more efficient than any other method of app development I've encountered. The official IDE is Android Studio, and the procedural programming used to develop apps for Android is Java. The home page of the developed mobile app is depicted in figure 6.

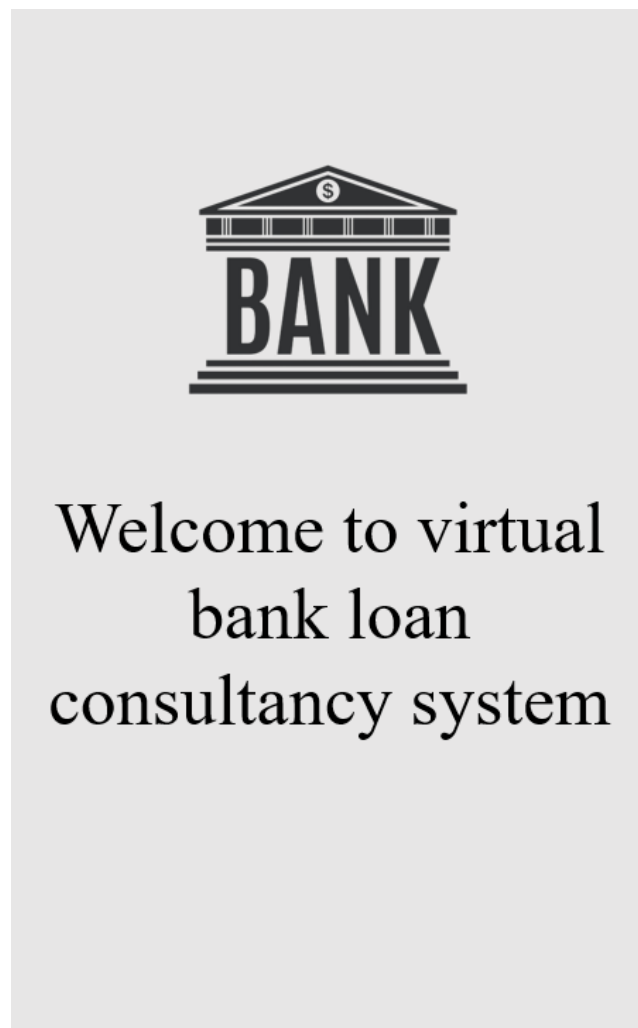


Fig. 6. Mobile app:Bank loan consultant

## VII. RESULT AND DISCUSSION

After pre-processing and training, the ML models are put through their paces and then evaluated. The experimental results of the categorization model are documented in this section. A model evaluation approach is a technique that is used to evaluate the quality of the model based on predetermined criteria. When evaluating the model, it is important to remember that the model cannot be underperformed or overfit. The performance of the model can be evaluated in a variety of ways, including accuracy, precision, recall, and F1 score, among others. The metrics of all 3 ML model is shown in figure 7.

- Accuracy tells how correct the model prediction is. This is the percentage of total correctly predicted loan approval status (containing both yes and no) from the total test sample. The percentage of accuracy obtained by LR, RF, and C4.5 are 87.09, 90.86, and 83.87. The RF gives a higher accuracy level for the prediction of loan approval.
- Precision (Pr) describes the perfectness of the model. This is the percentage of total correctly predicted loan approval (only yes) from total positive loan approval prediction. The method LR gives 91.3% precision, whereas the RF, and C4.5 attains 95.65% and 87.71%.
- The model's positive perfectness is described by the term recall (Re). This is the percentage of total accurately anticipated loan approvals (only yes) divided by total actual positive loan approvals, expressed as a percentage. The LR approach has an 88.23 % recall, however, the RF and C4.5 methods have 90.16 % and 86.20 % recall rates, respectively.
- The F1 score gives the harmonic mean of Pr and Re. The numerator of F1 is the twice product of Pr and Re, the denominator is the sum of Pr and Re. The F1 of the three ML such as LR, RF, C4.5 are 89.74%, 92.82%, and 86.95%.

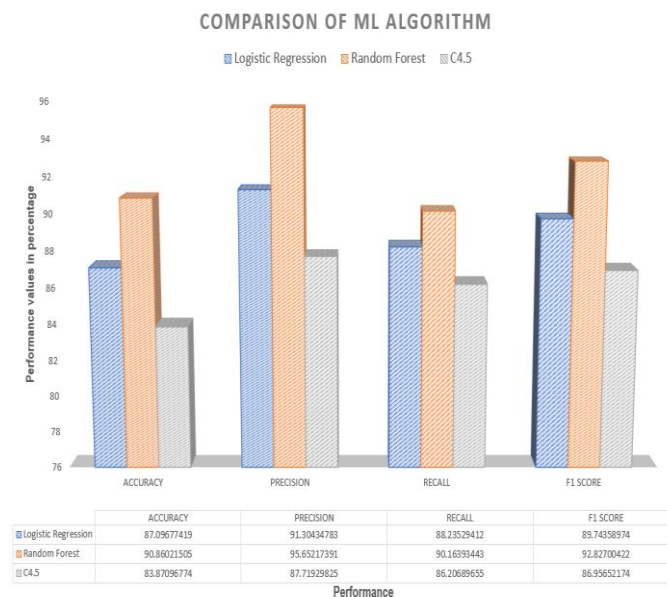


Fig. 7. ML model comparison

From the above figure 7, found that RF gives better results than the other 2 ML methods. The RF model is deployed on the mobile app. After developing a mobile app, testing is done and depicted in figure 8. After getting into the mobile app, the customer should enter the details like gender, marital status, employment type, education, income, loan,



past credit, and location. Next, the submit is used to know whether the candidate is eligible for a loan or not.

**BANK**

**Virtual Bank Loan Consultancy System**

Gender :

Married :

Education :

Self Employed :

Applicant Income:

Co-applicant Income:

Loan amount:

Loan amount term:

Credit History:

Property Area:

**SUBMIT**

**CONGRATS!!! You are eligible for loan**

Fig. 8. Mobile app testing

## VIII. CONCLUSION

Processing loan applications is one of a bank's most significant jobs. This study seeks to assess how likely it is that a client will be able to repay a loan using bank credit information. On the bank credit dataset, ML approaches such as LR and C4.5 were tried to determine which algorithms were most suited for the task. Based on a range of data and targets, RF is a viable technique for predicting if a loan will be authorized. There are 12 features and 926 samples in the loan approval dataset. To begin pre-processing, first, comprehend the data, clean the data, and then encode the data. Furthermore, the most important factors influencing a customer's creditworthiness were discovered. They trained and evaluated three distinct ML algorithms. The RF had a 90.86% accuracy rate, LR had an 87.09% accuracy rate, and C4.5 had an 83.87% accuracy rate. The RF model has been tested

and is now suitable for inclusion in a mobile app. Customers can use this app to check the status of the loan application without visiting the bank. Establish an automated loan acceptance system in the banking sector by evaluating consumer creditworthiness and this will reduce the work pressure of the bank employees. To summarise, the research efforts are aimed at the mutual benefit of the bank and its customers.

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Review

# Recent Advances in the Electro-Active Therapeutic Phytochemical-Based Sensors

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**Abstract:** Naturally occurring phytonutrients/phyto-components are likely to have therapeutic values. These phyto-derived naturally occurring components, such as polyphenols, phenolics, flavonoids and phenolic acids have a hydrocarbon background with a polyphenolic ring, an ester bond with a polyphenolic ring, etc. Their structures play a critical role in determining the chemical and physical attributes that define their activity/functions and roles. Owing to their chemical structure, most of them are electroactive. Thus, these phytochemicals can be used in the preparation of electrochemical sensors. Gaining an understanding of functional genotypical units using electrochemistry is a unique study. The feasibility of incorporating an array of biosensors into a fully-automated micro-electrochemical system is further explored. This review is intended to provide in-depth knowledge of biosensors' applications based on/for Plantae kingdom and varieties. The discussion focuses primarily on the fields associated with the fully-automated micro-electrochemical system and appropriate methods for its advancement. The intended approach is to provide a selective outlook including the setbacks/shortcomings and usefulness of opting for the concerned technique.

**Keywords:** phytochemical; therapeutic; electroactivity; biosensor



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## 1. Introduction

Herbal formulations made from phytochemicals with therapeutic values are currently an extensive topic of research. They represent quite a vast pool of biologically active, extensive sets of macro- and micro-elements. Surveys executed on the subject of the usage of medicinal plants have shown a rise of approximately 10% over the last 2 years. The appropriate mix of health and food has come into the mainstream due to the health emergency created by the emergence of novel/new viruses in our lives. The phytomedicines have re-emerged in advertisements, social media and retail outlets with the arrival of multinational pharmaceuticals attracted to these domains [1]. The past decade has witnessed the succession of nutraceuticals wherein phyto-constituents ensure additional health benefits for the long term [2]. The major distinguishing factor between nutraceuticals and phytomedicine is that, nutraceuticals have a nutritional role that arises from using them for a longer time frame (e.g., chemoprevention) [1]. Thus, this field has now encompassed pharmacognosy, phytochemistry and nutraceuticals. Plant cytochrome P450s are an essential part of the plant with the ability to catalyze many unfavorable chemical reactions and hence called “nature's blow torches”, ensuring high specificity and stereochemistry. Thus, the cytochromes have a pivotal role in the synthesis of a plethora of metabolic compounds that are involved in the functioning and survival of sessile organisms under stressful conditions. Bavishi et al. reported the mediator-free electrochemical investigation of plant cytochrome P450s and their NADPH P450 oxidoreductase [3]. Later, Udit et al. studied the direct electrochemistry



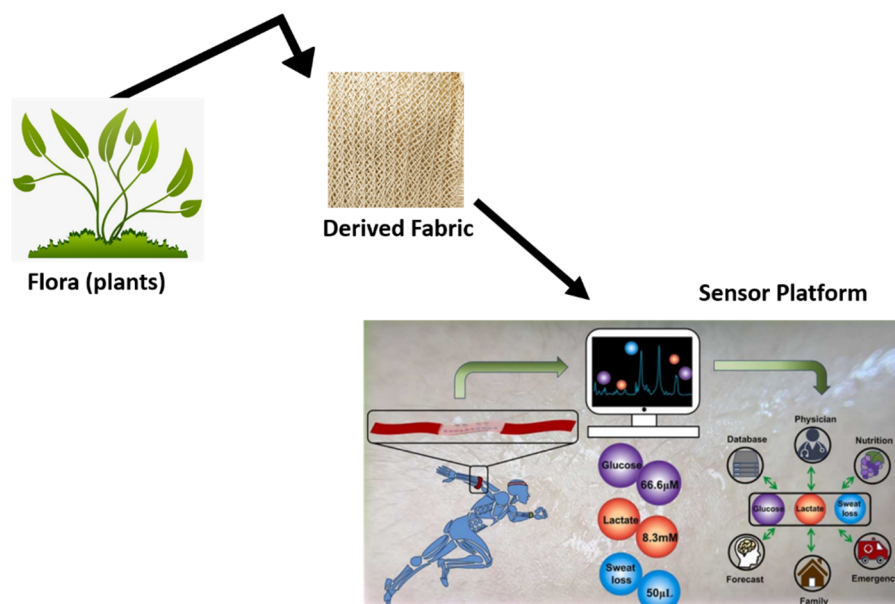
for cytochrome P450 BM3 heme protein using graphite electrodes [4]. The free radicals are an essential part of both phytomedicine and nutraceuticals.

Reactive oxygen intermediates, superoxide anions, alkoxy radicals, etc., are all examples of free radicals. They are special species containing more than one unpaired electron, and thus are chemically very reactive or unstable. Their high reactivity is termed “ ‘stress’ or the ‘unbalance between the oxidants and antioxidants causing damage to the individual’ ” [5,6]. Free radicals are essential for oxidizing nucleic acids and lipids, initiating degenerative disease generation, etc. Epidemiological evidence indicates the necessary association of a cardiovascular diet with fruits and vegetables [7]. Vitamin E and Vitamin C have the intrinsic ability to scavenge free radicals, thus protecting the body from being damaged. These antioxidants are preventive agents in relation to various diseases, such as cancer, mutagens, risk of cardiovascular diseases, etc., as they modulate the enzyme activities thereby reducing chances of carcinogenicity or oxidative stress-inducing agents [8]. Apart from their constructive nature, they can also be highly destructive with the presence of metal ions leading to cell disorders [9,10]. The reactive oxygen species (ROS) lead to cell injuries by disrupting the oxidative balance and contribute to the development of many diseases over time such as cataractogenesis, cancer, rheumatoid arthritis and ageing [11]. Hence, the widespread empirical demand for wild plant groups needs proper information based on their phytochemical and antioxidant abilities. Thus, many techniques are working together to complete their analysis. Many plant-based systems have to be engineered into various sensing platforms, after the incorporation of micro-reservoirs in the thread along with detection dots, to become a wearable sensor.

Various sensing platforms have been introduced for the detection of potential chemicals, moieties (proteins, molecules) and byproducts of any chemical reaction. The principle of detection is based on physical, chemical or biological changes. However, the major drawbacks observed in these sensors are the pre-sampling, the requirement for a skilled technician and the large amount of sample required with sophisticated instrumental set-ups, hence uneconomical. This has paved the way for electrochemical sensing which involves nano-quantities of analytes, without any pre-sampling and sensitive data output. Surachet et al. reported the detection of reactive oxygen species using fluorescence spectroscopy. He quoted that the “sensitivity and selectivity of both fluorescence and electrochemical techniques are similar. The simplicity of electrochemical methods has gained the attention to many researchers.” He also added that, “the electrochemical techniques have potential for real time detection using micro or nano-scale electrodes, readily available for real time sensing irrespective of the type or location” [12].

The electroanalysis field has brought in a new revolution to combat the activities of free radicals via the identification of the molecules responsible. An ideal electrochemical sensor should have key attributes such as accuracy, repeatability, sensitivity, least toxicity, biostatistical support, reliability, and easy sample preparation protocols, showing high specificity [13]. Figure 1 accounts an illustrative image portraying the various scopes that can be explored regarding the phyto based electrochemical sensor platforms. The antioxidants help in counteracting the free radicals by preventive chain reactions and disrupting the oxygen activation thereby preventing the damage caused by free radicals [11]. Antioxidants are present in fresh fruits and green leafy vegetables [14]. The reason behind all the parents and doctors asking for a higher intake of green vegetables and fruits may be to diminish/stop the incidence of chronic and degenerative diseases. The consumption of carrots, sweet potatoes and oranges are protective against cancer [15]. In addition, including onions, honey and grapes, i.e., sources of polyphenols, into the diet can help in preventing cardio-vascular and anti-viral activity in the body [16,17]. Many classical methods such as high-pressure liquid chromatography (HPLC), spectrophotometry, gas chromatography (GC), potentiometry, micellar capillary electrophoresis, etc., have been reported for the qualitative estimation of antioxidants [2,14,15]. The protocols are ORAC (Folin Ciocalteu assays, oxygen radical absorbance capacity), FRAP (the ferric reducing ability of plasma), DPPH (2,2-diphenyl 1-picrylhydrazyl) assays, lipid peroxide inhibi-

tion, DCHF-DA (dichlorofluorescein-diacetate), etc., for assessing their total antioxidant activity [18].



**Figure 1.** Illustrative image portraying the various scopes that can be explored regarding phytochemical and/or sensor platforms. Rights and permission, copyright [13].

The detection of disease biomarkers, sequencing of the genome, and quantitative recognition of specific metabolites are quite intellectually challenging parameters to be studied and equally relevant in the medical and healthcare sector. Due to the evolution of all organisms with time, micro-organisms have mutated as a “survival of fittest” concept to sustain in varied environmental conditions via a broad distribution of protein stabilities, variable denaturation temperature and subsequent growth temperatures, lack of evolutionary trade-off, obtaining a protective coat, etc. [19,20]. Balol et al. have studied in depth the various unusual sources of genetic variation, and provided tabulated data for the diversity value of various distinct plant viruses [20]. Due to these variations, genetic alterations have come into existence which have led to a huge loss in economic terms basically due to the acceleration in development of pests and pathogens witnessed by the agricultural sector. As of now, not much research has been processed to detect the existence of plant diseases using in-field instrumentation. Meanwhile, the off-field pathogens, volatile organic compounds (VOCs), etc., released by plants during unfavorable conditions (biotic stress) have been assessed.

Synergistic interactions among different antioxidant species present in plants make it a tough task for accurate antioxidant assays. The goal of this paper is to understand the correlations between electrochemical profiles that can reveal possible interactions based on the biological activity of plants [21]. This is highlighted via the presence of various characteristics (phytochemicals) of herbs and spices available in nature (different parts of plants are used to treat different diseases and conditions) as explained in Section 2. Following this, the understanding of phytochemicals (chemical class and its compounds) with their mechanism of action is highlighted. Furthermore, the role of these plant-based system have been amalgamated with electrochemical approaches viz-a-viz their electrochemical quantitative and qualitative estimation of compounds; phenotyping; establishment of an electrochemical platform having phytochemical properties for sensor development, and many more possible opportunities explained in Section 3.



## 2. Therapeutic Value

Herbs and spices have an old-fashioned reputation for their usage with sturdy roots within cultural territory and heritage. Moreover, they have an incomparable association with nourishment and its links. Validating their benefits by scientific means has always remained a challenging task, particularly when compared to the standards applied for gauging pharmaceutical agents. Food can be eaten in various combinations, and in relatively large and unmeasured quantities. Herein, the real challenge is not proving whether foods have health benefits but rather developing scientific methods to expose them qualitatively and quantitatively. Table 1 provides a listing of such phytochemical defense foods with the part of the plant consumed, advantages, and disadvantages.

**Table 1.** Accounts the name of various phytochemical defense compounds/foods used in our daily routines with tremendous benefits and few side effects.

S. No.	Name of Herbs	Part of the Plant	Treatment of Diseases	Disadvantages/Side Effects	Ref
1.	Chamomile (used as a tea)	Flower	Skin irritation due to radiation, anxiety and relaxation, wound healing	May interfere with some medicines	[22]
2.	Cinnamon	Bark	Diabetic treatment (insulin-enhancing activity)	Not to be used with medicines	[23]
3.	Crocus sativus	Petals	Antitumor, Anti-inflammatory and anti-oxidant	Can cause allergic reactions	[24]
4.	Echinacea	Leaf, Stalk, Root	Prevents infections, and promotes wound healing	Can be an allergen	[25]
5.	Feverfew	Leaf	Treatment of fever, migraines and arthritis	Digestive irritation	[26]
6.	Garlic	Clove, Root	Antimicrobial, cardioprotective, anticancer, anti-inflammatory, lowers blood pressure	Not to be used with warfarin	[27]
7.	Ginger	Root	Easing nausea and motion sickness. Anticancer agent	Bloating, gas, heartburn	[28]
8.	Gingko	Leaf	Treatment of asthma, bronchitis, fatigue	Can cause seizures, has ginkgo toxin	[29]
9.	Ginseng	Root	Tonic and aphrodisiac	High bp and tachycardia	[30]
10.	Goldenseal	Root, Rhizome	Treat diarrhea and eye skin irritation, antiseptic, diarrhea	Can be poisonous in high doses and can cause gastric irritation	[31]
11.	Kava	Rhizome, roots	Treat anxiety, nervous tension, and agitation and act as a sedative	Digestive issues	[32]
12.	Milk Thistle	Fruit	Liver conditions and high cholesterol reduces the growth of cancer cells	Uncertain about side-effects	[33]
13.	Saint John's Wort	Flower, Leaf	Antidepressant	Sensitivity to light	[34]
14.	Saw Palmetto	Fruit	Treat urine symptoms from benign prostatic hypertrophy	Digestive upset, headache	[35]
15.	Valerian	Root	Treatment of sleeplessness and reduce anxiety	Consultation with a doctor is necessary	[36]

Seasonings can be any part of the plant vis à vis the stigma of flowers (saffron), bark (cinnamon), roots (ginger), aromatic seeds (cumin), berries (peppercorns) and buds (cloves). Another interesting example is coriander which can be employed as an herb using its leaves

while its dried seeds are used as a spice. Plant-based systems (herbs and spices) have an extended past of both culinary use and of providing health benefits, as well as acting as preservatives [37]. In 1555 BCE, ancient Egyptian papyri recorded the use of coriander, fennel, juniper, cumin, garlic, and thyme [38]. In 5000 BCE, accounts were found of the Sumerians using thyme for its health attributes, followed by the farmers of Mesopotamia in 3000 BCE growing garlic [39]. The global trade in spices goes back to 4500–1900 BCE, chiefly in Ethiopia [40]. The early Egyptians worshipped garlic and its cloves, which were found in the tomb of King Tutankhamen. The records of Egyptians having wooden cloves of garlic in their tombs to preserve the future meals of the afterlife is an astonishing detail. In addition, Hippocrates (460–377 BCE) had a catalogue consisting of 300 medications that included garlic, cinnamon, rosemary, etc. They seemingly included garlic to cure uterine cancer, whilst mint was treasured for its positive effects on the intestinal system, and licorice was initially used as a sweet but can be utilized as an herb too, based on its anti-inflammatory actions for asthma, chest problems, and mouth ulcers. The herb, rosemary, was used to improve and strengthen memory. The Greeks published a book that encompassed more than 500 herb varieties unfolding the variability of choice while a store in Rome has a strong inspiration for herbal remedies that consists of complicated mixtures, consisting of more than a hundred ingredients from 162 CE. Another significant example of such herbs is cinnamon for treating colds and flu, galangal used to treat abdominal pain, and nutmeg for a cure for diarrhea.

Moreover, traditional Ayurveda evolved more than 5000 years ago in the Himalayas, with knowledge being transmitted orally until it was first jotted down in Sanskrit poetry in 1500 BCE as “The Vedas”. Ayurveda prospered in the 7th century and focused on disease demotion and health promotion, highlighting dietary habits. Ayurveda includes the protection of various organs and functions: basil → heart, cinnamon → stimulate circulation, turmeric → jaundice, mace → stomach inflection, and ginger → universal medicine, essentially to cure indigestion and relieve nausea. With the decline of the Roman Empire and built upon the knowledge of Galen (476 CE), the development of Arabic medicine (500–1300 CE) preserved some of the awareness of the surrounding health benefits [41].

The topic of functional foods includes scientific endeavors, technological advancements, advertising, and standard guidelines. Based on a scientific standpoint, functional foods have been defined as “foods that provide advantages beyond the basic nutritional needs” [42]. However, the food modules of today are not just limited to concepts of preventing clinical deficits and sustaining homeostasis but account for a budding recognition of how food constituents actively interact within the body to support health and check abnormalities and overt infections. Herbs and spices fit into this picture in numerous ways. In particular, with supplements, the emphasis may be on their role in a dietary regime rather than their usage as medicines. They provide a path for the future investigation and development of an appreciation of the potential assistance of herbs and spices to health and well-being. They incorporate a significant character in dietary flavonoid intake. Chamomile, rosemary, licorice, onions, thyme, rosemary, and sage have enriched flavonoid content [43]. Herbs have anti-carcinogenic effects from different stages. An example is diallyl sulfide (found in garlic) which is an effective inhibitor of cytochrome P450 (CYP)3 IIE1, i.e., a Phase I enzyme and providing considerable surges of a variety of Phase II enzymes, such as glutathione S-transferase, quinone reductase, and uridine diphosphate-glucuronosyltransferase, which are implicated in the detoxification of carcinogens [44,45]. Even animal models of cancers have achieved some success with basil, mint, lemongrass, rosemary, and turmeric. Herbs involve protection against oxidative stress and inflammation, both of which can lead to the risk of commencement and advancement of cancer. Parsley, a culinary herb, consists of myristicin, an inducer of phase II enzyme glutathione S-transferase as tested on albino mice [46]. Even sedatives may be replaced by *Passiflora incarnate*, as suggested by the German Commission E in 1987. Another sedative



is *Valeriana officinalis* which interacts with GABA systems (systems affected by amino butyric acid) in the brain [47].

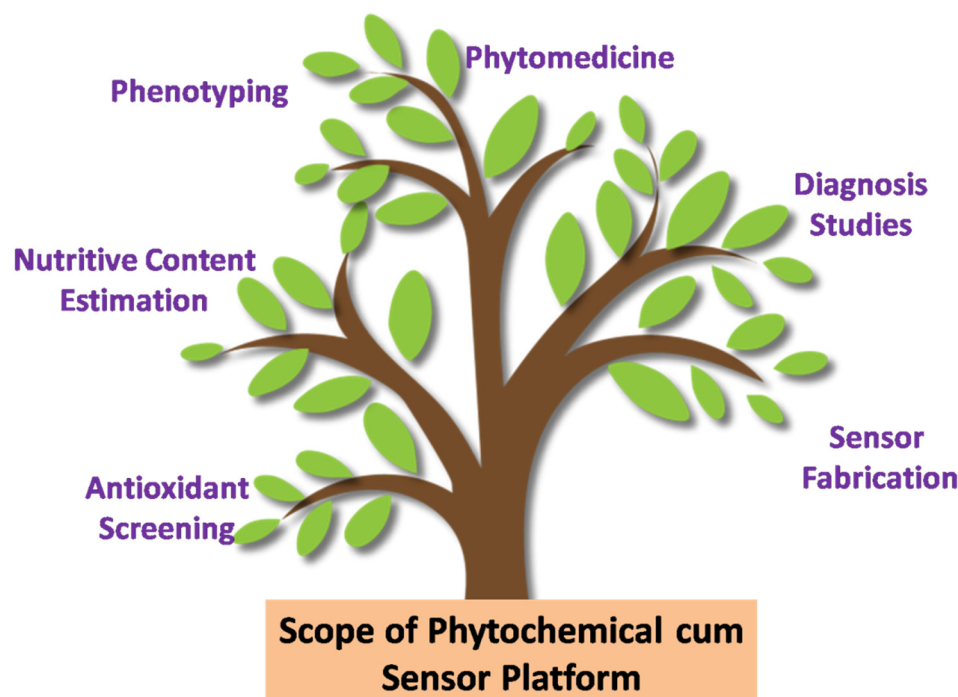
The major question that arises is why are these plant-based systems (examples as listed in Table 1) so advantageous. The plant-derived super foods consist of tannins, catechins, alkaloids, steroids, and polyphenolic acids. These play a crucial role in plant defense mechanisms against invasion by foreign agents [15,39,42]. Substances such as terpenoids are responsible for odour (quinones and tannins) plus the pigment of the plant. Many of these substances account for the plants' flavor (e.g., terpenoid capsaicin from chili peppers). Alkaloids include heterocyclic nitrogen compounds, an example being morphine isolated in 1805 from *Papaver somniferum* (opium poppy) [48]. The name morphine comes from the Greek word Morpheus, which means "God of dreams". Codeine and heroin are both derivatives of morphine. Flavones are phenolic structures containing one carbonyl group. They are hydroxylated phenolic substances that are linked to an aromatic ring. Dimethoxyflavone and bonducellin were isolated. Essential oils and terpenoids are the anti-microbial properties of aromatic volatility oils derived from medicinal, or other edible, plants that have a high content of phenolic derivatives such as carvacrol and thymol. Some traditional medicinal plants, their parts, and the name of compounds are enlisted in Table 2.

**Table 2.** Accounts of various traditional medicinal plants and their inhibitory activities.

S. No.	Scientific Names	Parts	Constituent Class	Compounds	Mechanism of Medicine	Ref
1.	<i>Baccharis grisebachii</i> Hieron	Resinous exudate	Diterpenes, p-coumaric acid, derivatives, flavones	3 and 3,5-Prenyl-p-coumaric acid	Argentinian traditional medicine showed activity toward dermatophytes and bacteria	[49]
2.	<i>Cassia podocarpa</i> Guill et Perr.	Leaf and Flower	Glycosides	Anthraquinone glycosides, free aglycone	Optimum laxative activity and reduced toxicity	[50]
3.	<i>Curcuma longa</i> L.	Rhizome	Flavonoids	Curcumin and curcuminoids	Several different molecules are involved in inflammation that is inhibited by curcumin including lipo-oxygenase, phospholipase and elastase.	[51]
4.	<i>Hydrastis Canadensis</i> L.	Whole plant	Alkaloid	Berberine-entrahydroberberine and 8-oxoberberine	Chinese herb, exhibited vasodilator activity, has been attributed to multiple cellular mechanisms. Its derivatives are attributed to the blockade of K <sup>+</sup> channels and exchangers.	[52]
5.	<i>Bougainvillea xbuttiana</i>	Leaf	Protein	Lysine	The inhibitor showed N-glycosidase activity on 25S rRNA of tobacco ribosomes, which interfere with virus multiplication through ribosome interaction.	[53]

Peptides are inhibitory to micro-organisms as first reported in 1942. Peptides called "cathelicidins" represent an important native component of innate host defense in mice and protect against necrotic skin infections caused by *Streptococcus* [54]. The broad spectrum activity displayed by anti-microbial peptides is considered as a "chemical condom" against

HIV infection and the Herpes simplex virus [55]. Tannins are a group of phenolic substances capable of tanning leather or precipitating gelatin from solutions, by a property known as “astringency”. The growth of fungi, yeast, bacteria, and viruses was inhibited by tannins, whilst the ones with mixtures such as neem (*Azadirachta indica*), verasingam pattai (*Zanthoxylum limonella*), and Indian Babool (*Acacia nilotica*) stick are widely used as toothbrushes [56]. Strawberry extracts are strong inhibitors of Salmonella bacteria [54]. The dried flower-heads of *Chrysanthemum morifolium* are an oriental drug as well as a popular herbal tea in China, and also used for the treatment of eye diseases in Japan [57]. The scopes of/for phytochemical-based sensors are illustrated in Figure 2.



**Figure 2.** Diagrammatic illustration of various scopes that can be explored regarding phytochemical and/or sensor platforms.

### 2.1. Phytomedicine

Due to their in-built abilities, traditional plants are recognized to be powerful ingredients that can substitute for anti-inflammatory, diuretic, and diaphoretic medicines [8]. They are well established with antioxidant and radical scavenging properties with minimal side effects compared to chemical therapeutics [58]. Thus, the use of traditional medicines for primary healthcare has steadily increased worldwide. Nearly 80% of the pharmaceutical-derived plants are used as anti-microbes for treating infectious diseases. These inhibit the germ and exhibit greater selectivity toxicity towards infecting germ and host cells. The beneficiary medical effects of plants are a result of their secondary metabolites [6]. The chemotaxonomic considerations and target-directed screening play a crucial role. These plant-based medicines have come as an end product in the market.

In comparison to synthetic pharmaceuticals based on a single constituent, phytomedicines exert a synergistic action of several chemical compounds acting at a single or multiple target site, thus eliminating the problematic/side effects associated with a physiological process. Williamson and his coworkers, in 1999, extensively documented how synergistic interactions imply underlying effectiveness [59].

### 2.2. Nutritive Content Estimation

A new interface with a class of sensors has been developed, such as a transistor-based sensor for sap fluid nutritive content analysis. However, the drawback observed



was the invasive approach that affects plant activities [60]. Based on differential pulse voltammetry (DPV) data, we can determine the peak height which reflects the concentration of species following the peak potential for identification of species. The shape of the peaks helps in distinguishing molecules having the same potential characteristics. Gandhi et al. involved a GCE/CB electrode for the qualitative and quantitative estimation of nutritive content—sesamol in various natural samples (sesame seeds and oil varieties)—in neutral media without any pre sampling [61]. Another real-time approach accounts for using a pencil graphite electrode for testing various tea assays [62]. Square Wave Voltammetry is one such technique which distinguishes diverse samples based on peak current values. The most important characteristic is the elimination of capacitive current values. A few of their examples are tabulated in Table 3.

**Table 3.** Tabulation of various sensors used for the nutritive estimation by various researchers.

S. No.	Chemically Modified Electrode	pH	Technique	Nutritive Estimation	Comment	Ref No.
1.	GCE/CB	PBS	DPV	Sesamol content	Direct analysis with buffer dilution	[61]
2.	Pencil graphite Electrode	PBS	DPV	1,2-, 1,3-, and 1,4-Di hydroxyl benzoic acid	Tea quality assays were calculated	[62]
3.	Pencil graphite electrode	PBS	DPV	Xanthine, Hypoxanthine and Uric acid	Fish freshness was accounted	[63]
4.	SPCE	HClO <sub>4</sub> <sup>-</sup>	CV	Caffeic acid	Tea samples	[64]
5.	Ion Selective Electrode	-	Based on ion-selective field emission transistors	Soil nutrient sensing	Suffer various interferences	[65]

GCE—glassy carbon electrode; CB—carbon black; SPCE—screen-printed carbon electrode; PBS—phosphate buffer solution; DPV—differential pulse voltammetry.

### 2.3. Therapeutic Protein Expression Using Plants

The beginning of recombinant DNA technology has provided the gears for generating recombinant proteins that could be employed as therapeutic agents [66]. Recently, advances can be developed involving phytosystems as bioreactors to harvest therapeutic proteins directed against infection and diseases. The unceasing hazard of disease-causing microorganisms is a serious apprehension/concern and has evolved a paradigm modification in the pharmaceutical and biotechnological enterprises, encouraging them to exploit the heterologous expression of amalgams in the living individuals. Plant-based biofactories promise rapid developments as biopharmaceutical agents and edible vaccines [67]. Table 4 shows the different recombinant types. These support the production of great yields, lower production cost and storage expenses, exclusion of pathogen contamination, few/limited processing steps, and the safe and secure distribution of oral vaccines that have boosted the practice of such systems in recent years. Nevertheless, bottlenecks lead to reduced expression, encouraging researchers to comprehensively investigate heterologous protein expression in phytosystems for the evolution of innovative approaches with a defined expression of biopharmaceutical peptides that encourage various immune responses. The advent of recombinant DNA manipulation in 1977 initiated the use of *E. Coli*, for therapeutic proteins, ground-breaking research, and manufacturing of recombinant DNA [68]. The US Food and Drug Administration (FDA)-approved Human Insulin was launched in 1982, followed by an *E. coli*-based insulin in 1979 [69]. However, very few plant-based expression systems excel in the manufacturing of vaccines, as suggested by Goeddel and his coworkers in 1979. Furthermore, they have tremendous growth potential as the basis of a modern discipline, providing immediate cures for infectious diseases. The expression of

an alien gene in a host plant cell is dependent on cumulative effects for several essential components required for cell transformation (including the coding sequence, promoter region, transcript termination, etc.). In the plant cell pH, the efficiency and accuracy of the transcriptional and translational machinery, plant cell biochemistry, and the ease of amino acid are required by the plant for recombinant protein, including the interaction between the storage of expressed proteins in the cellular environment. An elevated safety concern due to transgene contamination strongly discourages the commercialization strategy for plant-derived pharmaceutical production.

**Table 4.** Tabulation of distinct types of transformation in various host, their vector and the recombinant protein targeted.

S. No.	Type of Transformation	Host Plant	Vector/Promoter	Recombinant Protein	Ref
1.	Agroinfiltration	Tomato	Pepino Mosaic Virus PepMV	FMDV 2A catalytic peptide	[70]
2.	Chloroplast transformation	Sugar beet	Prn promoter-	GFP	[71]
3.	Nuclear transformation	Carrot	CaMV-35S	Heat labile enterotoxin B	[72]
4.	magniCON	Tobacco	pTBSV	HBc (VLPs), GFP	[73]
5.	Target Specific Expression	Rice endosperm	Tapur promoter	Human lysozyme	[74]
6.	Virus-induced gene silencing (VIGS)	Wheat Spike	Barley stripe mosaic virus	NA	[75]

### 3. Phytochemical Based Analysis and Sensor Systems

#### 3.1. Phenotyping Tool/Phylogenic Classification

The valuation of basic plant traits including architecture, growth, development, defense, physiology, tolerance, resistance, etc., is the primary characteristic of measurement for an individual's quantitative constraints that laid the foundation for complex trait evaluation.

An electrochemical platform can be employed to distinguish phylogenetic data based on the electrochemical approximation that can be used to build relationships in evolutionary patterns. The electrochemical combination is a rapid phenotyping tool accounting for the distinct combinations of mutants in plants, animals, and microbes. Tansil et al. gave a new outlook to DNA detection using a simplified electrocatalytic imidazole-naphthalene diimide-functionalized system as a threading intercalator [76]. The practicability of the proposed electrochemical platform incorporates a non-labelling procedure carried out at a neutral phosphate buffer. Carbo and his coworkers published an electrochemistry-based chemotaxonomy for different varieties of Plantae using microparticle methodology [77]. Even the infragenic identification based on petal tissue fingerprinting can be easily worked out using the electrochemical approach, as reported by Fu et al. [78].

The basic backbone of any nucleotide consists of phosphoric acids and base units that make the molecule heavily charged. This charge corresponds to the potential of the backbone unit which is generally negative.

A new approach related to an integrated chip-on-plant modified sensing-platform for the monitoring of gene expression has been explored by Pandey et al. [79]. Herein, the  $\beta$ -glucuronidase (GUS) expression bio-sensing is validated using chrono-amperometry in tomato and tobacco plants. The electrode microchip is fabricated to transduce the GUS enzyme expression based on genetic modifications.

#### 3.2. Antioxidant Screening

The growing interest of the population in the functional foods and health sector has invoked a wave of antioxidant studies increasing the understanding of the role of free



radicals in disease generation [11]. The vegetal kingdom and its associated products are the targets for the assays using various distinct methodological and protocols developed over time. Traditional approaches account for undefined reaction times with a separate setup required for each constituent with tedious protocols. The output of traditional approaches is quite specific for the concerned systems. Meanwhile, a universal criterion should be maintained for assay as it is not solely dependent on one specific reaction but many different mechanisms exist contributing to antioxidant ability. Thus, an electrochemical platform can be a potential approach to be applied for an overall antioxidant capacity determination as suggested by numerous reports using the solution-phase technique [80–83]. The lower the oxidation potential, the more intense the total electrochemical antioxidant power (TEAP). The non-essential compounds can be masked, reducing the chances of false or wrong information. The minor oxidation potential is recommended to understand the antioxidant concentration but sometimes that can cause trouble with wave broadening and shoulder-type signals. Quercetin is referred to as a standard antioxidant and has a peak potential of 0.16 V (oxidation) [84]. Table 5 is the culmination of such examples. The oxidation potential is a characteristic feature contributed by the free radical scavenging ability of plant extracts. Similarly, other phytochemicals can be accounted adequately based on various potentials with their subsequent current value; the higher the current “I”, the higher the antioxidant concentration. Arbelaez and his research group gave an elaborate review on an antioxidant capacity determination using Cyclic Voltammetry (CV), Chronoamperometry, differential pulse voltammetry (DPV), and square wave voltammetry (SWV) in various vegetable varieties including spinach, chicory, edible oils, cabbage, etc. [85,86]. Based on the CV data, peak separation, i.e.,  $E_p$ , was obtained which helps us to determine the number of electrons transferred ( $n$ ). Following the variation in potential is linear to the logarithmic activity of species. The peak correlates with the type of reductant, and the low oxidant potentials relate to greater strength for the electron-donating characteristics. Many reports also involve the estimation of oxidation peaks with that of ascorbic acid extendable to non-transparent samples [87]. Zegarac et al. have confirmed that the results compared between classical and CV techniques are reliable and comparable. The reason for the similarity is that, the mechanism is same incorporating one electron transfer. The electrochemical method is simple and two times faster than usual. They even stated a good correlation between the methods under the same conditions; however, the spectrophotometric technique was affected by color and turbidity constraints. Meanwhile, the electrochemical set-up was easier with no pre-requisites of color, stability, and pre-sampling steps.

**Table 5.** Tabulation for the determination of Antioxidant Assay by researchers in the last two decades.

S. No.	Chemically Modified Electrode	pH	Phytochemical/ Chemical Moiety	Technique	Comment	Ref No.
1.	GCE	Sodium acetate acid buffer	Labiatae family	CV; Amperometry	The pH of 4 was maintained and oxidation-reduction potential was used for antioxidant activity measurement	[88]
2.	SPCE/MWCNT	0.1 mol L <sup>-1</sup> HClO <sub>4</sub> <sup>-</sup>	Caffeic acid	CV	Tea and other samples were tested for total polyphenolic content.	[64]

Table 5. Cont.

S. No.	Chemically Modified Electrode	pH	Phytochemical/ Chemical Moiety	Technique	Comment	Ref No.
3.	Carbon Paste electrode	PBS	Cornelian cherry was tested against reducing peroxy radical	SWV; CV	Antioxidant potential was tested for Cornus mas extracts	[89]
4.	Carbon Paste Electrode	Acetate buffer	Infusions (green tea), beverages (red wine and coffee)	CV	Antioxidant activity via in situ formation of free superoxide radical was analyzed	[89]
5.	GCE/Ag-NCF	PBS	Glioma cells were tested	Amp i-t	Superoxide ions released from glioma cells were monitored	[90]
6.	GCE	Acetonitrile + Bu <sub>4</sub> NPF <sub>6</sub>	Venezuelan Propolis	CV	Total phenolic content and flavonoid content were determined	[91]
7.	SPCE/SWCNT-COOH	PBS	Chlorogenic acids and coffee extract	CV	Antioxidant activity based on pro-oxidant content (OH <sup>-</sup> ) quantification	[92]
8.	GCE	NaCl + NaOH	Chilean red wine, grape and raspberry	DPV	The pH of 3.6 was maintained (N <sub>2</sub> atm)	[93]
9.	Guanine/GNR/GCE	PBS	Fruit juices	SWV; CV	Antioxidant capacity was tested based on ascorbic acid oxidation of free radicals	[94]
10.	SPE/Tyr/HSA/GA	Acetate buffer	<i>Salvia microphylla</i> ; <i>Lippia dulcis</i> ; <i>Lippia alba</i>	Amp i-t; EIS	Indirect evaluation based on catechol formation using enzyme has been studied at 4.5 pH	[95]

NCF—Nitrogen-doped carbon nanofibers; GNR—Graphene nanoribbon; HSA—Human Serum Albumin; GA—Glutaraldehyde; Tyr—enzyme tyrosinase.

### 3.3. Sensor Platform Fabrication

Plant component- or phyto-nutrient-based sensing platforms can be a green initiative for the development of sustainable and eco-friendly sensors. Moreover, the involvement of a renewable source for the sensing element or the transducer (which converts one form of signal to another) can be an additional step to enhance the technological process which is significantly crucial for added advantages and eliminates the step for synthetic organic components. Table 6 shows various phytochemicals used for sensor development.

Hong et al. initiated qualitative nucleic acid investigation using the amperometric technique [96]. A gold bi-layer electrode was prepared with oligonucleotide and mercaptahexadendric acid on an Au electrode via a self-assembly approach. The fabricated biosensor involved 16S gene detection in a mixture of gene and PCR products at 0.15 V [96]. The presence of ascorbic acid was correlated proportionally to miRNA content [97].



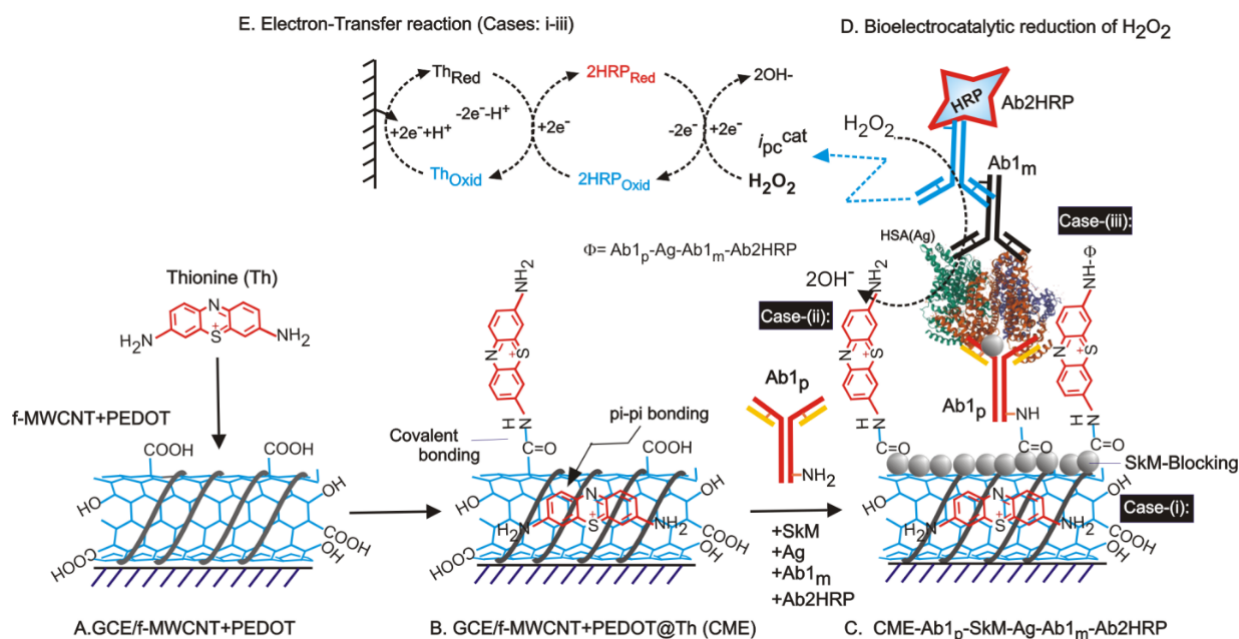
**Table 6.** Account for various sensing platforms used with different phytonutrients.

S. No.	Chemically Modified Electrode	pH	Reactant Moiety	Technique	Sensor Development	Ref
1.	GCE/ CB@Ses-Qn	PBS	Sesamol- phytonutrient- based	CV	White spot syndrome virus detection	[60]
2.	SPCE/HQ	PBS	Mustard protein via sandwich immuno-sensing	Amperometry	Trace mustard protein determination in food samples	[98]
3.	Ni foam/ curcumin-based	PBS	Curcumin-based phytonutrient for Amyloid $\beta$ oligomer detection	EIS	Alzheimer's disease detection	[99]
4.	GCE/GMC @Ginger-Cu <sup>2+</sup>	pH 2 HCl-KCl	Ginger/Gingerol- phyto compound	CV	H <sub>2</sub> O <sub>2</sub> sensing	[100]
5.	CB-SPE	0.1 mol H <sub>2</sub> SO <sub>4</sub>	capsaicin	SWV	Determination of capsaicin in pepper samples	[101]

Ses-Qn—Sesamol Quinone; HQ—Hydroquinone; CB—Carbon black; SPE—Screen-printed electrode.

### 3.4. Disease Diagnostic Functions/Disease Biomarker

The discovery and sequencing of disease via various methodologies involve understanding the valuable insight as to how diseases can be selectively diagnosed at a nano-scale level within a few minutes. It contributes to the early-stage detection and monitoring of the situation in a better way, so that the least destruction in terms of mortality and economic aspects is achieved. Current detection methods are based on meticulous experimental requirements, expensive setups with skilled technicians, and complicated understanding. However, to overcome the issues, electrochemical techniques pave an easy way for early diagnosis and are quite economical. Electrochemistry-based sensors will become more prominent diagnostic tools for various clinical assays, widening the bandwidth for genetic-related disorders and diseases. Gandhi et al. introduced a simple and novel concept of the phytochemical-based sensor for immuno-sensing of White Spot Syndrome Virus [61]. Figure 3 illustrates the preparation of electrochemical biosensor for human serum albumin (HSA) present in urine samples using a sandwich ELISA involving CME-Ab1<sub>p</sub>-SkM-Ag(HSA)-Ab1<sub>m</sub>-Ab2HRP modified electrodes (A–C) and its (D) bio-electrocatalytic reduction of H<sub>2</sub>O<sub>2</sub>. The Ab1<sub>p</sub>-Polyclonal primary antibody, Ab1<sub>m</sub>-Monoclonal primary antibody, SkM-Skim milk powder as a blocking agent, Ag = HSA, Ab2HRP = Horseradish peroxidase enzyme tagged Ab1<sub>p</sub>. Cases-(i–iii) are possible routes for molecular orientations of surface-confined-Th and its interactions with biological systems. The figure shows the mechanistic pathway observed for the development of an electrochemical biosensor for human serum albumin (HSA) in this regard. Another such immunosensor is fabricated for the detection of urinary human serum albumin (HSA) [102]. Table 7 shows the disease detection process using the electrochemical platforms in various conditions. Furthermore, Khatar and his coworkers have reported, using a citrus Tristeza virus, the detection of destructive viruses in plants using screen-printed carbon electrodes modified with gold nanoparticles in 0.01 M Phosphate buffer involving electrochemical impedance spectroscopy [103]. Fang et al. reported a bi-enzyme sensor for the detection of methyl salicylate (allelochemical; a volatile organic compound released by plants during pathogenic infection and infestation) via a molecular tethering technique [104].



**Figure 3.** Diagrammatic illustration for preparation of electrochemical biosensor for human serum albumin (HSA) present in urine samples using a sandwich ELISA involving CME-Ab<sub>1p</sub>-SkM-Ag(HSA)-Ab<sub>1m</sub>-Ab<sub>2</sub>HRP modified electrodes (A–C) and its (D) bio-electrocatalytic reduction of H<sub>2</sub>O<sub>2</sub>. The Ab<sub>1p</sub> = Polyclonal primary antibody, Ab<sub>1m</sub> = Monoclonal primary antibody, SkM = Skim milk power as a blocking agent, Ag = HSA, Ab<sub>2</sub>HRP = Horseradish peroxidase tagged Ab<sub>1p</sub>. Cases-(i-iii) are possible routes for molecular orientations of surface-confined -Th and its interactions with biological systems. Rights and permissions, copyright [102].

**Table 7.** Disease detection using the electrochemical platforms in various conditions.

S. No.	Chemically Modified Electrode	pH	Disease/Virus Targeted	Technique	Comment	Ref
1.	CM-GQD's-ITO-APO-e4-DNA sensor	0.1 mM PBS	Alzheimer's Disease (cardiac Troponin I biomarker)	EIS	No redox peak was associated with the CU modified platform	[105]
2.	Ab <sub>1</sub> /Luminol-Au@MoS <sub>2</sub> /Bi <sub>2</sub> S <sub>3</sub>	PBS	Amyloid-β-protein	EIS	No CV or redox studies	[106]
3.	PEG/TA/pDA	0.2 M PBS	(BRCA1) Mutation of breast cancer	EIS	No CV or redox characteristics	[107]
4.	Cur-oligonucleotide modified/PGE	Tris-EDTA	Interleukine-2	DPV	As the different contact time changes, the current value changes	[108]
5.	PtNP/Gr-IL-Chit/GCE	0.1 M PBS	Human Chronic Gonadotrophin	DPV	Ill-defined peak response	[109]

TA—Tannic Acid; PEG—Polyethylene glycol; DA—Dopamine.

#### 4. Bottlenecks/Drawbacks

The insolubility of essential oils and non-polar extracts makes it very difficult for them to be used in aqueous media [110].

Many VOCs are generated by plants due to unfavorable events; these are termed Green Leaf Volatiles (GLVs); they are non-specific to pathogenic attacks. The release of such GLVs can interfere with VOCs detection.



The electrochemical investigation studies are hampered due to the background noise caused by the fluctuations in the signal. These fluctuations need to be suppressed and a proper determination of changes in electrical potential, impedance values, current output, and kinetics change should be monitored after performing background correction.

The performance of any electrochemistry-based sensing platform is particularly dependent on affinity confined/initiated by the probe with the target molecules which are based on the exploitation of structure, design, amplification modules, technique, and environmental conditions. However, with the proper adjusting of the parameters, we can enhance the response of sensors [111].

The presence of metals with plant extracts can lead to their uptake and the transport mechanism. Their presence can put restrictions on the binding capacity or binding competition and can further deteriorate their transportation activity. Pawel et al. studied the electrochemical fingerprinting of plants rich in flavonoids and even studied the interaction of electro-active metal species, especially of iron (Fe) and copper (Cu). There is an inverse output between the flavonoids' detectability with increasing metal ions [112].

This instability is frequently encountered by a biosensor with miniature oligonucleotide detection probes (8–10 nucleotide long) that binds to the similar template of miRNA [96], thus paving a way toward chemical-electrochemical amplified assays.

The biggest challenge encountered is eliminating the non-specific redox process under a specific potential window, especially when the redox feature of a particular substrate is at the lowest potential which should be quite close to the thermodynamic potential of the substrate concerned [113].

The amperometric technique of electrochemistry is quite accurate, non-invasive, and selective. However, this Amp i-t accounts for the role of faradaic contribution only while the non-faradaic current can lead to a non-steady response of systems. Hence, to ensure a steady current response, constant potential amperometry (a technique called "Chronoamperometry") must be applied [104].

The use of intercalators is a substitute for labelling drawbacks but their usage leads to noise problems as the binding is only established between single strands and not the double-stranded DNA. An advancement in this field has been approached via designing new intercalators that offer better discrimination between DNA strands and segments with achieving a better signal/noise ratio [75].

Frequently, voltammograms show a similar response of several molecules that restricts their usage and even makes it a complication to differentiate between substances [87].

In enzyme-oriented assays for DNA detection, the background current is observed in the range of nanoamperes due to non-specific adsorption and electrostatic abrupt changes. These excess current/background current can be minimized with a bi-layer configured sensor.

## 5. Advantages

The same equipment, i.e., galvanostat/potentiostat and a few add-ons such as an electrode, stirrer, etc., is sufficient to gather diverse information with a choice of techniques inbuilt into the operating system.

Much enhanced sensitivity and accuracy were achieved using a biosensor and reducing the effort of running multiple replicates [114,115].

The electrochemical approach has in-built techniques to gather information based on various spheres as large quantities of data can be obtained within the same set-up (a voltammetric charge, electrode potential, diffusion current, electron transfer characteristics, half wave potential, etc.).

Usually, the DNA-based sensors have high Gibbs free energy (1 to 10 kcal/mol) based on the thermodynamics. Hence, the energy barrier had to be overcome vis à vis high temperature and ion concentration gradient in the bio-systems. The electrochemical sensors make this happen/realistic/easy as the electric field generated (one hundred millivolts) due to potentials, can overcome the energy barrier.

These nano-electrode systems are highly specific and extremely efficient concerning power consumption, recognition interval time, smaller electrical potentials, and have a low throughput process compared to traditional approaches.

This technique avoids the use of labelling, which makes the investigation complex and cumbersome with an alternative to using redox-active intercalators [75].

EC sensors are based on the simple manipulation of the molecules and their properties within the sample due to the electronic charge variation by the potentials and current parameter optimization. This enhances the homogenous mixing of the sample and their ability to achieve a precise output [61].

Low noise potentiostats/bi-potentiostats can monitor microampere currents which are far more economical compared to sensitive non-electrochemical instrumentation [111].

Amperometric detection at lesser negative or positive potentials squeezes the interferences and background variables, yielding a lower signal/noise ratio with an improved detection limit [62].

This provides a simple strategic concept that can enable portable, handheld electrochemical apparatus for in-field testing of samples [102].

## 6. Conclusions and Future Outlook

In this review, after introducing and classifying various phyto-based nutrient systems, we highlighted how they have been employed in subsequent fields, and described their potential appealing values that could be utilized in future. Because of the inherent and unique characteristics of the Plantae kingdom, interesting bioactive or nature-based compounds are used in a myriad of fields. They consist of a plethora of fascinating therapeutic properties which are utilized in the planned system and could be a boon for the living. Thus, a simple, low throughput process providing a novel set-up for phenotyping the plant models is set to become a new avenue for scientists. As explained in the review part of the article, the phytochemical defense compounds within plants can be an avenue to solve major health issues for mankind, as illustrated in Sections 1 and 2. This led us to their role as phytomedicine and their therapeutic usage in day-to-day lives. They can be further extended for therapeutic protein expression using plants. Various phytochemical-based analysis and sensor systems have been developed for phenotyping and classification along with antioxidant screening, which could be a boon to the healthcare sector. Apart from these, various systems have been extended as transducer elements for the development of biosensing systems for point-of-care setups. This plant physiological approach has a vital role to play in the burgeoning field. However, research is ongoing for bottlenecks related to sample handling and fluidic processing with detection on/using a well-defined platform. Thus, further research in this pattern should be allowed on a highly advanced platform for tailoring according to their structural properties and for expanding their potential applications.

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Review

# Electrochemical Profiling of Plants

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**Abstract:** The profiling, or fingerprinting, of distinct varieties of the Plantae kingdom is based on the bioactive ingredients, which are systematically segregated to perform their detailed analysis. The secondary products portray a pivotal role in defining the ecophysiology of distinct plant species. There is a crucial role of the profiling domain in understanding the various features, characteristics, and conditions related to plants. Advancements in variable technologies have contributed to the development of highly specific sensors for the non-invasive detection of molecules. Furthermore, many hyphenated techniques have led to the development of highly specific integrated systems that allow multiplexed detection, such as high-performance liquid chromatography, gas chromatography, etc., which are quite cumbersome and un-economical. In contrast, electrochemical sensors are a promising alternative which are capable of performing the precise recognition of compounds due to efficient signal transduction. However, due to a few bottlenecks in understanding the principles and non-redox features of minimal metabolites, the area has not been explored. This review article provides an insight to the electrochemical basis of plants in comparison with other traditional approaches and with necessary positive and negative outlooks. Studies consisting of the idea of merging the fields are limited; hence, relevant non-phytochemical reports are included for a better comparison of reports to broaden the scope of this work.

**Keywords:** Plantae kingdom; profiling; varieties; bioactive compounds; ecophysiology; electrochemical sensors; non-invasive detection



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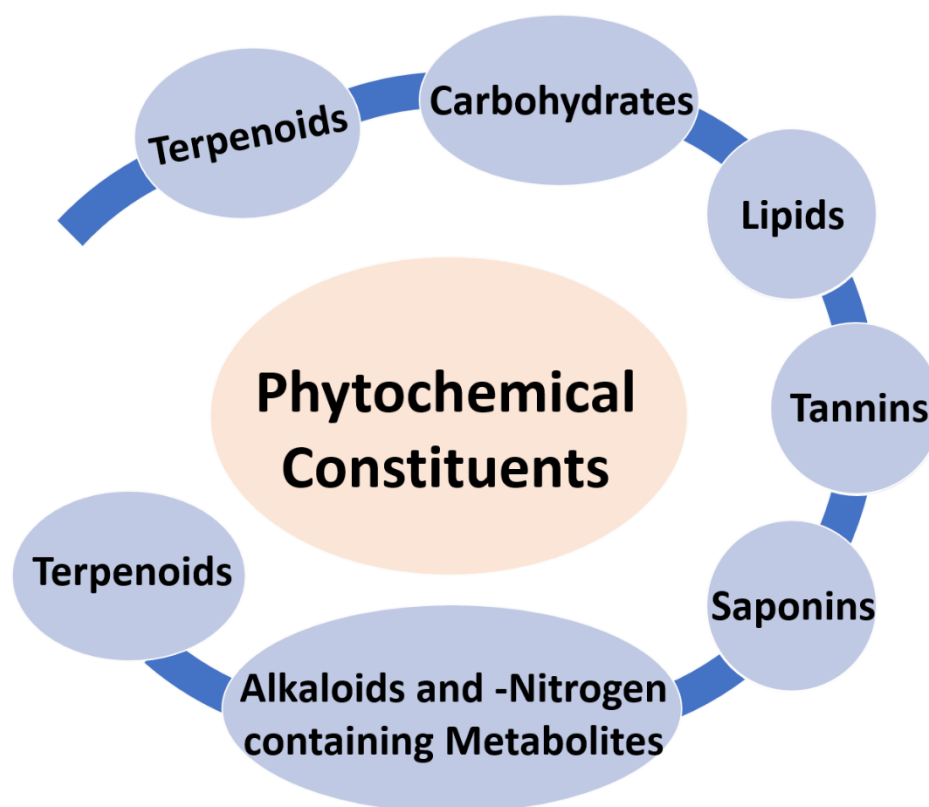
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## 1. Introduction and Background

The traditional approach to classifying living organisms is ‘uni’ or ‘multicellular’, wherein plants are defined to be majorly eukaryotic multicellular species. They are essential for nature and to maintain distinct domains of living species, with the ultimate ability of food and metabolite resource hubs. Along with representing a food supply to different strata of society, their products are equally vital for the sustenance of shelter, fuel, and innumerable counter-products. The use of sensor technology in agriculture is mainly focused on the soil [1], water [2], weed control [3–5], and nutrient analysis [6–8]. The platforms are designed to understand the external factors surrounding plants rather than the plant itself. The term “Internet of things” has evolved over time; in a recent report, the authors emphasized a nascent concept concerning plants [9]. Plants have signal communications similar to neurons, and these signals are the basis of sensors and cloud technology. The idea herein, is to understand or decode the signals and employ this knowledge for both agriculture and plant domain. These environmental factors are known to be proxies for estimating the well-being of plants. This further affects the food supply chain and the different stages of plant growth need monitoring to ensure proper yields. This paper focuses on plant profiling rather than surroundings to understand the basics of plants, their features, and characteristics. Plant-based sensor technologies provide all kinds of crucial understanding for the optimization of food production.



When plants are subjected to external stressors, various defensive compounds are released by their systems. These compounds are basically a mixture of secondary metabolites (terpenoids, alkaloids, phenolics, etc.), volatile compounds (ethylene, methylene chloride, salicylic acid, etc.), and chemical defenses (phenyl propanoids, glucosilones, etc.), which exert interesting functions, as shown in Figure 1. Automated pattern recognition is implemented to categorize the phytonutrients. Unambiguous identification and quantification are based on the bio-activity assay, modes of action in the body systems, target sites, and their source of cultivation. Categorizing wild varieties/species can cause potential problems in variety identification, as well as quality aspects, with catastrophic consequences. Taxonomy is one such study that offers a clear understanding of plant origins, relationships, and evolutionary patterns. The concept of the 'origin of species' is such an understanding wherein a phylogenetic relationship is derived for clear infragenic identification. Many other fields contribute to the achievements of botany, involving molecular biology, ecology, genomic understanding, cytology, bioinformatics, and biochemistry. These together are penetrated or interlinked with each other, providing directions for new explorations and modelling/untangling the core of life's existence.



**Figure 1.** A typical illustration of the presence of phytochemicals in plants and the right key choice for electrochemical studies.

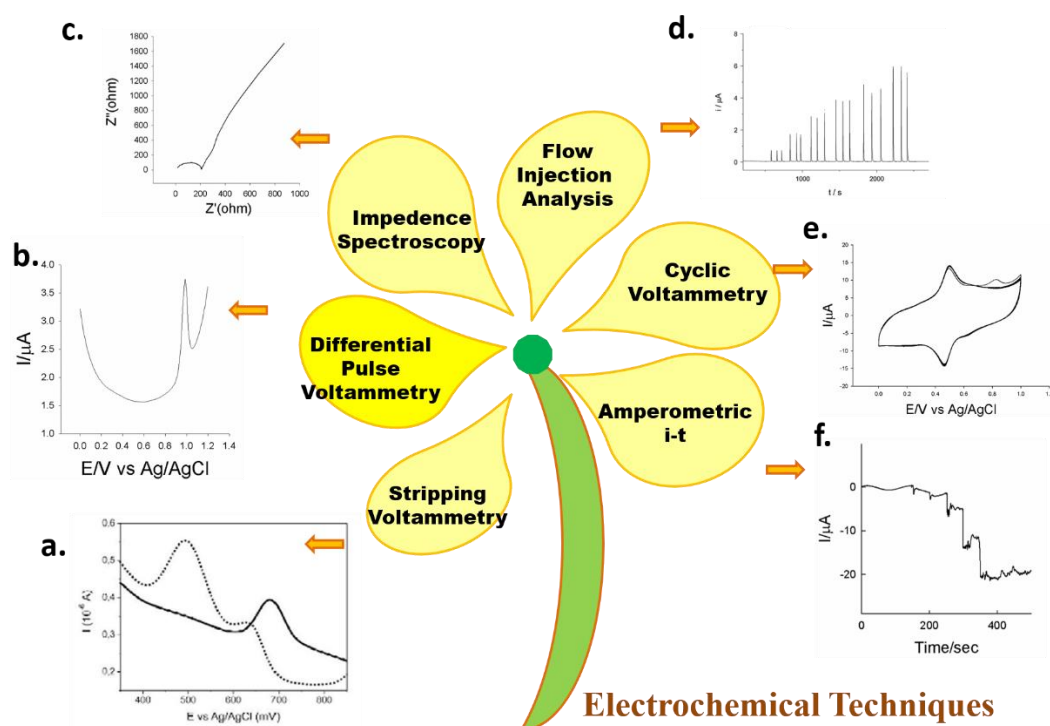
Plants consist of a mix of complexes or a rather soup of bio-synthesized nutrients as a part of their metabolic pathway. These metabolites are not easy to segregate for qualitative and quantitative estimations. The electrochemical approach involves detecting only a fraction called phyto-actives with response to the current–voltage plot, while the rest remain silent. Fortunately, the combination of these secondary metabolites is often taxonomically distinct and causes oxidative stress to plants; consequently, these are detectable using electrochemical approaches. An interesting example of the Herbivory group is the aggression-induced profile of undamaged pepper from chemical reactions due to beetles. The divergence in signals points towards the alterations in chemicals involved in defense mechanisms upon oxidative stress (polyphenols and terpenes). These changes were

regarded to be the chemical communications of plants using volatile organic compounds. The first report for a potentiometric sensor involving the tissue of the yellow squash plant variety was postulated by Kuriyama et al. for glutamate sensing [10]. Following this example, another report for dopamine using 'Banantrode' (a banana pulp modified Clark oxygen electrode) was demonstrated by Sidwell et al. in 1985 [11]. In continuation, new electrochemical platforms were developed based on corn kernels for the sensing of pyruvate [12], whereas a cucumber-plant-leaf-based system was used for cysteine detection [13]. Transgenic plants used as platforms themselves were reported in 2002 [14]. This includes the genetic alteration of genes with green fluorescent protein (GFP) via the use of an alcohol dehydrogenase promoter under variable conditions.

In the plant kingdom, many different ubiquitous secondary metabolites have an immensely positive impact on human individuals. These metabolites are responsible for herbivore defenses that exert cytotoxicity towards pathogens; and these features can be exploited in the pharmaceutical industry as antimicrobial supplements. Defensive products against neurotoxin activity can be potent muscle-relaxants, anti-depressants, etc. In continuation, many secondary products evolve and target molecular cells and tissues in competing trophic levels. Meanwhile, species of plants can contribute as defenses against insects and can be utilized as insect repellents. Ginkgo, an ornamental plant, improves peripheral and cerebrovascular circulation and the treatment of medical conditions associated with tinnitus and vertigo, because it is particularly effective in the scavenging of free radicals [15–17]. Kava, an indigenous species of 'Oceania' islands, is an intoxicating beverage used for treating tension, insomnia, anxiety, and agitation issues, because their profile includes benzodiazapines [18]. The property of radical scavenging is attributed to the 'antioxidant power' of a plant variety. With its widespread empirical use, the varieties necessitate accurate and reliable information on their phytochemical and antioxidant properties. However, there is no universal method for accurately and quantitatively measuring power. In electroanalysis, the total electrochemical antioxidant power (TEAP) of various plant material is dependent on the peak maxima [19]. The other established methods include 2,2-diphenyl 1-picrylhydrazyl (DPPH) assays, Folin-Ciocalteu assays, oxygen radical absorbance capacity (ORAC), lipid peroxide inhibition, dichlorofluorescein-diacetate (DCHF-DA), and the ferric reducing ability of plasma (FRAP) for assessing their total antioxidant activity. The electrochemical approach makes the total estimation relatively rapid, simple, and low cost.

Intelligent systems/studies are now being used to simplify and interpret large sets of plant profiles developed as a collaborative initiative to systematically classify the folk taxonomies based on plant characteristics. Stobiecki et al. reported the profiling of *Arabidopsis thaliana* of phenolic glycosidic conjugates [20]. Umoh et al. compiled a phylogeny based on subjective knowledge and through a multidisciplinary approach using metabolite investigations [21]. Berkov et al. recently elaborated the basic chemodiversity, chemotaxonomy, and chemoecology of Amaryllidaceae alkaloids, which were subdivided into 59 genera [21]. Herein, we present a basic method for 'taxa' characteristics in order to understand the phylogenetic relationships and profiling of plants using an electrochemical approach. This review accounts for various approaches accounted to date, including non-electrochemical techniques. A brief comparative study of techniques is presented in Figure 2.





**Figure 2.** Mind map of the techniques used for the analysis of phyto-constituents using the Electrochemical Workstation and their graphic output images. (a–f) These include the techniques such as Electrochemical Impedance Spectroscopy, Flow Injection Analysis, Amperometric *i-t* technique, Cyclic Voltammetry, Stripping Voltammetry and Differential Pulse Voltammetry. Each technique has its own characteristics, laws and theoretical relationships that helps in decoding the various aspects. Electrochemistry is a subject concerning the interlinking of chemical concepts based on the flow of electrons governing the product outcome. The field is generally based on green and environmentally friendly concepts, with the minimal use of reagents and organic solvents in the nano-scale operation of reaction protocols.

Electrochemistry has been used in the building of sensor platforms in order to detect biomolecules in a laboratory setting, which has been extended to miniaturized point-of-care kits [22]. Globally, it has changed the perspective of medical and clinical diagnostics [23]. The sample manipulation of biosensors enables a wide spectrum of bio-compound measurements [24]. The minute quantities of the sample sizes involve just a few microliters to several nanoliters and simple pre-treatment steps before the initiation of experiments. The investigation requires tens of seconds to few minutes, making it the best choice in establishing on-site or in-field biosensing and detection systems [25]. These sensors represent a perfect interface with the incorporation of methodologies establishing a bio-recognition system consisting of electrode coatings with distinct transducers [26], reporter molecules [27], probes [28], nanomaterials [29], polymers [30], dendrimers [31], bio-derived assemblies [32], etc., with the scope of improved biocompatibility [33], enhanced signal intensities [34], additional binding attributes [35], etc. The aim is to combine the two separate categories of nanomaterials and nanofabrication in a recent trend of designs including contributions of MEMS and portable platforms for the diagnostics of fluidic, gaseous, and solid interfaces. Thus, commercialized diagnosis devices are the end goal of these sensor developments, incorporating confined sample pretreatment, delivery, and analysis sections [36–38]. The signal readouts are an amalgamation of responses from the electrode, capture and/or reporter probe, molecular linkers, enzyme molecule, etc., which are integrated to enhance the performance [39]. The presence of a nanomaterial matrix on the electrode system is quite favorable in establishing a high surface-to-volume output, strengthening attachment to the target substrate, enhancing biological compatibility, uplifting signal intensity, and

establishing a better recognition base for adjoining molecules. Thus, the extensive use of nano-molecules has become a trend in the approach for developing electrochemical sensors. Many nano-molecules, such as carbon nano-materials (such as MWCNT, SWCNT, DWCNT, and graphene), metal nanoparticles (such as TiO<sub>2</sub>, Ag, and Ni), and bulk carbon (such as carbon black, mesoporous carbon, activated charcoal) have been developed. A number of extensive studies have been performed on these nano-molecules to establish well-defined relationships [40,41]. Carbon nanomaterials assure immobilization characteristics for enzymes, and at the same time, act as amperometric transducers [42–44]. There are a few key attributes of a potential sensor, including: the detection speed (complete recognition within a few minutes; easy signal readout), sensitivity (from several fM to aM, without any issues of sample amplification), specificity (stringency for recognition; single-base mismatch detection), convenience (MEMS + nanotechnology; portable in-field assays; point of care biosensing), and multiplexing (combination of different biomarkers/bio-molecules correlates better accuracy; simultaneous detection) [45]. These sensors enable device miniaturization with a choice of batch-fabrication and well-defined precision capabilities.

DNA oxidation via electrochemistry opens the door to powerful techniques assembling the kinetics and thermodynamic information on a same scale. This approach can be utilized for analyzing DNA damage [46]. Liang et al. reported ruthenium tris(bipyridine) immobilized on a tin oxide nanoparticle electrode to identify DNA oxidation based on each nucleotide [47]. The quest for DNA-specific binding agents is fueled by the desire to modulate gene expression, to search for new antitumor drugs, and to develop molecular probes for DNA. This feature review article is not intended to be an exhaustive review, but rather, a compilation of classical and new avenues for the profiling of various plants in recent studies. Additionally, a critical outlook towards the state of the art and important advancements for the electroanalysis field are the focus. The aim of this study was to provide a new outlook of merging the electrochemical setup with the Plantae kingdom and to exploit the various domains to extract inherent information.

## 2. Traditional Approaches for Profiling of Plants

The enormous potential of plant systems can only be prioritized based on completed analyses of bioactive compounds using different traditional protocols such as those detailed subsequently.

High-resolution proton and carbon nuclear magnetic resonance spectroscopy (NMR) measure for high concentrations of metabolites in simplified tissue extracts, biofilm, and intact tissue. It is quite insensitive to a wide range of genetic modifications, toxicology insults, and physiological stimuli using conventional <sup>1</sup>H NMR. This approach often suffers due to small chemical shift range, producing significant overlap in resonance, whereas <sup>13</sup>C NMR is intrinsically less sensitive due to the lower gyromagnetic ratio of the <sup>13</sup>C nucleus. Hence, an alternative is using hyphenated techniques such as GC–MS or LC–MS, thereby improving the sensitivity.

Gas chromatography–mass spectroscopy or liquid chromatography–mass spectroscopy represent better routes for lower concentrations of metabolic fingerprints [48]. Although the theoretical basis involves major detection advantages, no reports are available in the literature. LC in conjugation with coulometric arrays represents a much better technique for the identification of discrete serotypes. Furthermore, LC–MS or GC–MS help in directly importing the data into pattern recognition. Coupling HPLC with MS offers the analysis and quantification of folates present in their natural state, with the added benefit of analyte identification. It ensures the correct analyte identity when compared with a reference library. The confirmation of identities can be achieved by negative ion ESI–MS using authentic reported samples [49]. However, a robust technique to overcome issues of the non-selective extraction and determination of the comprehensive profiling of contents, i.e., folates, polyglutamated status, flavones, etc., has not yet been developed.



Thus, a combination of NMR, LC–MS, GC–MS, FT–MS, and HPLC electrochemical arrays for profiling can be used as a global analytical approach. These include non-directed and class-specific analyses.

Capillary electrophoresis is another alternative with different choices of detectors; analyses of the majority of compounds can be carried out for those of extremely high molecular weight with varying polarity and a thermo-labile nature [50].

Electron spray, ionization spray or atmospheric pressure chemical ionization can be used in conjugation with LC/MS for efficient metabolic profiling. This involves a step for the creation of ions (protonated or de-protonated), exhibiting low internal energy. Thus, the fragmentation of molecules is solely dependent on the potential difference applied between the capillary and entrance in the ion source.

Fluorescence-labeled oligonucleotides labeled with a fluorescent moiety in conjugation with surface modification techniques serve for high-density DNA sequence and gene expression analyses. The detection level can be up to sub-nanomolar.

Size exclusion chromatography–high-performance liquid chromatography coupled electrochemical detectors are a well-defined substitute for ensuring proper differentiation for small and larger molecular systems (especially secondary metabolites).

In contrast, cyclic voltammetry is a unique and simplified tool for characterization, providing specific oxidation–reduction potential based on the chemico-physical properties of molecules [51,52]. It is a relatively rapid technique with nano-concentrations of samples required for analysis. Most widely used is the technique with considerable output referring to thermodynamics with respect to redox properties and kinetics for chemical reactions or adsorbed processes. Various electrochemical techniques have been extended for sensing of plant based systems in Table 1.

**Table 1.** Tabulation of distinct types of electrochemical sensors and the scope of their applications reported by researchers in since 2000.

Chemically Modified Electrode	pH	Profile Constituent	Technique	Real Sample	Comment	Ref No.
GCE (acetone/chloroform extracts)	7, PBS	Quercetin, rutin, morin	SWV; $-0.25$ V to $+1.25$ V	Rosid varieties	Taxonomical studies; repeatability with 3 different harvests	[53]
Au (electrochemical chip fabrication)	5.8 and 7.1, PBS	$\beta$ -glucuronidase monitoring	CV, $-1$ V to $1$ V; chronoamperometry at $0.7$ V and $-0.4$ V	Tobacco and tomato varieties	Gene expression monitoring	[9]
Boron-doped diamond electrode (BDDE)	7, PBS	Flavone peaks	SEC–HPLC–ELC detector; $0.8$ V to $0.4$ V	Profiling of <i>Betula verrucosa</i> , <i>Equisetum arvense</i> , <i>Polygonum aviculare</i> , etc.	Relationships between Cu and Fe species and with flavonoids	[54]
GCE	7, PBS	Antioxidant power determination	CV; $-0.4$ V to $+0.8$ V	<i>Buxus hyrcana</i> , <i>Rumex crispus</i> , <i>Archillea millefolium</i> , <i>Zateria Multiflora</i> , <i>Ginkobiloba</i> , <i>Lippa citriodora</i> , etc.	The lower the potential, better the antioxidant power	[51]
Hanging Hg drop electrode	3.5, B-R	Indole butyric acid	Stripping voltammetry; $N_2$ atm	Direct bio-chemical	Plant hormone monitoring	[55]
GCE/CNT/AO-HRP	7.6, PBS	Methyl salicylate	Amperometry; $0.45$ V	Wintergreen oil	Volatile organic compounds	[56]

Table 1. Cont.

Chemically Modified Electrode	pH	Profile Constituent	Technique	Real Sample	Comment	Ref No.
GCE/GO-Fe <sub>2</sub> O <sub>3</sub> -CS	Methanolic 0.1 M NaClO <sub>4</sub>	Gallic acid; ascorbic acid	CV, DPV, −0.5 V to +1.5 V	Inflorescences variety	Antioxidant activity	[19]
SPE	7, PBS	-	DPV; −0.3 V to 1.0 V	14 Lycoris flowers	Fingerprinting petal tissues	[57]
Ag/AgCl	pH 2–7	H <sub>2</sub> SO <sub>4</sub> , HNO <sub>3</sub>	Potential difference	Soyabean seedling	Effects of uncoupler (FCCP) and acid rain are studied	[58]
SP/TiO <sub>2</sub> or SiO <sub>2</sub>	4, KPH	p-ethylene guaiacol	CV; −0.1 V to +0.4 V	Phytophthora catorum	Plant disease biomarkers	[59]
CF-UME	Bz + EtOH: H <sub>2</sub> SO <sub>4</sub>	-	SWV; 0.05 V	Edible oils	Antioxidant activity	[60]
GCE	9, Tris buffer	Scatter Pattern study	DPV; −0.2 V to 1.4 V	Chimonanthus praecox	Evolutionary studies	[61]
Ag/AgCl	In situ study	DNP	Potential Difference	Glycine Maxx Merrill	Interfacial ion transport	[62]
Hg drop electrode	5, acetate buffer	Cd(II) and Pb	DPV; −0.2 V to 1.2 V	Maize and sunflower seedling	Trace element contamination determination	[63]
SPE/Tyr/GA	4.5; acetate buffer	catechol	DPV; 0.2 V to 0.6 V	Mushroom tryosinase	Antioxidant capacity	[64]
-	-	PAH's	Electrochemical detectors	Pisum Satvium	Oxidative stress agent determination	[65]
Ag/AgCl	-	CCCP	Action potentials	Soyabean	Environmental biosensors	[66]
GCE/guanine, GCE/adenine,	4.8; PBS	AA, GA, coumaric acid, resveratrol	SWV;	Mayonnaise and margarine	Antioxidant capacity	[67]
CPE/stearic acid/DNA	7.4; PBS	herbicide resistance	SWV	Phosphinothricin Resistance	Barcode gene	[68]
Au wire/TGA	7; PBS	Binding site of plant protein	CV; 0.0 to 0.8 V	Planthacyanin (Blue Cu-protein)	Binding site of proteins	[69]
SPE/MWCNT-NH <sub>2</sub> /AG/PNPG-PVA	7; PBS	Inhibition of AG enzyme represents the potential for the plant to inhibit glucose production	CV; Amp i-t	Ehretis laevis; Micromelum pubescens; Spondias dulcis	Anti-diabetic potential of medicinal plants	[70]
Au	13; NaOH	Mg, Zn, and Mn	Electrochemical detector	Folium betulae; Folium menthae; Folium salviae; radix Valerianae; radix Taraxaci	Speciation of Mg, Zn, and Mn in plants	[71]
GCE	HClO <sub>4</sub>	Trans-resveratrol	Adsorptive stripping SWV	21 Rioja red wines	Phytoalexin determination	[72]
GCE/MWCNT	7; PBS	Sesamol	DPV	Sesame seeds and oils	Phytonutrient content analysis	[44]
GCE/CPE/PVP	3; B-R buffer	Kaempferol	CV; SWV	Spinach, cabbage, broccoli, and chicory	Total phenolic compounds	[73]
GCE/GNR/guanine	7.4; PBS	OH scavenging by AA	SWV	Fruit juices	Total antioxidant capacity	[74]
GCE/carbohydrate	0.1 M KCl	Quercetin	CV; SWV	Chemical compounds	Flavonoid determination	[75]



Table 1. Cont.

Chemically Modified Electrode	pH	Profile Constituent	Technique	Real Sample	Comment	Ref No.
Ir, Rh, Pt, Au, Ag, Cu, Co, Ni	4.8; K <sub>3</sub> PO <sub>4</sub>	Fruit juice characterization	Pulse voltammetry	Electronic tongue	Passiflora mollissima, Myrciaria dubia	[76]
GCE/Poly-CDDA	4; PBS	AA, dopamine, and UA	DPV;	Chemical compounds	Antioxidant capacity	[77]
GCE/MWCNT	7; PBS	Guaiacol	DPV	Whisky and brandy	Nutritive content	[78]
GCE	NaClO <sub>4</sub>	Standard reduction potential of OH.	CV	Melissa officinalis L, Fragaria L, Origanum majorana L, Salvia officinalis L, Equistum arvensis L, Calendula L, Alcea rosea L, Melilotus officinalis L	Total antioxidant potential	[79]
CPE	5; PBS	Electrochemical index	DPV	Red, white, and sparkling wine, and grape juice	Correlating DDPH with the electrochemical index	[80]
6B pencil graphite electrode	7; PBS	2,3;2,4;2,3,5 hydroxy benzoic acid	DPV	Commercial tea available	Tea quality testing	[43]
CPE	5; PBS	Electrochemical index; antioxidant power	CV; SWV; DPV	Coffee extracts	Total phenolic content	[81]
GCE	3.6; acetate buffer	delphinidin glucoside; cyanidin glucoside	DPV	Cabernet Sauvignon wine, raspberry	Antioxidant capacity	[82]
GCE	4.7;7; potassium phosphate	Normalized current plots	SWV	Potentilla Argentea; Sarcopoterium spinosum; Agrimonia eupatoria; Salvia valentina; Lavandula multifida	Fingerprinting of seeds	[83]

AO, alcohol oxidase; HRP, horseradish peroxidase; SP screen-printed; CF-UME, carbon fiber ultra-microelectrode; FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazone; DNP, 2,4 dinitrophenol; Tyr, enzyme tyrosinase; GA, glutaraldehyde; Pah, polycyclic aromatic hydrocarbon; CCCP, carbonyl cyanide 3-chlorophenyl hydrazone; AG,  $\alpha$ -glycosidase; PNPG, p-nitrophenyl- $\alpha$ -D-glucoopyranosidase; PVA, polyvinyl alcohol; CPE/PVP, carbon paste electrode with poly(vinylpyrrolidone); GNR, graphene nanoribbon; CDDA, 3-(5-chloro-2-hydroxyphenylazo)-4,5-dihydroxynaphthalene-2,7-disulfonic acid.

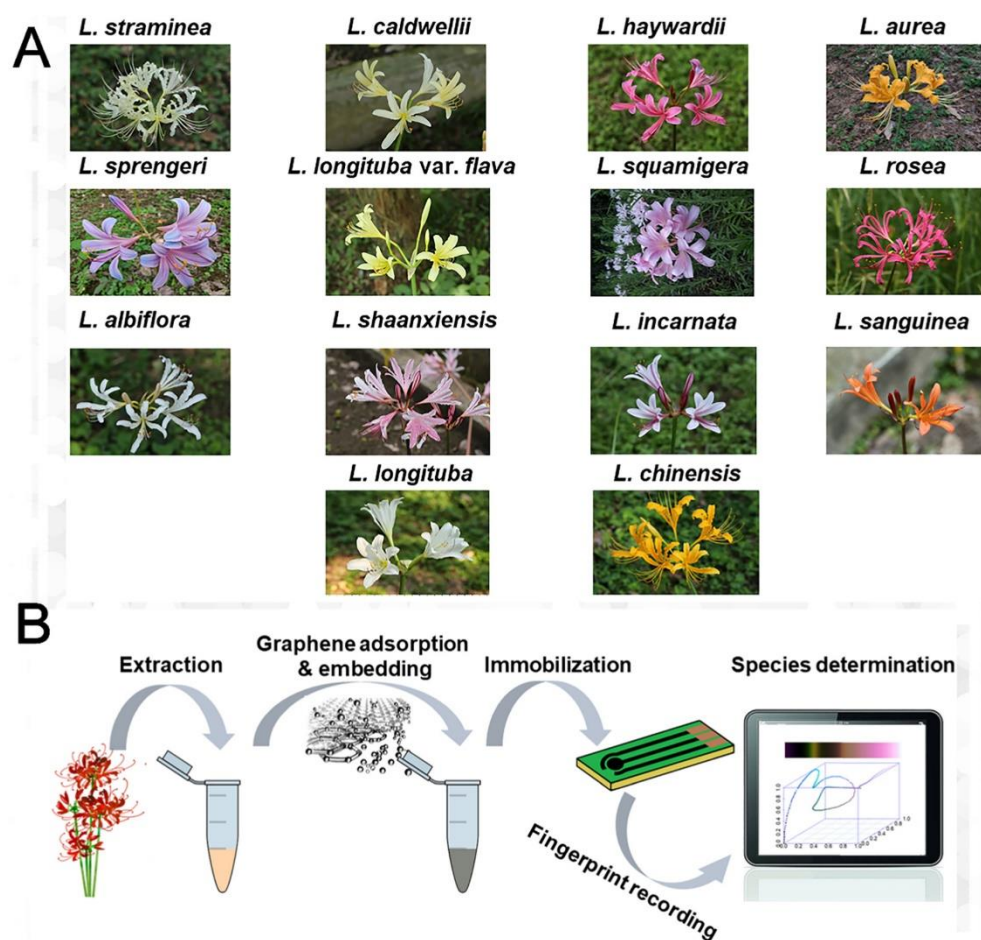
Flow injection analysis is a simplified chemical procedure performed with a flowing carrier stream and used in combination with the electrochemical system, and shows similar results to HPLC-coupled ECD.

### 3. Profiling of Biochemicals

Profiling details a whole plant cellular network constituting of transcription (DNA  $\rightarrow$  mRNA; the first step), followed by translation (mRNA  $\rightarrow$  nucleotide), and finally, post-translational modifications of the gene products. Each step of the analysis is extremely difficult due to the presence of a huge number of primary and secondary metabolites. Countless metabolites are present in cells based on the cell type, differentiation, and specialized type acquired. These essential bio-elements mix with organic moieties, forming natural complexes which are potentially important for survival. It is typical to identify the nucleotide sequences of nuclear and chloroplast regions. Hence, chemotaxonomy is one such classification that can further the understanding of chemical compositions [21,53,84,85].

The chemical analysis concept includes vast domains of estimating nutritional, pharmacological, and chemological properties. Alternatives for the identification and monitoring of chemical species are the “targeted approach” and the “non-targeted or fingerprinting

technique". Figure 3 shows the use of fingerprint recording for the species determination of 14 flowers species of *Lycoris*. Fingerprinting or non-targeted approaches intense techniques based on organic–inorganic plant interactions due to ignorance in understanding the exact mechanisms. Among such a plethora of complex molecules, ‘n’ is the number of phenolic acids, anthocyanins, procyanidins, and flavonoids which have been observed. The genetic profiles of variant species are directly proportional to their chemo-taxonomical studies; this approach is associated with inherited properties of natural products and synthetic analogues. The use of electrochemical approaches involves representations of simple plant extracts, eliminating the concept of prolonged storage under aerobic environments. The approach is quite simplistic due to the redox-active components; many identified chemical counterparts are non-electroactive in nature. Some examples of these phenolic molecules are rutin, vanillic acid, salicylic acid, morin, and quercetin. Isoflavone is one such phytoestrogen or secondary metabolite, particularly present in leguminous plants. Many constituents such as flavonoids, lignans, and coumestrans are fractions of phytoestrogens. These help plants to maintain resistance to diseases such as neurodegenerative disorders, cancer, Alzheimer’s disease, fungal attack, and hypertension. Carotenoids are the most abundant natural pigments and exert substantial pro-vitamin and antioxidant activities. Chlorophyll and its associated compounds have huge antioxidant potential, and thus inhibit lipid peroxidation and even protect mitochondria from oxidative damage [86]. The application of methods can be extended to mammalian tissues and organ domains. These have been further subdivided as follows.



**Figure 3.** (A) Digital photographs of 14 flowers species of *Lycoris* and (B) its schematic diagram for fingerprinting recording using its petals for species determination. Reprinted with permission from reference [57], Elsevier, 2019.

Genomic and metabolomic study: metabolites can vary across a wide range of modifications, making them a complex system to accept as unique biomarkers. Using metabolic engineering could improve the situation by enhancing the synthesis of valuable therapeutic agents. Wang et al. presented a comparative review on plant miRNA biogenesis and degradation, encountering stability issues upon 3' modifications and studied degradation upon translation [87]. Gao et al. developed a microRNA biosensor based on chemical ligation which involved the concept of electrochemistry for amplified detection [88]. They explained how Northern blots and cloning are the gold standard for microRNA validation; these assays are inappropriate because the consumption of RNA samples is a laborious phase. Subsequent high-throughput and sophisticated instrumentation with highly skilled technicians are necessary for quantification assays.

Electrochemical DNA interpretation involves the use of gold nanoparticles [89,90], the direct oxidation of guanine [91], DNA intercalations [92,93], DNA threading intercalators [94], etc., which were established in 1900 [11,95]. In addition, the sensitivity of metabolic studies alters with response to stress and toxicity investigations. The detection of nucleic acid by various means is an essential part of genomics studies. The use of electrochemical means to mark an effective biomarker involves the stepwise designing, synthesis, and characterization of probe molecules. Many reports have detailed the use of gold surfaces and nucleic acid motif functionalization in investigations of DNA barcodes, DNA hairpins, aptamers, enzymes, etc. [96]. Functionalized sensors for nucleic acid targets focus on a diverse repertoire of chemical readout mechanisms. Commonly, modifications using chromophores, click chemistry reagents, biotin, etc., are vital components of probes. Approaches for the site-specific attachment of thiolated DNA probes are being developed independently.

Transcriptomics: various biological and chemical amplification strategies are being upgraded using enzyme-amplified assays with minute quantities, without the need for PCR [97], such as Au-NP bilayer electrode systems [98], although electrochemical amplification strategies with electro-catalytic redox moieties [94] yielded quite an enhanced sensitivity. There is an ever-increasing demand to develop rapid, sensitive, and selective bioassay methods for various molecular diagnoses and the detection of infectious agents. Methylene blue is one such example of an end-labeled oligonucleotide used as a fluorophore/quencher that relates 'on' and 'off' stage switches based on the conformational state of methylene blue. The signal is directly proportional to the distance between the quencher and the fluorophore. The use of MB has been accepted in applications in molecular and cellular biology, pathogen detection, and biomedical diagnostics [99].

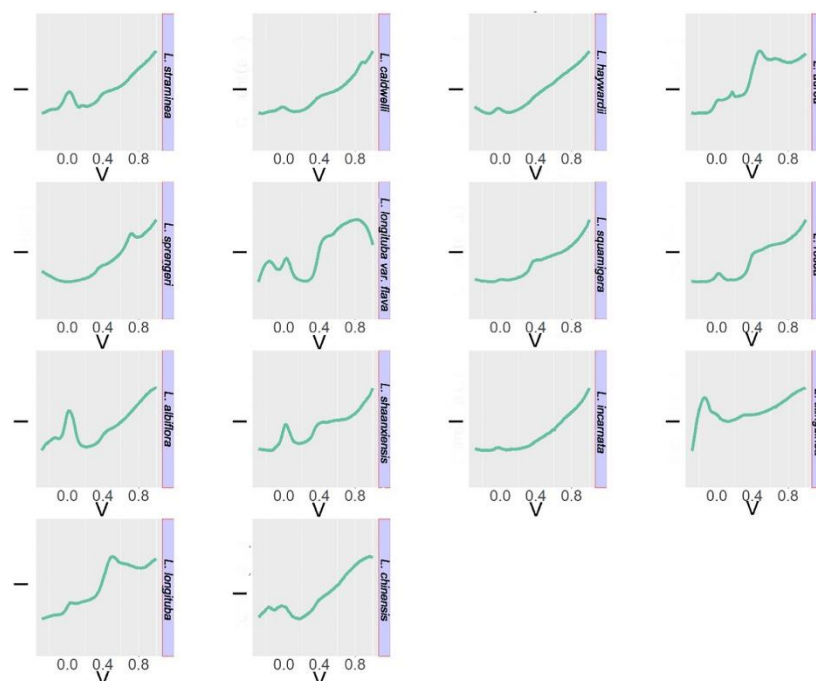
The use of traditional expensive instrumentation poses a setback in the research field due to the unavailability of instrumentation. The analysis protocols are time-consuming with tedious pre-concentration steps before the investigation. Conventional systems require complex processing steps performed by highly skilled technicians.

#### 4. Use of Electrochemistry

Electroanalysis reflects the fingerprints of pigments, plants, and cultural relics based on recordings of electrochemical signals [83,100]. The electrochemical detection of biological species requires reactions consisting of bio-recognition steps involving current or potential, impedance, redox kinetics, etc., or non-electrochemical properties involving van der Waal interactions, intercalation with matrix due to electrical fields, mass transportation beyond the Helmholtz's double layer, conformational changes, etc., inducing fluctuations in electrical responses. Miniaturized biosensors, due to their compatibility the advanced use of semiconductors, are combinations of accurate and inexpensive platforms. These sensors offer electron transfer reactions without labelling for ultrasensitive DNA detection [101]. Domenech-Carbo et al. used squarewave voltammetry to characterize tomato plants based on six diverse varieties [102]. They placed a glassy carbon electrode directly into fresh tomato varieties and estimated the ratios of vitamin C to total phenolic compounds [103–105]. Many other such plant constituents have been analyzed, and are



presented in Table 1. A plot for use of Differential pulse voltammetry as shown in Figure 4 has been highlighted to determine the relation between species.



**Figure 4.** The normalized current values of differential pulse voltammetry responses on screen-printed electrodes using digital photos of 14 flowers of *Lycoris* species vs. Ag/AgCl after ethanol extraction. Reprinted with permission from reference [57], Elsevier, 2019.

Many methods have been proposed for the application of electrochemistry as a tool to understand plant feedback mechanisms to stress via assessments of the redox activity of phytochemicals. The initial condition for determining species involves replicating experimental procedures on the chosen species type, followed by studies of voltammograms with increasing peak resolutions. Voltammograms display different values for different species based on the multiplication of current values ( $I$ ) with potential ( $E$ ), involving 'i' solvent extracts having 'j' electrolytes. Hence, the matrix dimension are  $I \times (ij)$ , referred to as PCA diagrams for wave voltammograms or dendrograms. We can distinguish and segregate species signatures in various phylogenetic classification based on electrochemical approaches; however, few similar species diverge from genetic similarities. However, overall, satisfactory correlations with phylogenetic trees at the level of taxonomical orders can be judged [53]. Fu et al. used a DPV technique for the electrochemical fingerprinting of *Lycoris* petal species of several varieties and confirmed the consistency of the PCA diagram with the dendrogram correlating the infragenic relationships between native *Lycoris* species [57]. As explained by Carbo et al., ramifications in the phylogenetic tree concerning the Rosaceae family lead to complications related to voltammetric analysis. The authors explained that the differences might be attributed to evolutionary tendencies due to the diversification of polyphenolics in them. In addition, they also related the advent and disappearance of the signals as temporary sequences due to genetic changes that help us in acknowledging the modifications; hence, this corroborates other classifications of phylotaxonomy [106,107]. The concept can be further extended to the subcellular analysis of plants, with outstanding data enabling further manipulation of the essential constraints for improving health and product outcomes using plants. Leon et al. compared electrochemical and conventional techniques for the determination of antioxidants used in the monitoring of DPPH radical capture [108]. On comparison between spectrophotometric and chronoamperometry techniques, sample dilution and pretreatments steps were easily eliminated in cases of amperometry without any issues of concentration and absorbance uncertainty. Table 1

shows the culmination of various electrochemically based sensor systems and the scope of their applications.

The voltammetry of immobilized particles using solvent extraction (ethanolic and methanolic) approaches uses the preferred parts of plants [55]. Cyclic voltammetry (CV) enables non-faradaic contributions that result in non-steady signal outputs. Hence, constant potential with amperometry or chronoamperometry is one such technique that ensures a steady state response. Scanning electrochemical microscopic analysis involves the direct imaging of plant systems and their internal transformations/modifications based on the electroactivity of phytonutrients.

### 5. Electrochemical Profiling of Biochemicals

Phenolic acids, flavonoids, and carotenoids are considered to be key compounds due to their electrochemical profiles [109]. The accessibility of techniques involving easy operation via portable in-field equipment with simple sampling protocols that ensure immediate analysis without prolonged maceration/extraction is highly beneficial. Most importantly, the intrinsic ability to select molecules such as sugars, cellulose, or carotenoids can be excluded because this refers to electrochemically silent moieties; only a limited number of components are active in a similar way to phenolic moieties. These non-conducting ingredients consume key parts of plant tissues, making it difficult to analyze the conducting counterpart ingredients. An embedding plant strategy has been designed to ensure selective and amplified responses using a matrix or polymers [25,110]. It is a relatively tedious job to summarize the protocols and resolve the varieties and species domains in the Plantae kingdom; hence, conventional systems are not applicable in real-world analysis. Considering electrochemical techniques, only voltammetric profiles did not exhibit any alterations in condition when studied. Differential pulse voltammetry is one such technique, which can be used to establish 'p' values for MANOVA tests: this helps in maintaining normalized wave signals of a particular species group. However, the 'p' values can be quite close to 0.5 for inter-species attributes; for the case of intra-species analyses,  $p \gg 0.5$  thus helps in establishing significant relationships [57]. This analysis can further be used for the detection of food and medicinal attributes exerted by phytonutrients [111–113]. The concept of correlating species with their successive voltammetric attributes has previously been studied [114].

Thin film cyclic voltammetry represents the optimal choice for the evaluation of P450 chromosome enzymes. This approach has momentum, especially in the field of deciphering electroactive constraints wherein specifically engineered enzymes are fused in conjunction with electroactive nucleotide chains. These fused molecules act as an interface component of electrodes modified with biomimetic systems; further techniques are employed for biosensing applications [115,116].

Nurmi et al. reported the profiling of phytoestrogens using hyphenated HPLC electroarray methods. These techniques have shown good sensitivity and reproducibility over various assays [117]. Similarly, Klejdus et al. initiated an electrochemical platform for isoflavone profiling by adsorptive transfer stripping voltammetry in soyabean plants at pH 9.2 [118]. Based on successive investigations, Gil et al. reviewed the use of electrochemistry for flavonoid profiling, with in-depth knowledge of electrode platforms and their buffer media [119].

An innovative approach for the identification of plant species based on the fingerprinting of petal tissue was reported by Fu et al.; the use of electrochemistry was emphasized, although the comparison of infragenic identification was based on a traditional botanist approach. Electrochemical fingerprints were analyzed after various solvent extractions and the contents of electroactive molecules were controlled by respective genes [57].

### 6. Conclusions and Future Prospects

This paper presents a culmination of all the different techniques and their probable outcomes for the profiling of plants. The basic comparison between other classical and

electrochemical techniques shows that electrochemically based platforms are optimum pathways because they are relatively rapid and simple. The results for profiling are comparable with other techniques. The main conclusions and future scope of this study focus on the identification of molecules produced within plants, and obtaining/extracting them on an industrial scale with the scope of using them further as selective pesticides. Based on the electrochemical behavior of plants, a lower potential is more suitable, i.e., the possibility of establishing an 'electrochemical taxonomy' will be soon realistic. The report by Fu et al. can be extended to other plant varieties and compared with other techniques to develop the PCA diagrams. The electrochemolomic methodology is a potential set of analysis techniques for assessing different vegetal species in order to establish taxonomical groups; it can be used as a complementary technique to molecular phylogeny, in tandem with existing genetic and chemical assays. The apparent potential of this technique may be an assistive technology for phytogenic studies in many other fields of application. The importance of preserving biosphere diversity is highlighted through the correct development of science and technology. The use of a rapid practical and reliable methodology with good analytical performance and suitable selectivity indicates the contemporary need for profiling systems. Clinical applications of these sensors are still distant, because major selectivity/specificity and performance issues need to be addressed.

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**“Qualitative /situational analysis of prevalence and practices to prevent anaemia among Adolescents of Telangana Social Welfare Residential Educational Institutions Society” –Stakeholder-principal**

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**ABSTRACT**

**Introduction:** Anaemia may be a condition characterised by a decrease within the concentration of haemoglobin in the blood that results from a reduced quality or amount of red blood cells that reduce Oxygen carrying capability to tissues. Red blood cells carry haemoglobin, an iron-rich protein that attaches to oxygen in the lungs and carries it to tissues throughout the body. Anaemia occurs when you do not have enough red blood cells or when your red blood cells do not function properly. It is diagnosed when a blood test shows a haemoglobin value of less than 13.5 gm/dl in a man or less than 12.0 gm/dl in a woman. Normal values for children vary with age.

**Aim:** To Study the current steps and practices on prevalence of anaemia among adolescents students studying in Telangana Social Welfare Residential Educational Institutions Society by different stakeholders perspective in Telangana state.

**Objectives:**

To Study the prevalence of anaemia among adolescent children’s in Telangana Social Welfare Residential Educational Institutions society by different stakeholders.

To know certain measures and techniques in the study

To analyse the current situation with the help of tools and techniques

**Methods:**

Selection of districts from Telangana state based on NFHS indicators. A focus group discussion (FGD) interviewed was conducted with the stakeholder. A semi-structured questionnaire was prepared. Verbal and written informed consent was taken from the stakeholder before interviewing them. The responses was recorded for further non-verbal observations.

**Results:**

This study shows the lacuna in Identification, Screening and Management of anaemia in the adolescents children studying in Telangana Social Welfare Residential Education Institution Society(TSWREIS) by the stakeholder.

**Conclusion:**The study gives the conclusion that There are many schemes initiated by the government but no proper standard operating procedures is followed.The students are not screened regular for the identification of anaemia. Problem with the identification and diagnosis of anaemia

**Keywords:** Anaemia,Haemoglobin,Nutritional status,Dietary supplements

**INTRODUCTION**

Anaemia may be a condition characterised by a decrease in the concentration of haemoprotein in the blood that results from a reduced quality or amount of red blood cells that reduce Oxygen carrying capability to tissues. Anaemia is caused by the many-sided interaction of things equivalent to diet; communicable diseases such as malaria, HIV, and soil-transmitted helminths significantly hookworm infestation; and socio-demographic and economic factors. Anaemia ends up in an impaired oxygen circulation in the blood, it's going to cause adverse maternal and birth outcomes, poor kid growth, impaired psychological feature capacity and learning ability, and reduced work productivity and financial gain earning throughout adulthood. As a result of the on top of mentioned causes, anaemia in later life will cause substantial economic loss, decrease gross domestic product (GDP), and increase treatment costs. Anaemia deeply will increase the chance of maternal and kid morbidity and mortality rate.[1]

Haemoglobin is the main protein in your red blood cells. It carries oxygen, and delivers it throughout your body. If you have anaemia, your haemoglobin level will be low too. If it is low enough, your tissues or organs may not get enough oxygen.[2]

Normal Haemoglobin (Hgb)-particular laboratory cut-offs will vary slightly, however in general, the regular levels are as follows:

- 13.5 to 18.0 g/dL in men
- 12.0 to 15.0 g/dL in women
- 11.0 to 16.0 g/dL in children
- Varied in being pregnant relying at the trimester, however commonly more than 10.0 g/dL[3]

Anaemia is a global public health problem affecting both developing and developed countries at all ages. According to the World Health Organisation (WHO), anaemia is defined as haemoglobin (Hb) levels <12.0 g/dL in women and <13.0 g/dL in men. However, normal Hb distribution varies not only with sex but also with ethnicity and physiological status.[4]

Anaemia affects a third of the world's population and contributes to multiplied morbidity and mortality, reduced work productivity, and impaired neurological development.[5]



The causes of anaemia are multiple. The major ones are a deficiency of haemopoietic factors, genetic disorders causing haemolytic anaemias, infections including malaria, and increased losses of blood caused inter alia by infections. The major haemopoietic factors are iron, folate and vitamin. Iron deficiency is widespread and is the most common cause of nutritional anaemia in women of reproductive age and young children; folate deficiency is often present during pregnancy and may also be present in young children. Hereditary haemolytic anaemias include sickle-cell anaemia where abnormal haemoglobin is present and Thalassemia which is caused by an abnormal haemoglobin metabolism.[6]

Some forms of anaemia cause specific symptoms, including:1)Aplastic anaemia: This can cause a fever, frequent infections, and skin rashes2)Folic acid deficiency anaemia: This can cause irritability, diarrhoea, and a smooth tongue.3)Haemolytic anaemia: This can cause jaundice, dark urine, a fever, and abdominal pain.4)Sickle cell anaemia: This can cause painful swelling in the feet and hands, as well as fatigue and jaundice.[7]

Anaemia is in particular due to the iron deficiency within-side the body. Iron is one of the critical minerals required by the body to perform several critical functions. The crucial feature of iron is to move oxygen from the lungs to cells in our body. Iron deficiency is a situation that arises because of a very much less quantity of iron present within-side the body. Iron is a crucial mineral which allows the manufacturing of haemoglobin through erythroblasts. Anaemia is found through the much less awareness of haemoglobin from the positive endorsed levels. According to WHO the recommended Haemoglobin level, for non-pregnant women(age 15 and over) is 120gm/L and for males (15 years and over ) it's far 130gm/L [8]

Iron is an essential element and is controlled primarily by dietary intake, intestinal absorption and iron recycling. Dietary iron can be found in two forms: haem and non-haem iron. Haem iron is easily absorbable and arises from haemoglobin (Hb) and myoglobin in the form of animal meat, poultry and fish. Non-haem iron is mostly found in plant food but is not as easily absorbable. Compounds such as phytate, oxalate, polyphenols and tannin, which are found in plants, diminish the uptake of non-haem iron, as do some drugs, such as proton pump inhibitors.[9]

Iron-deficiency anaemia is the most common trusted Source type of anaemia, and blood loss is often the cause. A shortage of iron in the blood leads to this form of the condition, and low iron levels frequently occur as a result of blood loss. When the body loses blood, it draws water from tissues beyond the bloodstream to help keep the blood vessels full. This additional water dilutes the blood, reducing the RBC count. Blood loss can be acute and rapid or chronic. Some causes of rapid blood loss include surgery, childbirth, and trauma. Chronic blood loss is more often responsible for anaemia. It can result from a stomach ulcer, cancer, or another type of tumour.[10]

Anaemia is referred to as low haemoglobin, which will cause you to feel tired and weak. There are many sorts of anaemia and every one has its cause. It can be temporary or long term and might vary from mild to severe, in most cases anaemia has over one cause. they're classified supported their morphology into Microcytic hypochromic anaemia, Normocytic

normochromic anaemia, and macrocytic anaemia. The signs and symptoms vary relying upon the causes, these embody weakness besides fatigue, the pale or chromatic colour of the skin, irregular heartbeats, shortness of breath, vertigo or light-weight headedness, chest pain, cold hands and feet, headaches, anaemia is gentle with an unremarked symptom, but over some time the condition gets worsens with symptoms.[11]

Anaemia is a serious global public health problem that particularly affects young children and pregnant women. WHO estimates that 42% of children less than 5 years of age and 40% of pregnant women worldwide are anaemic. The optimal haemoglobin concentration needed to meet physiologic needs varies by age, sex, the elevation of residence, smoking habits and pregnancy status[12]

In 2019, the anaemia prevalence was 29.9% globally, in the reproductive age of women half a billion women aged 15-49 years are affected [4]. regardless of its aetiology, WHO estimates over 1.62 billion people worldwide. mostly the pre-school children( 47%), accompanied by pregnant women(41%),non-pregnant women(30%), school-age children(25%) and those older than 60 years of age (24%), men are the least affected group(12%).[13]

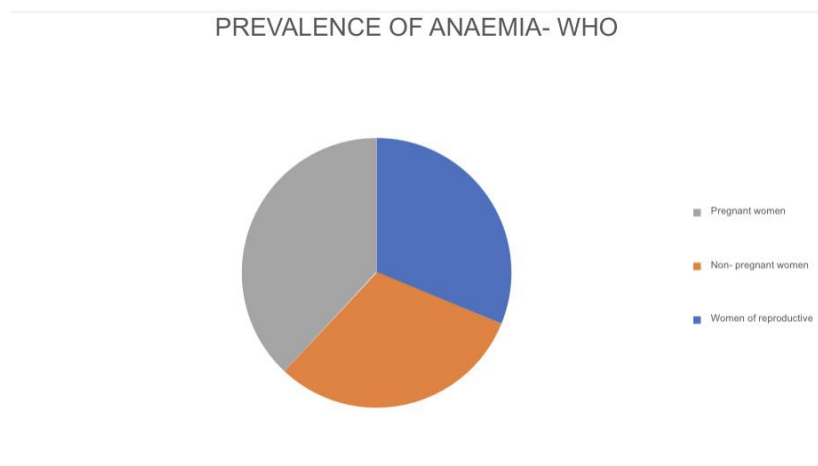


Figure-1

The maximum occurrence of anaemia is determined in Asia. About 1/2 of of all of the anaemic girls stay with-inside the Indian subcontinent in which 88% of them expand anaemia in the course of pregnancy.It is related to bad perinatal outcomes.[14]

India is one of the country in thenations with excessiveincidence of anaemia .It's farextensivelyuniversal in all age groups,being specificallyexcessivemost of themaximum vulnerable; almost 58% in pregnant women,50% amongst non-pregnant ,non-lactating women,56% amongst adolescent girls,30% in adolescent boys and round 80% in youngsters below 2 years of age. Adolescents (age 10-19 years) are at excessivedanger risk of iron deficiency and anaemia because ofmultiplied increase in necessities for iron,lownutritionalconsumption of iron, excessivecharge of contamination and worm infestation in addition to the social norm of early marriage and, adolescent pregnancy.[15]

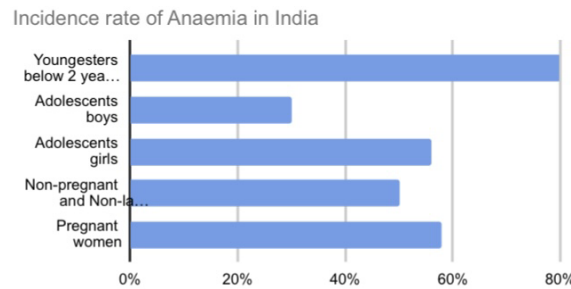


Figure-2

The national prevalence of anaemia in children is 28.8%, where the prevalence of anaemia among mothers is about 24.2%. The long-term effects of these conditions negatively affect the health of adults throughout their life, as well as their potential productivity in the work force and possible economic contribution to the nation..According to NFHS-5, the anaemia in adolescent girls increased between 54.1% and NFHS- 59.1%.[16]

In Telangana state, the Prevalence of anaemia was high. The prevalence of anaemia was observed in females (44.04%) compared to males (57.2%). The prevalence was more in reproductive age group (58.6%). Moderate anemia (58.6%) was more prevalent followed by mild anemia (36.6%). Microcytic hypochromic blood picture predominates with the incidence of 53.6% followed by normocytic blood picture 42.2%.[17]

Anaemia, as a result poses a first-rate hazard to maternal and baby survival, contributes to low delivery weight, diminished resistance if infection, poor cognitive improvement and reduced work productivity. The magnitude of anaemia collectively with the related detrimental fitness, improvement and economic consequences, spotlight the want for an intensified motion to deal with this public health problem. [18]

Anaemia has been a first-rate public health problem over the last few years. According to N Milman, "The maximum common nutrition deficiency in each growing in addition to in advanced countries is iron deficiency"[19]

Adolescence is a transitional duration from childhood to adulthood, for the duration of which positive health issues and change behaviour established among children may also have an impact on their future fitness. According to WHO, Adolescent (early life) is described as the period among 10 years and 19 years. Adolescent of each sexes are in particular prone to growing anaemia due to fast growth, in girls and women moreover due to the onset of menstruation.[20]

Adolescent girls have a higher risk of anaemia due to an increased requirement, low intake of hematopoietic nutrients and low intake of a nutrient that enhance absorption of these hematopoietic nutrients.[21]

Based on WHO guideline, adolescents are said to be anaemic when the haemoglobin level is less than 12mg/dl. But, this might be affected due to the increased iron requirement, decreased iron intake, rapid physical growth, menstrual loss, and high iron demand for haemoglobin (Hb) formation. Adolescent girls are at higher risk of anaemia due to a period of physical growth, reproductive maturation, and cognitive transformations which demands high macro and micronutrients including iron[22]

Anemia has its variety of consequences. Direct effects are on growth and indirect effects are like impaired concentration, lack of attentiveness, poor memory, defective performance in the academics, and decreased attendance in the schools. Menarche is delayed, immune system is affected which leads to more episodes of infections. Increased fetal morbidity and mortality, low



birth weight, perinatal risk, increased infant mortality, and maternal mortality are some of the future consequences of anemia among the adolescent girls.[23]

Sickle cell diseases (SCDs) is an rising public health challenge ,now no longer only in India however all over the globe. It has been predicted that, among 2010 and 2050 approximately 14.2 million toddlers might be born with sickle cell anaemia. Thus ,in 2006 WHO recognised SCD as international public health problem.[24]

Anaemia is described as a reduction in haemoglobin(Hb) concentration, hematocrit ,or number of red blood cells per to litre beneath the reference interval for healthful people of comparable age ,sex, and race, below comparable environmental situations According to the WHO , for below five years kids, the edge Hb stage for being anaemic is much less than 11.0g/dl .Anaemia is a common in medical circumstance characterised via way of means of reduced Hb ranges that are inadequate for the body's demand .Anaemia proven to be a public health problem that influences low,middle,and high income countries at different ranges and incidence varies with socioeconomic status. The maximum dependable indicator of anaemia on the population stage is blood haemoglobin concentration. Anaemia as a consequence of iron deficiency has been pronounced to well increase morbidity and mortality in preschool-elderly kids and pregnant women. numerous elements make contributions to the prevalence of anaemia and almost 1/2 of of 43% the anaemia instances in formative years are because of iron deficiency. The deficiency may also end result from insufficient nutritional consumption of iron,malabsorption of iron multiplied iron demand for the duration of rapid growth in kids and persistent blood loss. different reasons of anaemia consist of folate,vitamin B12 deficiencies,malaria,intestinal helminthes,viral infections.[25]

WHO worldwide information show, that anaemia because of iron deficiency impacts about 30% of world's population and approximately 37% of school children. In Indian children ,excessive occurrence of anaemia various from 27% to 90% has been stated in unique studies.[26]

Situational analysis helps develop a basis of understanding of the environment in which a plan is delivered. It provides a common reference point for the planning process and prioritises actions. The analysis can provide an appreciation of the risks and benefits to the project and the organisations involved from the way in which the communication process is implemented. It takes a snapshot view of an organisation or situation and where things stand at a certain point in time. It is sometimes accomplished by means of a SWOT analysis (Strengths, Weaknesses, Opportunities and Threats), which examines all aspects in relation to the success or results of the project in question.[27]

Situation Analysis is a preliminary assessment of a particular situation which is inter-connected with a project to be implemented in an area. It is a process which helps to understand the situation and also to identify the gaps in the situation. Situational Analysis helps to define the nature and scope of a problem. It helps to identify the current practices and strategies to overcome the problem. It also helps to understand the opinions and experience among the stakeholders.[28]

Current anaemia control programme focusing on prophylactic iron supplementation and facility-based screening with haemoglobin estimation is inadequate to reduce the high prevalence of anaemia in India.[29]

Certain key programmes are initiated by the Government of India to eradicate the prevalence of anaemia in pregnant and lactating women under the ICDS Scheme of MWCD (Ministry of women and child development) by providing supplementary nutrition and for childrens the provision of Iron and Folic acid (IFA) along with the supplementary diet has been a part of Government of India over a three decades of time.[30]

Government run programmes like Integrated Child Development Schemes (ICDS), National Nutritional Anaemia Control Programme (NNACP), Weekly Iron and Folic acid Supplementation

(WIFS), National Iron Plus Initiative (NIPI) to combat anaemia. Some of the steps done by the government to eradicate anaemia include communication campaign, testing and treatment of anaemia using Digital methods and mandatory provisions of IFA fortified foods in government programmes.[31]

Under National Rural Health Mission, Rashtriya Bal Swasthya Karyakram (RBSK) is an important initiative aiming at early identification and early intervention for children from the time of birth to upto the age of 18 years to cover 4 main aspects like - Defects at the time of birth, Deficiencies, Diseases and Development. Under RBSK, activities like confirmation, management, referral, tracking and follow up - are planned according to the age group of the child. It is important to note that the children under the age of 0 - 6 years of age group will be specifically managed at District Early Intervention Centre (DEIC) whereas, for the age group of 6-18 years, management of the conditions will be done through existing public health facilities. DEIC will act as a referral linkages for both the age groups. Child Health Screening and Early Intervention Services under RBSK are to cover 30 selected health conditions for Screening, early detection and free management. Deficiencies include Anaemia, especially severe anaemia, Vitamin A, D deficiency, Severe acute Malnutrition and Goitre.[32]

### RBSK Screening Process:



TSWREIS society is running 500 residential institutions in Telangana under the department of Social Welfare, Government of Telangana. The society institutions run residential education from 5th class to intermediate. The top goal of the society is to offer great residential training to the communities. They additionally preserve great meals through presenting accurate nutritious meals to the children. To reveal the every day Menu, great and widespread of the meals of the schools, the society added up software program like ANNAPURNA, A Menu Monitoring Software (MMS).[33]

Child Screening below RBSK is at levels - Community degree and Facility degree. Facility degree is primarily based totally on new child screening at public fitness centers like PHC's may be performed through Medical officers, nurses and ANM's. The Community degree screening may be carried out through the Mobile fitness groups at Anganwadi Centres and Government aided Schools. Clinical Tests are done by the team of RBSK in these government and government aided schools[34]

The RBSK team will be coming to the school and they will conduct clinical tests among the students. The results will be updated in SIF note by the officers. After updating the results, if they find any problem with the student like the student is not feeling well - they again the student will be tested in the nearby PHC and will take the advice of the doctor of Synergy India Foundation as well as the doctor in the PHC. As per the doctor's advice they give a special diet to the student like they include jaggery based food items, sesame seeds laddoo, chikkies, peanuts, ragi java. Fruits like pomegranate, apple, banana - given daily, watermelon, oranges and some seasonal fruits are given. Vegetables like beetroot, carrot, green leafy vegetables, potatoes, brinjals, cabbage etc., are given. Animal sources like egg - given daily, milk - given daily, chicken - 4 times in a month, mutton - 2 times a month are also provided.[25] A holistic approach is required with special emphasis on community awareness about consequences of anaemia and benefits of Iron Folic Acid (IFA) supplementation, uninterrupted supply availability of Iron folic Acid tablets and syrups, hard to reach areas and monitoring and review system to find out adequacy in terms of participation and frequency of meetings and problem-solving.[35]

National Iron plus Initiative (NIPI) : NIPI was introduced in 2013 to reduce the incidence and prevalence of iron deficiency anemia across all life stages including adolescents and women in reproductive age group who are not pregnant or lactating. Health care providers at all levels had been identified under NIPI framework for providing comprehensive package of continuum of care along with treatment and management of anemia.[36]



The Anemia Mukht Bharat strategy is being implemented in all villages in blocks of the districts through existing delivery platforms as envisaged in the National Iron Plus Initiative (NIPI) and Weekly Iron Folic Acid Supplementation (WIFS) programme Since 2018. Anaemia Mukht Bharat Scheme is implemented in Tamilnadu in order to reduce the prevalence of anemia by 3 percentage points per year, It is a universal strategy and it focuses on the following interventions: Prophylactic Iron and Folic Acid supplementation Deworming Intensified year-round Behaviour Change Communication Campaign (Solid Body, Smart Mind) focusing on four key behaviours Improving compliance to Iron Folic Acid supplementation and Deworming, Appropriate infant and young child feeding practices, < Increase in intake of iron-rich food through diet diversity/quantity/frequency and/or fortified foods with focus on harnessing locally available resources. Ensuring delayed cord clamping after delivery (by 3 minutes) in health facilities. Testing and treatment of anemia, using digital methods and point of care treatment, with special focus on pregnant women and school-going adolescents Target group Children of 6 – 59 months age Children of 5 – 9 years age Adolescent Boys & Girls Women of Reproductive Age Pregnant women Lactating Women [37]

To achieve the targets of World Health Assembly of 50% reduction of anemia in women of reproductive age by 2025 and POSHAN Abhiyan (2018-2022) to reduce the prevalence amongst young children (6-59 months), adolescents and women of reproductive age groups (15-49 years) by three percent per year, Anemia Mukht Bharat has been designed. This has been built upon the existing framework of NIPI with special focus on intensive behaviour change communication, vulnerable geographies, procurement and supply chain management issues and others. The operational guidelines were launched by Honorable Prime Minister Shri. Narendra Modi on 14th April, 2018 in Bijepur, Chhatisgarh. The beneficiaries are children 6-59 months, children 5-9 years, adolescent boys 10-19 years, adolescent girls 10-19 years, women of reproductive age (20-49 years), pregnant women and lactating women (0-6months). The key interventions proposed to be carried out in this program are IFA supplementation and deworming; intensive IEC/BCC about nutrition awareness, appropriate IYCF practices and intake of iron rich foods: awareness, screening and treatment of malaria in endemic pockets; special focus on pregnant women and school going adolescents; iron and folic acid fortified foods in all public health facilities; delayed cord clamping after delivery (3 Minutes).[38]

Prevention practices to eradicate anaemia in Adolescent Boys & Girls (10 – 19 ) Year: Weekly 1 Iron and Folic Acid tablet is provided to the Adolescent Boys & Girls of 10 – 19 years age (School going / non school going children) in the age group of 5 to 9 years for 50 weeks in a year with biannual deworming with one tablet of Albendazole tablet 400 mg during the National Deworming days conducted once in 6 months (February and August of every year)[39]

The mid-day meal provided by the government to rural children is not enough to meet their nutritional needs. Low nutrition in children makes them anaemic and hence reduces the oxygen supply to the brain. This affects their concentration and grasping power and lowers their education level.[40]

The current study is dealing with the stakeholder - The principal. Principal are the people look after the students health, academic performance and nutritional needs of the children at these residential schools. They see the students health checkup, screening problems, and the grocery supplementation to the school, conduct meetings with the students, initiate lunch programme (mid meal programmes) and see through whether the surroundings are kept clean. They also look after the children whether they're eating properly or not, sometimes they also check whether the children are wasting any of their food in their meal and also look after the students mental health status.

**AIM:** To Study the current steps and practices on prevalence of anaemia by different stakeholders.

**OBJECTIVES :**

- 1.To Study the prevalence of anaemia among adolescent childrens in Telangana Social Welfare Residential Educational Institutions society by different stakeholders.
2. To know certain measures and techniques in the study
3. To analyse the current situation with the help of tools and techniques

**MATERIALS AND METHODS**

**Study design:** It is a situational-based qualitative study

**Studyarea:** The study is conducted at the Residential Institutions under the Telangana Social Welfare Residential Educational Institutions Society.

**Inclusion criteria:** principals who are working for Telangana social welfare residential educational institutions society

**Exclusion criteria :** principals who are working in private institutions are excluded . Studypopulation : principals working in the residential institutions which are under the Telangana Social Welfare Residential Educational Institutions society.

**Ethical consideration:** The opinion given by Ethical committee is justification of sample and written informed consent.

**Sample size:** Qualitative studies can reach saturation at relatively small sample sizes. 4-9 interviews can be conducted to reach saturation[41].

The sample size used in qualitative research methods is often smaller than that used in quantitative research methods. This is because qualitative research methods are often concerned with garnering an in-depth understanding of a phenomenon or are focused on meaning (and heterogeneities in meaning)—which are often centered on the how and why of a particular issue, process, situation, subculture, scene or set of social interactions.[42]

The number of people interviewed are 9 principals from 7 different districts of Telangana.

**Sampling technique :** All the principals who satisfy the above inclusion criteria will be included in the study,after obtaining a written or verbal informed consent clearly explaining the purpose significance ,use of study, expected benefits of the study and potential aims of the study and that no answer is taken into consideration.

The written informed consent consists of two questions, whether the principal understand the aims of the project and agree to it.

**METHODOLOGY**

Anaemia happens when you do not have enough red blood cells.The cells travel with iron and haemoglobin, which is a protein that helps carry oxygen through the blood stream to your organs all through the body.In the country India,Anaemia is the most common deficiency of iron which causes the occurrence of many maternal deaths,still births,increase the rate of mortality and motality of the infants during labour period.Anaemia is mostly seen in adolescents girls.

To know the gap and root causes of anaemia in adolescents girls studying in Telangana Social Welfare Residential Educational Institutions Society (TSWREIS) the following methods has been conducted in this study.

1. Identification of selected districts - 33 districts of Telangana are graded as good, moderate, poor for prevalence of anaemia based on NFHS-5 indicators, 20% from each is selected to form a target district.

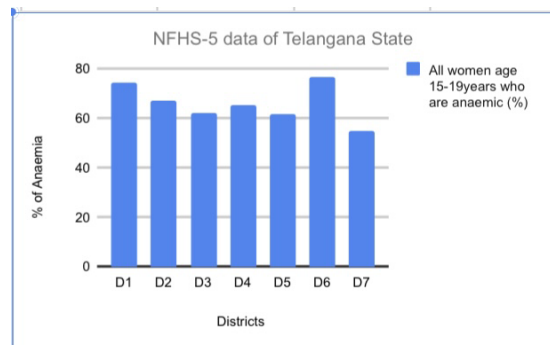


Figure-3

2. Approach to stakeholders( PRINCIPALS)-obtaining an informed consent on securing information from stakeholder.
3. A detailed interview was conducted with the stakeholder(principals of selected districts) by Focus group discussions (FGDs) method.
4. Recordings were taken as needed for non-verbal observations
5. The stakeholders were generally probe to discuss topics related to Anaemia monitoring ,individual perceptions , procedures,treatment and care ,prevention and practices, suggestions and innovations. From this discussions,we got to know about their roles and responsibilities and the initiatives they have adopted in the schools. The challenges they faced were also asked and noted.
6. The interview conducted with the stakeholders by FGD method helped in to know the right cause of anaemia in adolescents girls and boys.
7. The interviews were expected to last between 15 questions. Only researchers and the study participants present for the interviews
8. Semi-structured,open-ended exploratory questions (designed to address the research question) were used to obtain relevant context information.
- 9.Responses were recorded by audio recording device and hand-written field notes to capture each response to fullest.
10. After completion of the interview,the information is gathered based on the current situation provided by the stakeholders.
11. The data was recorded and transcribe into a word document and the risk factors was noted in a excel sheet.
12. All the data was analysed using qualitative analysis by tools (interventions , innovations )
13. Conceptualise the information by the data gathered on the current prevalence.
14. Transcripts are readed by researchers and new information is obtained, and data saturation is reached.
15. Then qualitative analysis is done identifying the Strengths, Weakness, Opportunities and Threats - SWOT analysi

## RESULT

As the interview is completed with the stake holders (principals),the study got many different answers form the respondents.The data collected is divided into three parts-identification of anaemia, management of anaemia ,and follow up.The study is also applicable for boysstudents so a stakeholder from boys schools said there is no cases of anaemia but some may rarely show sickle cell anaemia cases.The results are then followed by answers quoted by the stakeholders (respondents) their problems and a probable solutions are given.

**CURRENT PRACTICES:** Ongoing current practices in Telangana social welfare residential educational institutions society are the following:



- Monthly check up of the students
- Students are ought to do morning exercises and yoga.
- Management of diet and providing supplementation of special food groups

#### **Early identification:**

From the Focus group discussion (FGD) which was conducted with the school principals, their roles and responsibilities were known in identifying anaemia in students, issues they are facing, their challenges, suggestions and innovations have to be made to eradicate anaemia in students. In the perspective of school principals, the identification test of anaemia in childrens is conducted before joining the school the parents were asked to do all the required blood test of students and handover the reports to the school principal from these they got to know the actual count of anaemic students and after the admission is done the students are screened and do weekly or monthly health checkups by the health supervisor present in the school. The students are easily identified by their physicals signs and symptoms like fatigue, weakness, poor academic performance and their clinical indications like pale skin color, sunken eyes, tongue and nails are easily screened to know the anaemia in childrens.

#### **Challenges:**

- The main problem is lack of knowledge and skills in identifying anaemic students in these particular stakeholders. Even when there are symptoms seen in the children, principals cannot identify the children with only the symptoms.
- Screenings are not done regularly which is one of the main reasons leading to delayed identification of anaemia.

#### **Probable Resolutions :**

- Training has to be given to the assistant care takers to know more about the symptoms, causes, complications and importance of proper management of anaemia in girl students.
- There should be standard operating procedures to empower anaemia.
- More screenings need to be done from either the PHC's, NGO's , or RBSK.

#### **MANAGEMENT:**

They conduct life saving group meetings (LSGs), Healthy Tuesday in the schools.

Special diet is given to the students who are anaemic. Principals will look after the students whether they are eating the food completely or not. They assigned mess leaders to check if the students are eating or wasting the food. They are leaders at each table to look after the students. Vegetables like beetroot, carrot, green leafy vegetables, potatoes, brinjals, cabbage are given. Fruits like pomegranate, apple, banana - given daily, watermelon, oranges and some seasonal fruits are given. Ragi java, peanuts, jaggery based food items, sesame seeds laddoo, chikkis are also given. Animal sources like egg - given daily, milk - given daily, chicken - 4 times in a month, mutton - 2 times in a month are also provided.

Many Principals mentioned that there were no such challenges they faced. One of them also mentioned that they will send the student to their house if they are severe and will only allow them once they check their blood report. Some mentioned that since HS are present, they will see through the students and HS inform principals to give a special diet to those students who are anaemic. Provision of healthy and nutritious diet to the students

- Intervening immunity booster ,inclusion of seasonal fruits, non-vegetarian food sources , iron-rich food sources
- Supplementing IFTs and folic acid tablets ,iron injection to the anaemic students
- Life saving groups (LSGs) meetings is conducting every week
- Blood transfusion is suggested by the doctors for the very severe anaemic students
- Anaemic students are identified by blood tests and their performance in the class
- There won't be anaemia cases seen in boy's school, but there will be some minor accidents and skin allergies are mostly found in boys and house teachers manage to take them to hospital
- Mental health of the students is also taken into consideration Challenges :
- Their academic performance gets disturbed
- Intake of iron supplements on empty stomach can be problematic and worsen the health of the students
- Students feel uneasy and tired, poor performance in academics
- Lack of interest by Students in food eating There is no knowledge to the students about

- anaemia, where they give up certain foods which are good sources of iron.
- Some students won't participate in any games/programmes.

#### **Probable Resolution :**

- There should be proper standard operating procedures for the management of these anaemic students.

#### **Follow up**

The follow up is conducted through meetings and interacting the students weekly once at the assembly. Healthy Tuesday and life saving groups meetings is also being conducted in the schools, counselling with parents and students, providing the knowledge of nutrition and initiating health programmes in the schools are done as a part of follow up. They look after the health reports of the students.

- In LSGs meetings the health status is discussed with the house teachers and health supervisors
- Balancing proper food maintenance and physical activity can improve the health of the students.
- There are camps held for every 2-3 months, a CBP is done to the students
- Special diet is provided including carrot, pomegranate, boiled egg, peanut chikki weekly
- once
- Every student will be given same diet but extra care is taken for anaemic students
- Counselling the students, educating and informing their parents about their health status
- Assistant care taker health supervisors are specially take care for anaemic students and
- look after their diet
- Challenges:
- Ignorance from parents due to poor family background
- Their academic performance gets disturbed
- Students feel uneasy and tired, poor performance in academics

#### **Probable Resolutions :**

- There should be some programmes creating awareness among students regarding anaemia, causes, symptoms, complications etc., which makes the students understand more about the condition.
- In the special diet, there should be enough iron rich foods. The students need to exercise daily by showing them some drills of exercising and also should create awareness about the importance of exercising daily.
- The students need to participate in some of the extra-curricular activities, games etc., to make them stay active and healthy.
- There should be awareness programmes on anaemia to inform the students about the disease condition so that the students themselves will wash their hands, cut their nails and will be clean and tidy.
- Some of the respondents suggested that they tell the students to eat proper, healthy and nutritious food, so that they are not affected with any diseases.
- One respondent suggested that they tell the students to be clean and tidy and make them exercise daily.

#### **Discussion**

The main aim of this study is to know the current practices and prevalence of anaemia among children belonging to Telangana Social Welfare Educational Institutional Society in the Principal perspective's. . With the data collected from Principals,, it is prominent that anaemia is prevalent among children during their adolescence and it has been increasing due to lack of awareness. As there is no proper standard operating procedures for the management of these anaemic students- principals are not able to identify whether the students are anaemic or not visually. As the number of screenings not done this year, there is no particular number of anaemic students. The results showed that screenings are not done regularly where there is no special care given to those who are anaemic. As we all know that anaemia is a severe public health problem, we need to stop the prevalence at the very starting stage among the children. In future, these children will grow upto adults which causes complications in their maternal health and their offspring's health if they are anaemic. So the right way

to avoid the prevalence is firstly to create awareness among the students. To make them understand about the causes, symptoms and complications of the disease condition. To guide them about the foods rich in iron. To make them wash their hands regularly to avoid any infection. Exercise daily to be active and healthy. To make sure the students eat all kinds of fruits and vegetables. More screenings are to be done by the PHC's, NGO's, RBSK team to identify the students who are anaemic.

## CONCLUSION

The aim of the study is to know the certain gaps in the prevalence of anaemia in adolescents children studying in the residential school of Telangana state.

From these study it is concluded that the followings reasons:

- There are many schemes initiated by the government but no proper standard operating procedures is followed.
- The students are not screened regular for the identification of anaemia.
- Problem with the identification and diagnosis of anaemia
- PHC advises is not properly taken up by the incharges, negligence and in consent by parents

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मध्य भारती

मानविकी एवं समाजविज्ञान की द्विभाषी शोध-पत्रिका



# मध्य भारती

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संरक्षक

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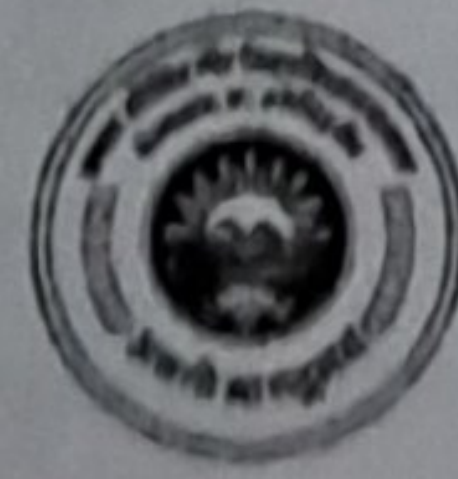
प्रो. अम्बिकादत्त शर्मा

सम्पादक

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**Introduction:**

Learning is the best path towards a successful and happy life, as it is conducive to a better understanding of life, and all acquired knowledge can be used towards fulfilling one's ambition. The magnitude of the importance of education in life is huge as well as multi-fold that helps to refine our character, strengthen morality, broaden the outlook and it prepares a nation for a better tomorrow. Well defined and futuristic education policy is essential for a country at school and college levels to be implemented by the governments in order to shape the future of the nation for its overall development.

A new chapter in education policy has begun after Indian independence. The implementation of previous policies on education has focused largely on issues of access and equity. The unfinished agenda of the National Policy on Education 1986, modified in 1992 (NPE 1986/92), is appropriately dealt with in this policy. A major development since the last policy of 1986/92 has been the Right of Children to Free and Compulsory Education Act-2009 which laid down legal underpinnings for achieving universal elementary education.

The National Education Policy (NEP) - 2020 replaces the National Policy on Education of 1986. In January 2015, a committee under former Cabinet Secretary T. S. R. Subramanian started the consultation process for the New Education Policy. Based on the committee report, in June 2017, the draft NEP was submitted in 2019 by a panel led by former Indian Space Research Organization (ISRO) chief Krishnaswamy Kasturirangan. The Draft New Education Policy - 2019 was later released by Ministry of Human Resource Development, followed by a number of public consultations. After a gap of 34 years, the Indian government consolidated feedback from 2.5 lakh village-level stakeholders to two national parliamentary level committees, over more than 50 months of consultations and workshops.

**National Education Policy-2020:**

National Education Policy 2020 is the first education policy of the 21st century and aims to address the many growing developmental imperatives of our country. This Policy proposes the revision and revamping of all aspects of the education structure, including its regulation and governance, to create a new system that is aligned with the aspirational goals of 21st century education, including Sustainable Development Goal, while building upon India's traditions and value systems. NEP lays particular emphasis on the development of the creative potential of each individual. It is based on the principle that education must develop not only cognitive capacities - both the 'foundational capacities' of literacy and numeracy and 'higher-order' cognitive capacities - such as critical thinking and problem solving - but also social, ethical, and emotional capacities and dispositions.

Built on the foundational pillars of Access, Equity, Quality, Affordability and Accountability, this policy is aligned to the 2030 Agenda for Sustainable Development and aims to transform India into a vibrant knowledge society and global knowledge superpower by making both school and college education more holistic, flexible, multidisciplinary, suited to 21st century needs and aimed at bringing out the unique capabilities of each student.

The Union Cabinet approved the National Education Policy (NEP) in July 2020 under the Prime Ministership of Narendra Modi, government of India. This policy will usher in sweeping changes to the education policy of the country, including a renaming of the Ministry of Human Resource Development as the Education Ministry. The Union Cabinet has approved the new National Education Policy 2020 with an aim to introduce several changes in the Indian education system - from the school to the college level.



- > Its aims at making "India a global knowledge superpower".
- > The Cabinet has also approved the renaming of the Ministry of Human Resource Development to the Ministry of Education.
- > The New Education Policy cleared by the Cabinet is only the third major revamp of the framework of education in India since independence.
- > The two earlier education policies were brought in 1968 and 1986.

**Background of NEP-2020:**

- The Ministry of Human Resource Development formed a committee chaired by Dr.K.Kasturirangan for preparing the National Education Policy. The Committee was constituted in June 2017. The Committee submitted its report on May 31, 2019. The National Policy on Education covers elementary and university education in urban as well as rural India.
- > The very first policy for education was promulgated in 1968 with the second one following in 1986.
  - > The first NEP was based on the recommendations of the Education Commission (1964-66). This policy sought to have a 'radical restructuring' of India's educational system and equalizing opportunities for education for all, to accomplish national integration and better economic and cultural development.
  - > The NEP also called for realizing compulsory education for every child until the age of fourteen, as mentioned in the Indian Constitution.
  - > It also aimed at providing enhanced training and improving teachers' qualifications

**Recommendations of National Education Policy-2020:**

- > **Early Childhood Care and Education:** The NEP-2020 recommended that early childhood care & education be developed in a two-part curriculum consisting of:
  - i. Guidelines for Parents & Teachers of students up to 3 years of age
  - ii. An educational framework for students between the ages of 3-8 years.

The NEP talks about the implementation of these recommendations by expanding and improving the quality of the Anganwadi system and co-locating them with primary school.

- > **Right to Education Act, 2009:** The NEP recommended extending the range of the Right to Education Act,2009 to include the following education levels:

- i. Early Childhood & Secondary School
- ii. Secondary School

This will allow coverage of RTE to all children between the ages of 3-18 years. In addition, it suggested the elimination of detention of children until class eight.

- > **Curriculum Framework:** Reforms in the framework of the current curriculum of school education are based on the development needs of the students. The NEP recommends the 5-3-3-4 pattern explained in the table below:

Years	Stage	Curriculum
5	Foundational	3 years of pre-primary followed by class 1 and 2
3	Preparatory	Classes 3 to 5
3	Middle	Classes 6 to 8
4	Secondary	Classes 9-12

- > **School Exam Reforms:** Reforms in the school exam recommended by the NEP include tracking the progress of the students throughout their school experience.
  - i. It includes State Census Exams in class 3, 5 and 8.



- ii. Another important recommendation was the restructuring of the 10th board exam 2022 would mainly focus and test only the skills, core concepts and higher-order thinking & capacities.
- **Regulatory Structure and Accreditation of Higher Educational Institutions:** In terms of Accreditation and Regulatory structure, the NEP recommended the following changes:
  - i. Setting up NHERA (National Higher Education Regulatory Authority),
  - ii. Separating NAAC from UGC into an autonomous and independent body.
- **National Research Foundation:** In order to improve the quality of research in India, the NEP recommended:
  - i. Establishment of a National Research Foundation.
  - ii. It would be an autonomous body that would administer the mentoring, funding and capacity building for quality research in India
- **Education Governance:** The NEP recommended establishing an apex body for education headed by the Prime Minister under the name Rashtriya Shiksha Aayog or National Education Commission. It also suggested changing the name of the Ministry of Human Resources & Development to the Ministry of Education
- **Financing Education:** Doubling the public investment for education was one of the important recommendations of the NEP 2020.
  - i. NEP 2020 insisted on the expenditure of 6% of the GDP on education.
  - ii. Doubling the current 10% of total public expenditure to 20% in the next decade was recommended.
- **National Mission on Education through Information and Communication Technology:** The NEP suggested setting up an autonomous body that would facilitate decision making on the deployment, induction and use of technology. NEP said that this would be achieved by implementing the following measures:
  - i. Establishment of National Education Technology Forum. The recommended autonomous body would be administered under this mission.
  - ii. It will also include virtual laboratories in various disciplines providing remote access.
- **Vocational Courses:** Recommendations of NEP 2020 with respect to Vocational courses can be listed as follows:
  - i. Students in classes 9 to 12 must receive vocational education on at least one vocation.
  - ii. Schools should build expert curriculum delivery methods that are aligned with National Skills Qualifications Framework (NSQF) competency levels.
  - iii. Higher Education Institutes must also provide vocational courses that are integrated into undergraduate education programmes.
- **Three Language Formula:** The Policy recommended that the three-language formula be continued and flexibility in the implementation of the formula should be provided. The three-language formula states that state governments should adopt and implement the study of a modern Indian language, preferably one of the southern languages, apart from Hindi and English in the Hindi-speaking states, and of Hindi along with the regional language and English in the non-Hindi speaking states.

**Challenges of NEP - 2020:**

- **Opening universities every week is a herculean task:** Doubling the Gross Enrollment Ratio in higher education by 2035 which is one of the stated goals of the policy will mean that we must open one new university every week, for the next 15 years which is an undoubtedly massive challenge.
- **The numbers are no less daunting in reforms to our school system:** The National Education Policy 2020 intends to bring 2 crore children who are currently not in schools back into the school system. Whichever way you look at it, accomplishing this over 15 years requires the setting up of around 50 schools every week.



states even if the medium of education for children is not in mother tongue or regional language.

➤ **Funding is a big challenge in the Covid era:** The NEP-2020 envisages an increase in education spending from 4.6% to 6% of GDP, which amounts to around INR 2.5 lakh crores per year. What makes things tricky is that this policy comes into being at a time when the economy has been battered by Covid-19 related lockdowns, government tax collections are abysmally low, and the fiscal deficit was high even pre-Covid.

➤ **Current focus on healthcare and economic recovery to lower the execution speed:** Economists have been calling for large stimulus packages amounting to double-digit percentages of GDP, despite the strain on the exchequer.

➤ **Need to create a large pool of trained teachers:** In school education, the policy envisages a sweeping structural re-design of the curriculum a very welcome step. But in order to deliver this curriculum effectively, we need teachers who are trained in and understand the pedagogical needs. Many of the curricular changes require substantial mindset shifts on the part of teachers, as well as parents.

➤ **Inter-disciplinary higher education demands for a cultural shift:** In higher education, the National Education Policy 2020's focus on inter-disciplinary learning is a very welcome step. Universities, especially in India, have for decades been very silo-ed and departmentalized. The NEP-2020 has many initiatives to improve the quality and the broadness of the education system in India. The objectives of this study on National Education Policy 2020 are:

- a. To highlight and overview the policies of the newly accepted higher education system (NEP 2020).
- b. To compare National Education Policy 2020 with the currently adopted policy in India.
- c. To identify the innovations in new national higher education policy 2020.
- d. To predict the implications of NEP 2020 on the Indian higher education system.
- e. To discuss the merits of Higher Education Policies of NEP 2020.
- f. Suggestions for further improvements for the effective implementation of NEP 2020 to realize its goal.

#### Opportunities of NEP 2020:

New education Policy begins with the unfinished agenda of NEP-1986. NEP-1986 was rooted in a very different India. Over the years, remarkable strides have been made in terms of access and equity. Near universal levels of enrolment at primary levels, and subsequent increase in enrolment at higher education levels (GER: 26.3%) have been achieved. However, there has also been a drop in the quality of learning in public school systems, followed by an exodus of elite and middle classes. This also led to the weakening of accountability mechanisms. Despite poor returns on learning, the pay-structures in public systems have seen a gradual increase.

#### School Education:

- a. Revamping of 10+2 structure to 5+3+3+4. New pedagogical and curricular structure to include pre-primary years. It's a good departure as this was ignored in education policy documents, and referred to in informal sense.
- b. NCERT will focus on the development of new curricular and pedagogical structure for ECCE. Policy also delves deep into the development and training of Anganwadi trainers through short-term and long-term programs. A positive thrust towards formalisation, of ECCE structure and delivery.
- c. Focus on attaining foundational numeracy and literacy by grade 3. Ministry of Education will strengthen this, and run it in a mission mode through a separate national mission.



- d. A separate national book policy to develop libraries around the country and instill love of reading in children. Public libraries in India are scarce. If this could be strengthened through the public education policy, it's a plus.
- e. Mid-day meals to see an upgradation in nutrition component, wherever possible, local alternatives to be provided. Eggs are still a contentious policy issue; policy plays it safe by steering clear to avoid any unnecessary controversy.
- f. Design of programs and interventions to alleviate issue of dropouts in conjunction with the Ministry of Social Justice and Empowerment.
- g. Medium of instruction section for some reason has received lot of undue attention. However, the section remains fairly flexible to avoid all sort of controversy. Half-baked understanding and market push towards English and paternal perception of 'quality' could've led to this flexibility. Policy also doesn't thrust/force/prefer any particular language over the other and encourages learning multiple languages. It also recommends teaching foreign languages at secondary level, Korean, Japanese, Thai, French, German, Spanish, Portuguese, and Russian.
- h. Policy inserts a new term called SEDGs (socio-economically disadvantaged groups). This hitherto hasn't been used as a social category in technical documents. Though later sections highlight categories as caste, tribe, disability, transgender and have passing references to term minority. Technical criticism aside, policy envisages ample initiatives to be targeted at these groups to increase enrolment and retention.
- i. PARAKH, a new body to focus on assessments as NAS (National Achievement Survey) and SAS (State Achievement Survey). PARAKH could be an important instrument to look at learning gaps and support targeting of various ministry goals and programs.

#### Higher Education:

It's important to view the policy in context of what has been happening in public universities, and recent debacle of universities of eminence. There has been continuous erosion of university autonomy by the state. Though the document highlights regulatory autonomy, it would be worrisome if the document also meant financial autonomy.

- This 'imagined' autonomy is envisaged through replacement of UGC (University Grants Commission) and AICTE (All India Council for Technical Education). It would be body Higher Education Commission of India is based on the idea of division of functions and separation of activities.
- Policy also argues against commercialization of education. New breadth allows for foreign universities to come to India. However, in the same increase in number of private universities to come to India. There has been significant increase competition, it makes sense. However, insertion of the statement doesn't integrate technology in institutions is a necessary direction.
- National Research Foundation in institutions is another great idea. However, if these spaces get filled by individuals who are driven by ideological agendas, little could be expected.
- Indian Universities will be allowed to set up campuses elsewhere in the world there is a strong potential for this to develop in gulf-markets. There is a huge demand for quality education by Indian diaspora.

#### Suggestions for New Education Policy - 2020:

- Its implementation must contribute to improved realization of the RTE Act, lead to the strengthening of public education.
- State and national governments should push the envelope on ensuring educational equity and non-discrimination, address the needs of children below six and above 14, confront privatization and commercialization of education, tackle the rising commercialization of education, and stem the rising commercialization of education.



- tools.
- They must put in place a change management process for the upcoming governance reforms and strengthen the administrative backbone of the education system.
- Further, the government must ensure adequate resources for its implementation and amplifying mechanisms for citizen participation.
- Improve public schools for quality and social integration
- To improve the schooling system, budgetary allocations have to be increased.
- The next logical step for government should be to universalize secondary education.
- To implement NEP successfully at all levels the government will need to create stakeholder incentives so that the implementation is smooth and uniform.
- Formulate instruments in the form of legal, policy, regulatory and institutional mechanisms
- Build reliable information repositories
- Develop adaptability across Higher Education Institutions, regulatory bodies and government agencies
- Develop credibility through transparent actions and participation of all stakeholders;
- Develop sound principles of management

**Conclusion:**

The new National Education Policy – 2020 which has been approved by the central government to change the Indian education system to meet the needs of 21<sup>st</sup> century India, if it is implemented successfully, this new system will make India one of the world's leading countries. The aim of the NEP-2020 which came after 34 years, is to provide higher education to all students, which aims to universalize pre-primary education by 2025. NEP-2020 is marching towards achieving such objective by making innovative policies to improve the quality, attractiveness, affordability, and increasing the supply by opening up the higher education for the private sector and at the same time with strict controls to maintain quality in every higher education institution. By encouraging merit-based admissions with free-ships& scholarships, merit & research based continuous performers as faculty members, and merit based proven leaders in regulating bodies, and strict monitoring of quality through biennial accreditation based on self-declaration of progress through technology-based monitoring, NEP-2020 is expected to fulfil its objectives by 2030.

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# Arduino and UV Sensors Embedded Assistive Hat for Visually Impaired

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## Abstract:

The World Health Organization (WHO) reported that there are 285 million visually-impaired people worldwide. Among these individuals, there are 39 million who are totally blind. There have been several systems designed to support visually-impaired people and to improve the quality of their lives. In this paper, we present an Arduino and UV sensors embedded hat to provide a 3-dimensional guidance for the blind which is wearable and portable in order to show the progress in assistive technology for this group of people. We thus contribute in assisting this population and highlight the improvements, advantages, disadvantages and accuracy. Our aim is to address and present to researchers in this area to design devices that ensure safety and self reliable mobility to visually-impaired people.

**Keywords:** assistive devices, visually-impaired people, obstacles detection, Arduino UNO , Ultrasonic sensor *HC-SR04*, vibrator.

## 1. Introduction

---

The World Health Organization (WHO) fact reported 100,000 students, who are visually impaired according to the American Foundation for the Blind [1,2] and National Federation for the Blind [3]. Over the past years, blindness that is caused by diseases has decreased due to the success of public health actions. However, the number of blind people that are over 60 years old is increasing by 2 million per decade. Unfortunately, all these numbers are estimated to be doubled by 2020 [4].

The need for assistive devices for navigation and orientation has increased. The simplest and the most affordable navigations and available tools are trained dogs and the white cane [5]. Although these tools are very popular, they are not wearable and adds to the discomfort of carrying by engaging one of the hands. The present work involves a wearable hat which is embedded with an Arduino UNO and three ultrasonic sensors to provide a 360 degrees pseudo view to avoid an obstacle hitting the person who is wearing it.

### 1.1. Assistive Technology

All the systems, services, devices and appliances that are used by disabled people to help in their daily lives, make their activities easier, and provide a safe mobility are included under one umbrella term: assistive technology [6].



In the 1960s, assistive technology was introduced to solve the daily problems which are related to information transmission (such as personal care) [7], navigation and orientation aids which are related to mobility assistance [8,9,10].

Visual assistive technology is divided into three categories: vision enhancement, vision substitution, and vision replacement [10,11]. This assistive technology became available for the blind people through electronic devices which provide the users with detection and localization of the objects in order to offer those people with sense of the external environment using functions of sensors. The sensors also aid the user with the mobility task based on the determination of dimensions, range and height of the objects [6]

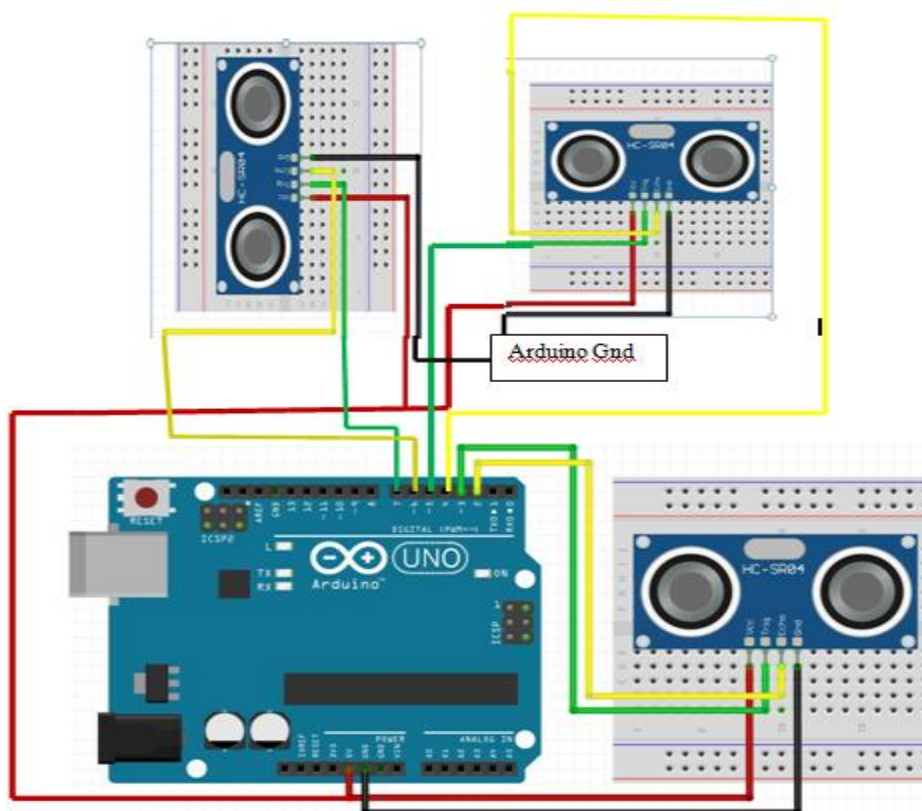
## 2. Components and Specifications

### 2.1. Physical Assembly

Figure 1a shows the schematic of the circuit employed in the assistive hat along with the components namely ultrasound sensors, Arduino Nano, vibrators and buzzer. It also comprises of the battery holder and a reset button . The circuit design is implemented in Proteus simulator and the Arduino nano is programmed in embedded C using the Arduino IDE 1.8.13

### 2.2. Circuitry Design and Implementation

The Arduino Nano [8] microcontroller, in which the copper wires from the sensor pins of the ultrasonic sensor (Figure 2a), and reset button were soldered with a 9 V battery supplying 3.3 to 5 V to the circuit (Figure 1a).



*Figure 1a Circuit Schematic showing the Arduino UNO microcontroller and UV sensors*



*Figure 1b & 1c The physical picture of the assistive hat and consisting of: Ultrasonic sensor HC-SR04, reset button, Arduino Nano board, vibrators and buzzer.*

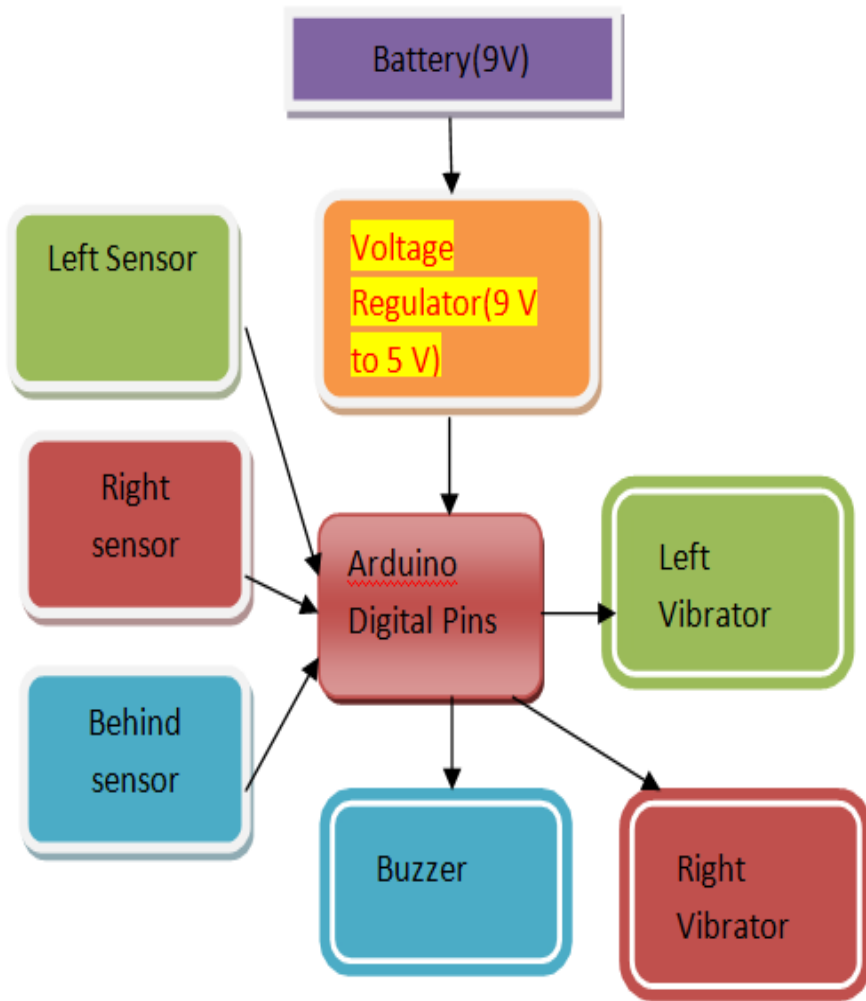
### 3 Experiment

To make an assistive hat for the blind we used an Arduino Uno, 3 Ultrasonic sensors, 2 vibrators. An ultrasonic sensor has trigger and echo pins as shown in figure 1c. The first sensor echo pin is connected to Arduino digital pin number 2 and the Trig is connected digital Pin 3. The second sensor echo is connected to Arduino digital pin number 4 and the Trig is connected to digital Pin 5. Similarly, the third sensor echo is connected to Arduino digital pin number 6 and the Trig is connected digital Pin 7. Left vibrator Vcc is connected to digital pin 9 and ground to the Arduino ground. Similarly right vibrator Negative is connected to digital pin 10 and ground to Arduino ground. The third sensor output is given to a buzzer in order to differentiate the direction of the obstacle. After making all these connections our circuit is ready. The code using embedded C language is developed in Arduino IDE 1.8.13 and uploaded using the USB to AB cable in order to dump into the Arduino board.

#### 3.1 Working

Ultrasonic sensors send ultrasonic waves. These waves are absolutely invisible and come back after hitting an obstacle there by activating the Trig pin of the respective sensor. The Trig pin activates (D3 or D5 or D7) to which a left vibrator, right vibrator and a buzzer are connected and thereby gets activated through embedded C program. The obstacle closeness is fixed to 10 cm so that the buzzer or the vibrator gets activated depending on their directional position if the obstacle comes closer than this distance.





*Figure 1d Flowchart of the algorithm fed into the Arduino UNO*

## Arduino Sketch for the assistive Hat

```

HAT_code $
const int trigPin_1=2;
const int echoPin_1=3;
const int trigPin_2=4;
const int echoPin_2=5;
const int trigPin_3=6;
const int echoPin_3=7;
long duration;
int distanceleft;
int distanceright;
int distancebehind;
void setup() {
  // put your setup code here, to run once:
  Serial.begin(9600);
  pinMode(trigPin_1,OUTPUT);
  pinMode(echoPin_1,INPUT);
  pinMode(trigPin_2,OUTPUT);
  pinMode(echoPin_2,INPUT);
  pinMode(trigPin_3,OUTPUT);
  pinMode(echoPin_3,INPUT);
}

void loop() {
  // put your main code here, to run repeatedly:
  digitalWrite(trigPin_1, LOW);
  delayMicroseconds(2);
  digitalWrite(trigPin_1, HIGH);
  delayMicroseconds(10);
  duration =pulseIn(echoPin 1,HIGH);

Done compiling.

Sketch uses 2918 bytes (9%) of program storage space. Maximum is 32256 bytes.
Global variables use 190 bytes (9%) of dynamic memory, leaving 1858 bytes for local variables. Maximum is 2048 bytes.

HAT_code $
if(distanceleft<10)
{
  digitalWrite(2,HIGH);
  digitalWrite(3,HIGH);
}
else
{
  digitalWrite(2,LOW);
  digitalWrite(3,LOW);
}

if(distanceright<10)
{
  digitalWrite(4,HIGH);
  digitalWrite(5,HIGH);
}
else
{
  digitalWrite(4,LOW);
  digitalWrite(5,LOW);
}
if (distancebehind<10)
{
  digitalWrite(6,HIGH);
  digitalWrite(7,HIGH);
}

Done compiling.

Sketch uses 2918 bytes (9%) of program storage space. Maximum is 32256 bytes.
Global variables use 190 bytes (9%) of dynamic memory, leaving 1858 bytes for local variables. Maximum is 2048 bytes.

```

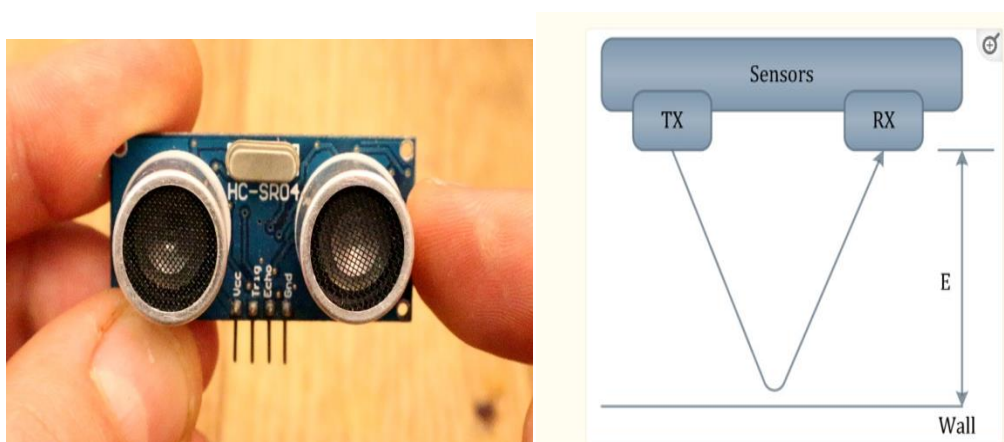


## 4 Sensor considerations

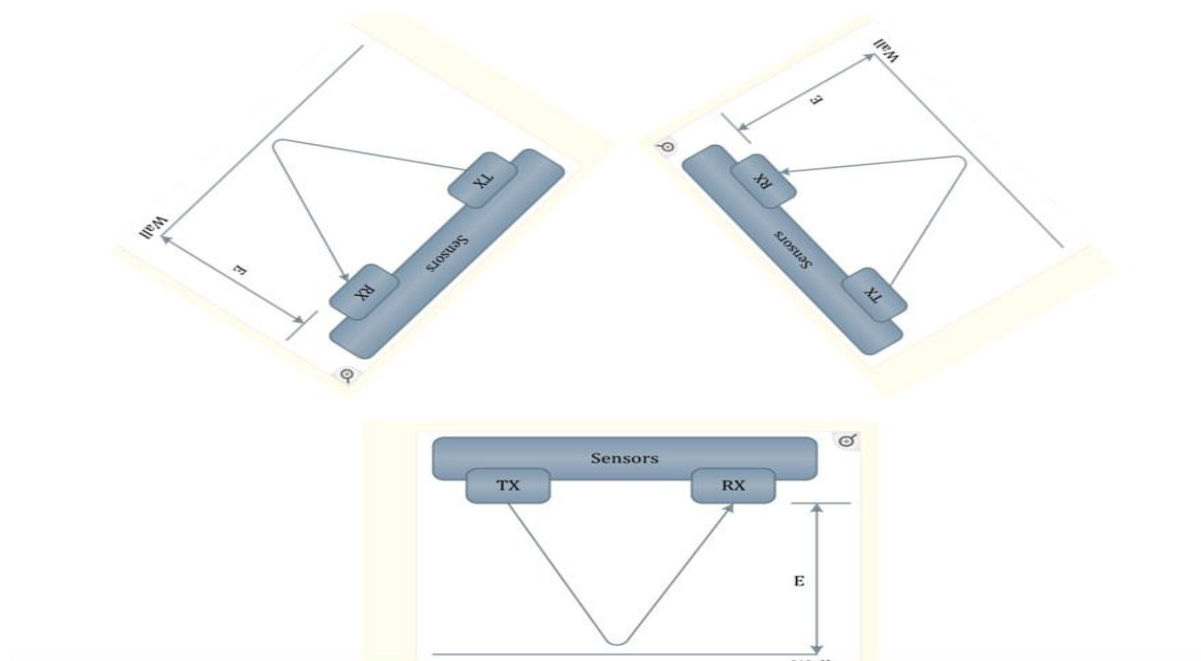
### 4.1. Ultrasound Sensor

The ultrasound sensor (figure 2a) consists of a transmitter sending an ultrasound wave and a receiver detecting the reflected wave by the targeted physical object. The time taken between the transmission and detected wave is registered for the calculation of the distance from the speed of ultrasound waves at 330 m/s by the programmed Arduino Nano.

The sensors sends a sequence of ultrasonic pulses. If the obstacle is detected, then the sound will be reflected back to the receiver as shown in Figure 2b. The micro-controller processes the readings of the ultrasonic sensors in order to activate the vibrators by sending pulse width modulation. It also provides a low power consumption [15]. Figure 2c shows the arrangement of the three sensors embedded in to the wearable hat in order to have a 360<sup>0</sup> obstacle finding range



*Figure 2a and 2b Ultrasonic sensor HC-SR04 component range of signal*



**Figure 2c Arrangement of the three sensors within the Hat for a 360 degrees view**

## 5 Results and Discussions

The experiment is performed on 10 undergraduate students by covering their eyes in order to study the accuracy of the unit. The observations were recorded by placing an obstacle of 3 to 4 ft height on a concentric circle of radius 12 cm and then of 10 cm. The observations were found to be quite satisfactory where in the buzzer alarmed and the vibrator gave a signal depending on the position of any obstacle for this distance giving a feel of presence of an obstacle for the subject wearing the assistive hat.

## Conclusions

We thus conclude the successful working of a prototype of an assistive hat for visually handicapped people which is economical, long lasting, reliable and portable at the comfort of just wearing as a hat there by enhancing their physical appearance.

## Acknowledgements

The authors acknowledge the sincere support obtained from the Head of the Institution and also the Department of Physics and Electronics, St. Ann's college fro Women, Mehdiapatnam, Hyderabad for their constant support in completing the project.

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Theoretical Article | Published: 16 December 2022

# Duality for fractional interval-valued optimization problem via convexificator

B. Japamala Rani & Krishna Kumhari

OPSEARCH 60, 481–500 (2023) | [Cite this article](#)

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## Abstract

In the current study, non-differentiable fractional interval-valued optimization problem (NFIVP) is studied by considering generalized invex functions. By using the optimality conditions, we investigate both types of dual problems namely Wolfe type and Mond-Weir type duals for (NFIVP) concerning invexity. Moreover, the suitable duality theorems are ascertained for both types of dual problems. Numerical examples are deliberated to substantiate the present work.

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## Research Article

Gurram Beulah, Divya Deepthimahanthi, Gullipalli Simhachalam, Anjani Devi Chintagunta\*, Machiraju Venkata Naga Sravya, and Nune Satya Sampath Kumar\*

# Purification and characterisation of phytochemicals extracted from *Rhizophora mucronata*: Their efficacy against *Pseudomonas aeruginosa* infection in *Catla catla*

<https://doi.org/10.1515/revac-2022-0050>

received December 28, 2021; accepted November 02, 2022

**Abstract:** The aim of this work is to purify and characterise bioactive molecules from various solvent extracts prepared with the leaves of *Rhizophora mucronata*, a mangrove plant. *In vitro* assessment of antibacterial activity of *n*-hexane, diethyl ether, ethanol, and aqueous extracts (AEs) against *Bacillus subtilis* ( $0.4 \pm 0.1$ ,  $1.1 \pm 0.3$ ,  $0.9 \pm 0.1$ , and  $1.2 \pm 0.7$  mm), *Staphylococcus aureus* ( $1.2 \pm 0.1$ ,  $2.3 \pm 0.5$ ,  $3.2 \pm 1.2$ , and  $4.4 \pm 1.7$  mm), *Klebsiella pneumoniae* ( $0.5 \pm 0.3$ ,  $1.7 \pm 0.4$ ,  $3.9 \pm 1.4$ , and  $3.9 \pm 0.9$  mm), and *Pseudomonas aeruginosa* ( $0.2 \pm 0.1$ ,  $2.5 \pm 0.6$ ,  $3.7 \pm 0.5$ , and  $4.2 \pm 1.3$  mm) has proven to act as potent similar to that of commercial antibiotic. Corresponding to antibacterial activity result, antioxidant activity was also higher for AE ( $75.97\% \pm 0.5\%$ ) compared to remaining extracts ( $73.77\% \pm 2.5\%$ ,  $69.99\% \pm 0.4\%$ , and  $65.24\% \pm 1.8\%$ ) at a concentration of  $500 \mu\text{g}\cdot\text{mL}^{-1}$ . Considering both the activities, AE was selected for separation of bioactive molecule by deploying two purification techniques – column chromatography

and preparative thin layer chromatography consecutively. After purification, the sample was injected into mass spectrometer and the presence of *N-p*-coumaroyltyramine and luteolin with excellent antibacterial and antioxidant properties ( $13.1 \pm 2.2$  mm and  $80.9\% \pm 2.3\%$ , respectively) were recognised. Further, *in vivo* investigations on *Catla catla* fingerlings infected with *P. aeruginosa* indicated that partially purified extracts cured the infection and resulted in a 100% survival rate.

**Keywords:** *Catla catla*, *Rhizophora mucronata*, column chromatography, mass spectrometry

## 1 Introduction

Aquaculture is an important sector in India as it meets food security and employment by contributing 1.07% in national GDP and 5.30% in agricultural GDP [1]. Freshwater aquaculture accounts for 95% of 5.77 million tons of aquaculture production according to FAO 2021 [1]. Techniques like polyculture, induced carp breeding, composite carp culture on Indian major carps and exotic carps led to the development of freshwater aquaculture. But on the other hand, they became a major bottleneck as they induces stress among cultured fishes which makes them more susceptible to diseases [2].

Bacterial diseases in fishes are highly infectious and result in significant financial losses [3]. Traditionally bacterial infections are being treated with synthetic antibiotics which has escalated antibiotic resistance among various bacterial species. Moreover, biomagnification of residual antibiotics in the cultured fishes has also discouraged the farmers to continue the usage of synthetic antibiotics [4]. To counteract the situation, medicinal plants owing to their non-toxic and eco-friendly properties are being investigated as a possible treatment option

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for bacterial infections [5,6]. Bioactive compounds derived from medicinal plants such as alkaloids, tannins, flavonoids, steroids, saponins, and triterpenes have therapeutic potential to control pathogenic microbes in animals and plants with minimal side effects [7].

In India, there are around 1,600 mangrove plant species which are used as ethnomedicine to treat diseases like dysentery, diarrhoea, malaria, fever, bacterial infections, diabetics, and leprosy inflammation [8]. Among them *Rhizophora mucronata* is a small- to medium-sized tree found in Indo-Pacific region, belonging to the family Rhizophoraceae. To evaluate the medicinal properties of *R. mucronata* leaves, phytochemicals were extracted using various solvents. The extracted phytochemicals were purified using chromatographic techniques and characterised using mass spectrometry. Further, the efficiency of crude and partially purified extracts was assessed against *P. aeruginosa* infected *Catla catla* fingerlings.

## 2 Materials and methods

### 2.1 Plant material

*Rhizophora mucronata*, a mangrove plant was amassed from Gilakaladindi, Machilipatnam (16°10'N latitude and 81°10'E longitude), Andhra Pradesh, India. The specimen was identified and confirmed at the Botanical Survey of India (BSI/DRC/2016-17/Tech/204). Leaves of the plants were collected and rinsed with distilled water before processing. The leaves were then cut into small pieces, shade dried, pulverised to a fine powder, and stored in an airtight container.

### 2.2 Chemicals

Various chemicals of analytical grade such as acetic anhydride, ascorbic acid, acetonitrile, chloroform, diethyl ether, ethanol, ferric chloride, formic acid, glacial acetic acid, *n*-hexane, methanol, sodium hydroxide, sulphuric acid, and potato dextrose agar media were procured from Merck India Ltd.

### 2.3 Extraction of bioactive compounds

Solvents such as *n*-hexane, diethyl ether, ethanol, and distilled water have been individually used for the extraction of

phytochemicals from the leaves of *R. mucronata*. The leaf powder was mixed with solvent (1:10 w/v) in a Soxhlet device for 6 h. The extraction process using *n*-hexane, diethyl ether, ethanol and distilled water has been carried out at 70°C, 35°C, 80°C, and 100°C, respectively. The extracts were filtered using Whatman No. 1 filter paper, concentrated using rotary evaporator, and further screened for phytochemicals using standard protocols [7].

### 2.4 Antimicrobial and antioxidant activity

Antimicrobial activity of all the four extracts against *Bacillus subtilis* (MTCC 441), *Klebsiella pneumoniae* (MTCC 3384), *Staphylococcus aureus* (MTCC737), and *Pseudomonas aeruginosa* (MTCC 1688) was determined through agar well diffusion method. Approximately, 100 µL of the mentioned bacterial cultures (12 h) were spread uniformly on to the surface of the solidified medium and dried for 5 min. Further, wells of 6 mm diameter were created in the agar and 40 µL of each extract was loaded into the wells besides amikacin (20 µg·mL<sup>-1</sup>) as positive control. After incubation for 24 h at 37°C the antibacterial efficacy of *R. mucronata* leaf extracts was calculated by measuring the zone of inhibition around the well [9]. Similarly, the antioxidant activity of *R. mucronata* leaf extracts was determined using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging assay and ferric reducing antioxidant power (FRAP) assay.

For DPPH assay, the reaction mixture (5 mL) was prepared with DPPH solution at different concentrations of extract (31.25, 62.5, 125, 250, and 500 µg·mL<sup>-1</sup>). The mixture was incubated in dark for 30 min and absorbance was measured at 517 nm. Methanol and ascorbic acid have been used for the preparation of blank and standard, respectively [10]. DPPH radical scavenging activity of the given sample has been calculated using the following equation:

$$\begin{aligned} \text{\% DPPH radical scavenging activity} \\ = [(A_0 - A_1) / A_0] \times 100 \end{aligned} \quad (1)$$

where  $A_0$  is the absorbance of the control and  $A_1$  is the absorbance of the test samples.

For carrying out FRAP assay, a reagent was prepared by mixing 300 mM acetate buffer (25 mL), 10 mM 2,4,6-tri(2-pyridyl-s-triazine) (2.5 mL) solution, and 20 mM FeCl<sub>3</sub> solution (2.5 mL). All the four plant extracts (10 µL) and standard ascorbic acid at varying concentrations (15.62, 31.25, 62.5, 125, and 250 µL·mL<sup>-1</sup>) are added to FRAP reagent (200 µL) followed by vigorous mixing and incubation at 37°C for 15 min. Further, the absorbance of the



samples was measured at 593 nm and the results were represented as  $\mu\text{g}\cdot\text{mL}^{-1}$  [11].

## 2.5 Purification of *R. mucronata* leaf extract by column chromatography

Active extracts of *R. mucronata* was subjected to column chromatography where the cylindrical glass column was packed with silica gel of 100–200 mesh size (Merck India Ltd). After passing the extract through the column, elution was carried out with varied proportions of *n*-hexane chloroform mixture and chloroform methanol mixture. The components of the extracts are separated based upon polarity and the separated fractions are collected and labelled. The fractions with similar colour were pooled and tested for antibacterial and antioxidant activities. The active fractions were further purified using preparative thin layer chromatography (TLC).

## 2.6 Preparative TLC

Approximately 30 g silica gel and small amount of calcium sulphate were homogenously mixed with 60 mL of distilled water and spread uniformly on glass plates (20 cm × 20 cm) with 250  $\mu\text{m}$  thickness. The plates were placed at room temperature for 10 min, further incubated at 105°C for 1 h, and then placed in the desiccator for 2 h. The sample from active fraction of *R. mucronata* was spotted on the silica gel plate and then placed in a lidded tank containing butanol:acetone:water (12:6:3) solvent system. The sample travels along with the solvent due to the capillary action and eventually the components in the sample gets separated and forms bands which can be observed under the UV light at 254 nm. TLC was run for 20–25 times and bands were scrapped carefully from the plates and used for assessing the antibacterial and antioxidant activities.

## 2.7 Characterisation of phytochemicals by mass spectrometry

The phytochemicals present in the crude and partially purified extracts of *R. mucronata* were characterised by subjecting to Agilent 1100 LC/MS System with separate ChemStation Rev.A.09.01 (1206) software. Approximately

20  $\mu\text{L}$  of sample was injected along with the isocratic mobile phase (0.1% formic acid in 50% water and 50% acetonitrile) at a flow rate of 0.5  $\text{mL}\cdot\text{min}^{-1}$ . The electro-spray ionisation was set in negative ionisation mode at 60–200 V and capillary voltage at 4,000 V. Nitrogen is used as nebulising gas at 350°C and 30 psi with flow rate of 8–10  $\text{L}\cdot\text{min}^{-1}$  [12].

## 2.8 *In vivo* studies

### 2.8.1 Experimental design and confirmatory tests for *P. aeruginosa*

*Catla catla* fingerlings of  $8 \pm 0.5$  cm length and  $10 \pm 0.9$  g weight were procured from local hatchery and screened for infections. Before 10 days of experimentation, these fingerlings were maintained in aerated tubs containing 40 L of freshwater at  $28^\circ\text{C} \pm 2^\circ\text{C}$  under 12 h day light. The fishes were given pellet diet and nearly 30% of water was changed regularly so as to reduce the ammonia toxicity. The parameters such as pH, temperature, total dissolved solids, oxygen, electrical conductivity, total hardness, ammonia-nitrogen, nitrate-nitrogen, and orthophosphate that have influence on the growth of the fingerlings have been maintained as per the standards set by FAO [1], and Beulah et al. [13]. Then, these fingerlings have been divided into five groups, each group containing nine fingerlings. Group 1 is the control, group 2 is the negative control, group 3 is the positive control, group 4 is treated with crude extract of *R. mucronata*, and group 5 is treated with partially purified extract of *R. mucronata*.

To start the study, all the fishes were allowed to starve for 24 h, and then pellet diet containing *Pseudomonas aeruginosa* ( $10^3$  CFU, 1 mL) is fed twice to the all the groups except group 1 which was fed twice with regular feed. After 24 h of feeding, the groups 3, 4, and 5 were treated with amoxicillin, crude extract of *R. mucronata* ( $25 \text{ mg}\cdot\text{g}^{-1}$  of body weight), and partially purified extract of *R. mucronata* ( $10 \text{ mg}\cdot\text{g}^{-1}$  of body weight), respectively, along with feed for 5 days [1]. The residual feed was cleared off before addition of fresh feeding. During the experimental period, pathological lesions, behavioural changes, and mortality in the fishes were observed and noted carefully. Further, the microbes from the moribund fish fingerlings have been isolated and cultured on agar plates. The characteristics of the isolated microbes were studied through Gram's staining, catalase test [14], methyl red test, superoxide dismutase activity [15] indole test, gelatine hydrolysis test, and citrate utilisation test.

## 2.9 Statistical analyses

The experiments were performed in triplicates and the results were expressed as mean value  $\pm$  standard deviation. The results were statistical analysed using IBM SPSS statistics for windows, 20.0 software (IBM Corp., Armonk, NY, USA).

## 3 Results

### 3.1 Screening of secondary metabolites in *R. mucronata* leaf extracts

The crude extracts of *R. mucronata* leaves produced with *n*-hexane, diethyl ether, ethanol, and distilled water were subjected to preliminary phytochemical screening. Tannins are present in ethanol extract (EE) and aqueous extract (AE)

**Table 1:** Phytochemical screening of various solvent extracts of *Rhizophora mucronata*

Phytochemicals	<i>Rhizophora mucronata</i>			
	HE	DEE	EE	AE
Tannins	—	—	+	+
Saponins	+	—	+	+
Flavonoids	—	—	—	—
Glycosides	+	+	—	+
Terpenoids	—	—	—	+
Steroids	—	+	+	—
Phenols	—	—	—	+
Proteins	+	+	+	+
Phytosterols	—	—	—	+
Anthraquinones	—	+	—	+
Cardiac glycosides	+	+	+	+
Reducing sugars	—	—	—	+

HE – hexane extract, DEE – diethyl ether extract, EE – ethanol extract, AE – aqueous extract.

“+” indicates presence and “–” indicates absence.

**Table 2:** Antibacterial activity (mm) of crude *R. mucronata* leaf extract

Test organism	Amikacin	HE (mm)	DEE (mm)	EE (mm)	AE (mm)
<i>Bacillus subtilis</i>	1.9 $\pm$ 0.6	0.4 $\pm$ 0.1	1.1 $\pm$ 0.3	0.9 $\pm$ 0.1	1.2 $\pm$ 0.7
<i>Staphylococcus aureus</i>	5.1 $\pm$ 0.4	1.2 $\pm$ 0.1	2.3 $\pm$ 0.5	3.2 $\pm$ 1.2	4.4 $\pm$ 1.7
<i>Klebsiella pneumonia</i>	4.6 $\pm$ 0.01	0.5 $\pm$ 0.3	1.7 $\pm$ 0.4	3.9 $\pm$ 1.4	3.9 $\pm$ 0.9
<i>Pseudomonas aeruginosa</i>	4.3 $\pm$ 0.9	0.2 $\pm$ 0.1	2.5 $\pm$ 0.6	3.7 $\pm$ 0.5	4.2 $\pm$ 1.3

Results are expressed as mean value  $\pm$  standard deviation;  $n = 3$ .

and saponins are present *n*-hexane extract (HE), EE, and AE. Glycosides are available in HE, diethyl ether extract (DEE), and AE. Besides, cardiac glycosides and proteins are found in all the extracts. On the other hand, terpenoids and phenols are exclusively present in AE (Table 1). Further, these four extracts were considered for determination of antioxidant and antibacterial activities.

### 3.2 Antibacterial activity of *R. mucronata*

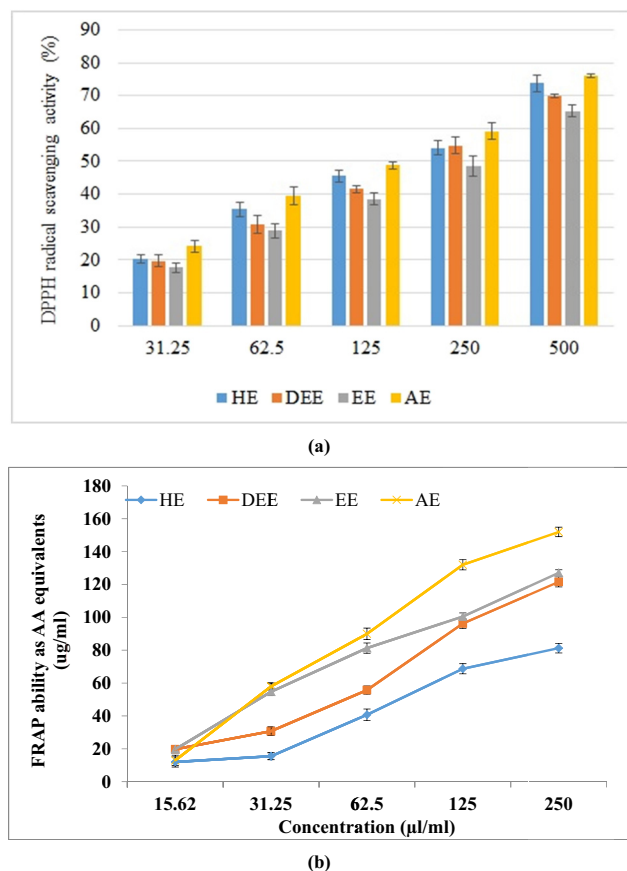
The antibacterial activity of all the four extracts of *R. mucronata* was assessed against *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. The antibacterial activity of HE was measured in the range from 0.2  $\pm$  0.1 to 1.2  $\pm$  0.1 mm, whereas DEE was measured in the range from 1.1  $\pm$  0.3 to 2.5  $\pm$  0.6 mm. Similarly, the bactericidal activity of EE ranged from 0.9  $\pm$  0.1 to 3.9  $\pm$  1.4 mm. Although all the extracts were effective in inhibiting the growth of the bacteria tested, the AE had the largest inhibitory zone against *Staphylococcus aureus* (4.4  $\pm$  1.7 mm), *Bacillus subtilis* (1.2  $\pm$  0.7 mm), *Klebsiella pneumoniae* (3.9  $\pm$  0.9 mm), and *Pseudomonas aeruginosa* (4.2  $\pm$  1.3 mm) and these values were in proximity with the activity of amikacin, a synthetic antibiotic (Table 2).

### 3.3 Antioxidant activity of *R. mucronata*

Using the DPPH radical scavenging and FRAP tests, the antioxidant activities of all four *R. mucronata* leaf extracts were studied at varied dosages. The DPPH radical scavenging activity was measured at concentrations ranging from 31.25 to 500  $\mu\text{g}\cdot\text{mL}^{-1}$ . The ability to scavenge radicals improves with concentration, the maximum activity (Figure 1) being 73.77%, 69.99%, 65.24%, and 75.97% for the 4 *R. mucronata* extracts at 500  $\mu\text{g}\cdot\text{mL}^{-1}$ .

FRAP assay for *R. mucronata* extracts has revealed excellent antioxidant potential ranging from 81.25  $\pm$  1.2





**Figure 1:** DPPH radical scavenging activity (a) and ferric reducing antioxidant power ability (b) of *R. mucronata* leaf extracts.

to  $152.3 \pm 2.3$  μg of ascorbic acid equivalents (AAE) per mL at  $250 \mu\text{L}\cdot\text{mL}^{-1}$  concentration for HE, DEE, EE, and AE (Figure 1). All the extracts showed increase in the activity with increasing concentration and least activity was recorded for HE and highest activity was recorded for AE. At  $15.62 \mu\text{L}\cdot\text{mL}^{-1}$  concentration, the AE was able to react with ferric tripyridyltriazine complex and convert it into a violet blue coloured ferrous tripyridyltriazine and the same was read as  $13 \pm 2.1$  μg AAE per mL of conversion. Whereas at the same concentration, DEE and EE were able to reduce  $19.75 \pm 2.3$  μg AAE per mL. But at high concentration, i.e.,  $250 \mu\text{L}\cdot\text{mL}^{-1}$  concentration these recorded lower value than AE.

### 3.4 Purification of AE of *R. mucronata*

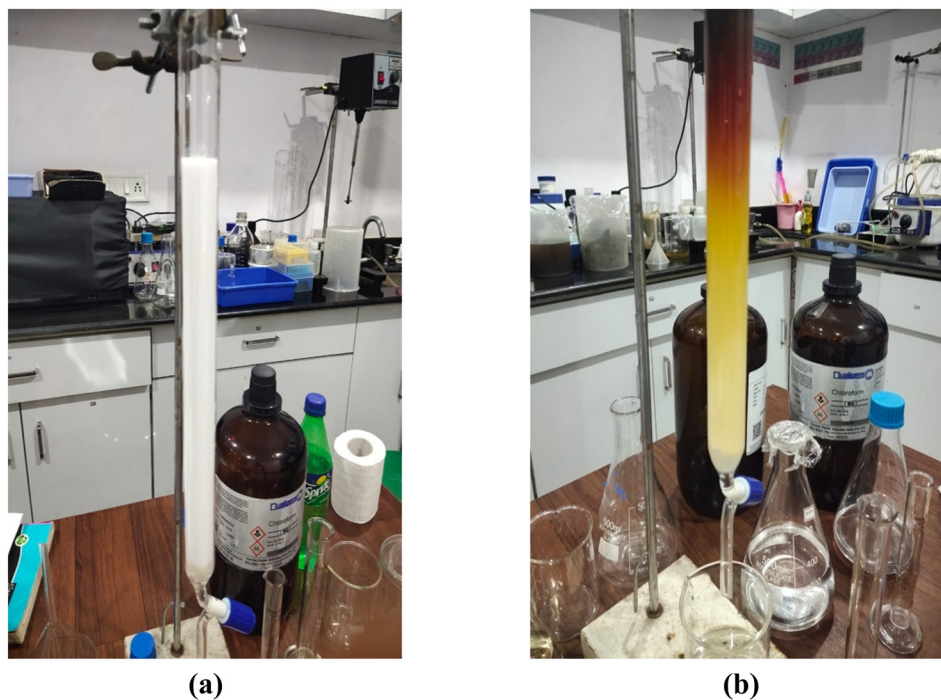
Using the mobile phase, AE of *R. mucronata* was loaded onto a glass column packed with silica gel and allowed to interact with the stationary phase (Figure 2). The molecules in the *R. mucronata* extract interacted efficiently and dispersed across the column in few hours. To enhance

the resolution of separation and improve degree of elution, two gradient systems were employed wherein initial elution is by *n*-hexane and chloroform, followed by chloroform and methanol as shown in Table 3. Higher polarity compounds need solvents of greater polarity and lower polarity compounds can be eluted with low polarity solvents. In the present study, elution was initiated with non-polar *n*-hexane and gradually shifted to its chloroform ratio and ended with methanol giving scope for elution of all polar compounds present in the extract.

Gradual change in the ratio of mobile phases has clearly separated compounds from crude mixture and each fraction based on colour shade difference was successfully pooled and labelled. Fractions collected from column chromatography were categorised into nine sets (A–I) and each fraction was assessed for antibacterial and antioxidant activity. Only *P. aeruginosa* was selected for determining antibacterial activity as *in vivo* studies are planned to challenge the healthy fishes with the same. So, upon testing nine fractions against *P. aeruginosa*, four fractions namely B, E, G, and H showed zone of inhibition in the range of 1.8–7.5 mm. Similarly, all the fractions were assessed for antioxidant activity using DPPH assay and 5 out of 9 fractions were successful in scavenging DPPH radical in the range of 11.5–67.9% (Table 3). On comparing both the results, fraction “G” had better results than remaining fractions. Hence, it was selected for further purification after repeating the column chromatography for couple of times to increase the quantity.

### 3.5 Final purification of extract by preparative TLC

Sample from fraction “G” collected from column chromatography was loaded onto the glass plates coated uniformly with silica gel and placed in the mobile phase chamber. The sample was separated gradually and each spot was pooled out and labelled as AR1, AR2, and AR3. TLC was repeated for number of times, each band was carefully scrapped and pooled together, and tested for antibacterial and antioxidant activities. After assessing, it was found that all the three fractions AR1, AR2, and AR3 have exhibited both antibacterial and antioxidant activities but with different potentials (Table 4). The antibacterial and antioxidant activities of AR1 are  $13.1 \pm 2.2$  mm and  $80.9\% \pm 2.3\%$ , respectively. Comparatively, AR1 showed activities increased threefold than the other fractions.



**Figure 2:** Fractionation by column chromatography: (a) column packed with silica gel and (b) column loaded with crude AE of *R. mucronata*.

### 3.6 Phytochemicals profiling by mass spectrometry

The existence of 11 and 2 bioactive compounds, respectively, was confirmed by mass spectrometry profiling of crude and partially purified aqueous leaf extracts of *R. mucronata* (Table 5). Phytochemicals identified in the crude extract were *N-p*-coumaroyltyramine ( $m/z$  148.89), *N-trans*-caffeoyltyramine ( $m/z$  120.87), quercetin 3-*O*- $\beta$ -D-glucoside ( $m/z$  180.89), angelicoidenol-2-*O*- $\beta$ -D-apiofuranosyl-(1  $\rightarrow$  6)- $\beta$ -D-glucopyranoside ( $m/z$  355.08), lotusine ( $m/z$  237.05), kaempferol-*O*-hexose-*O*-deoxyhexose ( $m/z$  429.12), luteolin ( $m/z$  132.93), syringaresinol-4-*O*- $\beta$ -D-glucopyranoside ( $m/z$  149.90), taxifolin ( $m/z$  150.98), hispidulin ( $m/z$  298.97), and ellagitannin ( $m/z$  783.70) (Figure 3).

These compounds were found to belong to classes such as hydroxycinnamic acid, cinnamic acids, flavonoid-3-*O*-glycosides, terpene glycosides, tetrahydroxyflavone, flavonoid, phenolic acid, and hydrolysable tannins. When the partially purified sample of AR1 of *R. mucronata* was loaded onto mass spectrometer, two major molecules *N-p*-coumaroyltyramine ( $m/z$  148.89) and luteolin ( $m/z$  132.93) were identified (Figure 4).

### 3.7 *In vivo* studies of antibacterial and antioxidant activity

The effectiveness of *R. mucronata* leaf extract in recovering *P. aeruginosa*-infected *Catla catla* fingerlings was

**Table 3:** Antibacterial and antioxidant activities of various fractions separated from AE of *R. mucronata*

Solvent system	Fractions	Code	Characteristics	Antibacterial activity (mm)	Antioxidant activity (%)
<i>n</i> -Hexane:chloroform (100:0)	1–4	A	White	—	—
<i>n</i> -Hexane:chloroform (90:10)	5	B	Green	2.6 $\pm$ 0.5	—
<i>n</i> -Hexane:chloroform (60:40)	6–7	C	Light green	—	—
<i>n</i> -Hexane:chloroform (40:60)	8–10	D	Orange	—	51.6 $\pm$ 2.8
<i>n</i> -Hexane:chloroform (20:80)	11–12	E	Pale orange	3.1 $\pm$ 0.9	—
<i>n</i> -Hexane:chloroform (0:100)	13–15	F	Yellowish orange	—	23.8 $\pm$ 3.1
Chloroform:methanol (80:20)	16–18	G	Yellow	7.5 $\pm$ 1.3	67.9 $\pm$ 2.4
Chloroform:methanol (50:50)	19–22	H	Dark yellow	1.8 $\pm$ 0.7	47.9 $\pm$ 1.7
Chloroform:methanol (0:100)	23–26	I	Honey	—	11.5 $\pm$ 0.9

Results expressed as mean value  $\pm$  standard deviation;  $n = 3$ .



**Table 4:** Retention factor (Rf) values and their corresponding bioactivities of bands collected from preparative TLC of *R. mucronata*

Fractions	Rf value	<i>P. aeruginosa</i> (mm)	DPPH radical scavenging activity (%)
AR1	0.12	13.1 ± 2.2	80.9 ± 2.3
AR2	0.29	2.7 ± 0.9	71.4 ± 3.9
AR3	0.41	0.9 ± 0.1	53.3 ± 3.2

Results expressed as mean value ± standard deviation;  $n = 3$ .

tested. All the experimental animals showed symptoms such as change in skin colour and haemorrhages on the abdomen, gills, and fins within a few hours of infection. Within 24 h of *P. aeruginosa* exposure, fish fingerlings in group 2 (negative control group) began behaving strangely with erratic movement and reached 100% death. Post-mortem was performed on dead fish fingerlings, which revealed a bloody fluid-filled intestine with dispositional internal organs. There were haemorrhages throughout the colon, liver, and gills. Bacteria from dead fingerlings were carefully collected and cultivated on a culture plate to corroborate the clinical symptoms observed in these fingerlings caused by *P. aeruginosa*. The bacteria in the culture were found to be rod-shaped Gram negative bacteria. It also showed positive for catalase, gelatine, and citrate utilisation tests, as well as negative for indole and methyl red tests, indicating that the bacterium was *P. aeruginosa*.

Fish from groups 3, 4, and 5 that were given a synthetic antibiotic, crude AE, and partially purified AE of *R. mucronata*, respectively, showed considerable recovery after 12 h of infection. Group 3 had a survival rate of just 67% and overall recovery did not occur within the first 7 days of the trial, but groups 4 and 5 had survival rates of 88% and 100%, respectively. Following the *in vivo*

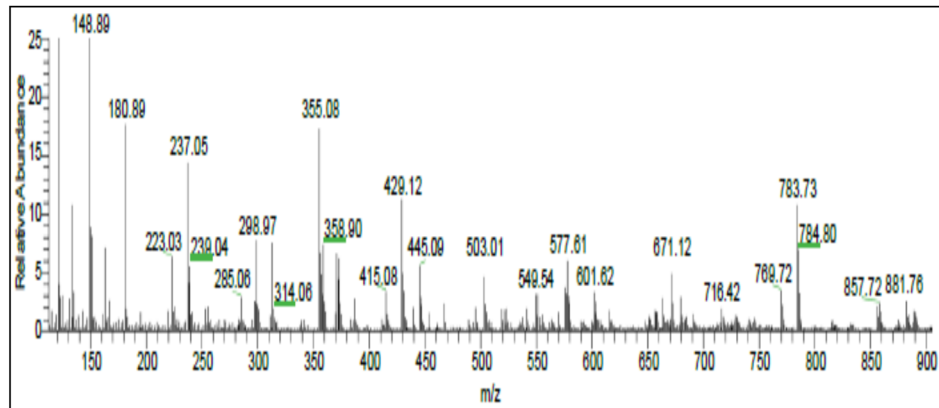
experiment, liver samples from all five groups were taken and antioxidant enzyme levels, such as CAT and SOD, were measured. Both enzyme levels were lower in group 2, indicating that bacterial infection can trigger a free radical chain reaction, resulting in the depletion of antioxidant enzymes. This might be the plausible reason for less recovery in the group 3 fishes, though amoxicillin is potential antibiotic but it cannot bring homeostasis in liver. In case of groups 4 and 5 fishes, the CAT and SOD levels are in close proximity to group 1 fishes.

## 4 Discussion

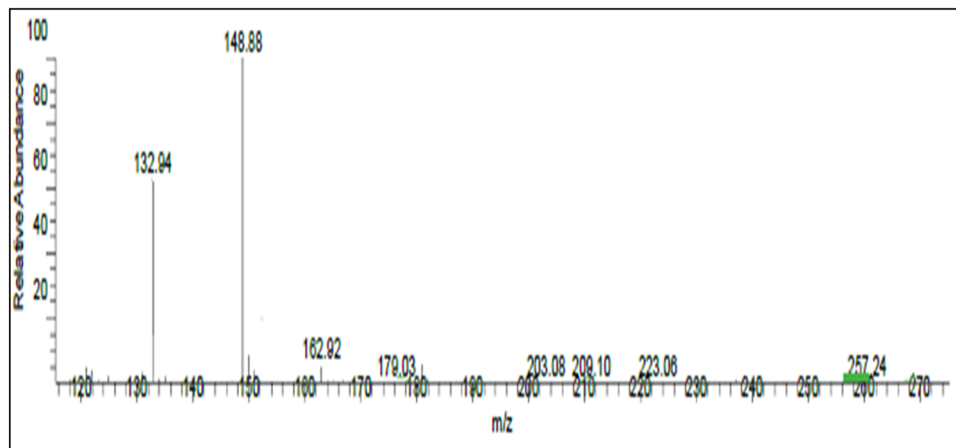
In aquaculture, a significant amount of investment is towards treatment of pathogenic diseases and maintenance of fish health. The use of synthetic/commercially accessible antibiotics has been hampered due to multi-drug resistance among bacteria [16,17]. As a result, the current research focuses on isolating diverse molecules from *Rhizophora mucronata* solvent extracts in order to find an appropriate and natural multi-potent chemical to treat fish diseases. Although various secondary metabolites were found in HE, DEE, EE, and AE, but majority of active components were found in the AE (Table 1). Among the available phytochemicals, glycosides and cardiac glycosides have antibacterial and anti-tumour activities besides potential in treating heart diseases, whereas tannins are used in treating haemorrhoids and skin eruptions. Terpenoids and phenols play an important role in defence mechanism of the plants, whereas anthraquinone possess antibacterial, antifungal, and antiviral properties [18]. Besides, antibacterial assay was conducted for all the extracts of *R. mucronata* against four bacterial species and all the extracts exhibited appreciable antagonistic

**Table 5:** Phytochemicals profiling of crude and partially purified extracts of *R. mucronata*

<i>m/z</i>	Compounds in crude extract	Compounds in partially purified extract	Structure
148.89	<i>N-p</i> -Coumaroyltyramine	<i>N-p</i> -Coumaroyltyramine	C <sub>17</sub> H <sub>17</sub> NO <sub>3</sub>
120.87	<i>N-trans</i> -caffeoyltyramine	—	C <sub>17</sub> H <sub>17</sub> NO <sub>4</sub>
180.89	Quercetin 3- <i>O</i> -β- <i>D</i> -glucoside	—	C <sub>21</sub> H <sub>19</sub> O <sub>12</sub>
355.08	Angelicoidenol-2- <i>O</i> -β- <i>D</i> -apiofuranosyl-(1 → 6)-β- <i>D</i> -glucopyranoside	—	C <sub>16</sub> H <sub>28</sub> O <sub>7</sub>
237.05	Lotusine	—	C <sub>19</sub> H <sub>24</sub> NO <sub>3</sub>
429.12	Kaempferol- <i>O</i> -hexose- <i>O</i> -deoxyhexose	—	C <sub>27</sub> H <sub>30</sub> O <sub>16</sub>
132.93	Luteolin	Luteolin	C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>
149.90	Syringaresinol-4- <i>O</i> -β- <i>D</i> -glucopyranoside	—	C <sub>28</sub> H <sub>36</sub> O <sub>13</sub>
150.98	Taxifolin	—	C <sub>15</sub> H <sub>12</sub> O <sub>7</sub>
298.97	Hispidulin	—	C <sub>16</sub> H <sub>12</sub> O <sub>6</sub>
783.70	Ellagitannin	—	C <sub>44</sub> H <sub>32</sub> O <sub>27</sub>



(a)

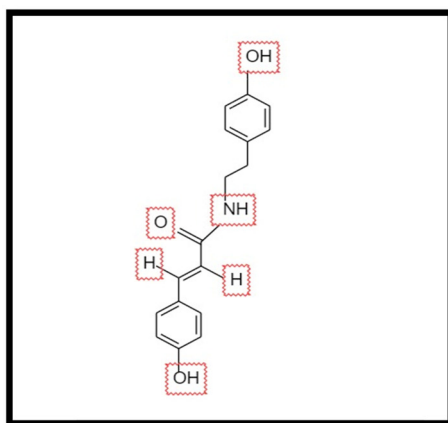


(b)

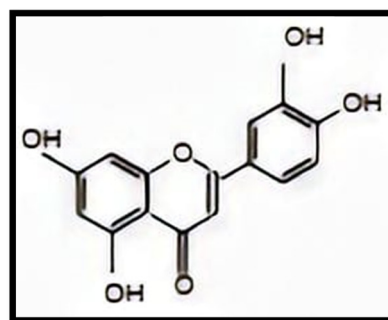
**Figure 3:** Phytochemicals profiling of (a) crude and (b) partially purified leaf extracts of *R. mucronata*.

activity, whereas highest activity was recorded by the AE of *R. mucronata*. The rationality for selecting the mentioned bacteria was availability, pathogenicity, and multidrug resistance properties. *Bacillus subtilis* is rod shaped, non-

pathogenic bacteria that resides in humans and other livestock. *Staphylococcus aureus* is a gram positive opportunistic food transmitted bacteria known to cause skin, lung, heart, and bone infections in various fishes [19]. *Klebsiella pneumoniae*



(a)



(b)

**Figure 4:** Structures of (a) *N-p*-coumaroyltyramine and (b) luteolin.



is a Gram negative bacterium that causes various health ailments like pneumonia and meningitis in humans, skin discolouration, ulcers, and red spottiness on skin, and haemorrhages. *Pseudomonas aeruginosa* is a common Gram negative bacterium that infects humans, animals, and plants. Its pathogenicity is very high during nosocomial infections and infected organs include lungs, ears, eyes, skin, and blood [20]. *Pseudomonas* sp. belongs to common micro-flora of aquaculture and during stressful condition its pathogenicity is already reported in rainbow trout, ayu, eel, tilapia, and Indian major carps [21]

The antioxidant activity of all four extracts was assessed using two antioxidant assays and both the experimental results have confirmed that AE of *R. mucronata* had maximum antioxidant activity compared to the remaining extracts (HE, DEE, and EE; Figure 1). This additional property for the extracts provides added advantage during formulating the drug because bacterial infections are one among the several reasons to increase free radical chain reactions inside the body [22]. During such diseased state, *in vivo* antioxidant enzymes cannot contribute fully in breaking the chain reaction of free radicals [23]. Usually, this natural system fails in the fishes grown in intensified culture systems due to various stressful conditions and in such cases, supplementation of antioxidant potent molecules' support to maintain homeostasis of the organisms plays a crucial role.

DPPH is a synthetic radical, stable at room temperature, and predominantly used for assessment of antioxidant activity [24]. The DPPH assay method purely measures the molecular ability to transfer electrons and in return turns violet solution into yellow or colourless solution based on its efficiency. DPPH assay is widely used due to its simple, fast, and user friendly procedure involved and large number of samples can be evaluated spectrophotometrically in a short span of time which was one of the reasons for selecting it throughout the study [25].

At doses ranging from 31.25 to 500 g·mL<sup>-1</sup>, the antioxidant properties of all *R. mucronata* extracts were investigated. All the extracts were successful in proton donation, with maximal abilities of 73.77%, 69.99%, 65.24%, and 75.97% for four *R. mucronata* extracts at 500 g·mL<sup>-1</sup>. In an earlier study on a mangrove plant, *Sonneratia caseolaris* extract prepared by ethyl acetate and 70% ethanol was also analysed for antioxidant activity and found to possess quite appreciable results [26]. Earlier reports also confirmed the antioxidant activity of ethanol leaf extract of *R. mucronata* as 127.5 µg·mL<sup>-1</sup> using DPPH assay method [27].

Ferric reducing antioxidant power assay is also one of the popular antioxidant assessing technique used to measure the samples' ability to reduce ferric ion (Fe<sup>3+</sup>)

to ferrous (Fe<sup>2+</sup>) and the reaction is supported with 2,4,6-tripyridyl-s-triazine [11]. The experimental solution turns into violet-blue colour upon Fe<sup>2+</sup> reaction with 2,4,6-tripyridyl-s-triazine and the same is measured at 593 nm [28]. Due to its simple procedure, FRAP assay is widely used and abundant literature was available conforming the antioxidant potential of various molecules extracted from microbes, plants, and animals [28]. *R. mucronata* AE has revealed excellent antioxidant potential of 152.3 ± 2.3 µg AAE per mL at 250 µL·mL<sup>-1</sup> concentration. The speciality of this procedure is that it can be used for measuring the activity of single antioxidant or total antioxidant activity without compromising the sensitivity of the results [11].

Purification of crude extracts to separate active molecules is obligatory because the genuine potential of a molecule may be shrouded with impurities [29]. Several techniques are employed to achieve that either by single technique or combination of multiple techniques. Generally, secondary metabolites are separated by fractionation, solid-liquid extraction, gel chromatography, precipitation separation, high performance liquid chromatography, and others [30]. Chromatography is one of the important bio analytical techniques used for the separation of phytochemicals on the basis of size, shape, charge, mass, solubility, and adsorption properties of phytochemicals with stationary and mobile phase [31].

Out of all the available purification processes, column chromatography is exceptional, user friendly, and cost-effective technique which facilitates loading and elution of large amount of sample. In the present study, column chromatography is generally deployed to separate individual compound from the crude mixture based on the differential adsorption considering their rate of mobility. Molecules with lower affinity/adsorption for stationary phase travel faster when compared to that with higher affinity/adsorption with the stationary phase. Molecules that move swift are eluted first, whereas the molecules that move slow are eluted out last. In the present study, nearly 9 fractions, 4 fractions showed zone of inhibition in the range of 1.8–7.5 mm and 5 fractions showed DPPH radical activity in the range of 11.5–67.9%. Among the fractions from A to I, fraction G showed better antibacterial and antioxidant activities (Table 3). Purification protocol adopted for the present work was analogous to that of Aguete et al. [32] wherein isolation of toxins from cyanobacteria extracts was reported.

As a next step of purification, sample from fraction G was loaded with four different concentrations on the TLC plate and placed it in the mobile phase chamber. Among the three fractions, AR1 showed threefold enhanced antibacterial and antioxidant activities (Table 4). This

fraction was scrapped off and used for phytochemical profiling. As in the present study, Simlai *et al.* [33] employed preparative TLC for purification of compound with antimicrobial activity from mangrove plant *Ceriops decandra* and found the improvement of activity.

Mass spectrometry is an extensively used, contemporary, and sophisticated analytical technique for characterisation of secondary metabolites from crude and partially purified extracts [34]. Xiao *et al.* [35] stated that out of all the existing technologies, mass spectrometer connected to GC or LC is the best approach for identification of secondary metabolites. In this study, LC-MS technique was used for identification of metabolites before and after purification of *R. mucronata* (Table 5). Crude and partially purified extracts showed 11 and 2 active compounds, respectively. The two major molecules in partially purified extract are *N-p*-coumaroyltyramine ( $m/z$  148.89) and luteolin ( $m/z$  132.93), where *N-p*-coumaroyltyramine belongs to hydroxycinnamic acid and mostly responsible for antioxidant property of the partially purified fraction [30]. On the other hand, luteolin is a flavone, which has the capacity to exhibit both antioxidant and antibacterial activities [36]. Thus, these two molecules are responsible for the recorded antibacterial and antioxidant activities of the partially purified sample.

Finally, *in vivo* studies on the *Catla catla* fingerlings infected with *P. aeruginosa* was undertaken since bacterial infections are one of the major risks recognised in aquaculture intensification. *P. aeruginosa* is one of the top three bacteria responsible for significant mortality among Indian major carps [3]. If not treated with appropriate drug, being an opportunistic bacteria *P. aeruginosa* is capable of creating huge losses by damaging fish and fish products [37,38]. Similar adversity was observed in fingerlings of group 2 wherein neither synthetic antibiotics nor *R. mucronata* extracts were used for treating. The crude and partially purified extracts of *R. mucronata*, on the other hand, were effective in curing the bacterial infection with minimal or no mortality. *Avicennia marina* leaf extract used in such recovery after bacterial infection of *Labeo rohita* was previously documented [39–41]. The antibacterial activity of herbal extracts was not limited to treating fish; *R. mucronata* and *Sonneratia alba* were also successful in treating tiger prawns [42].

## 5 Conclusion

Until now, a variety of synthetic antibiotics have been employed to treat infections caused by pathogenic bacteria.

Eventually, these microorganisms develop antibiotic resistance and actively transmit horrible illnesses that cause massive losses in aquaculture. As a result, biological molecules should be used instead of synthetic medications to treat various illnesses. The leaf extract of the mangrove plant *R. mucronata* was discovered to be high in phytochemicals with antibacterial and antioxidant properties. In this investigation, crude and partially purified extracts were found to be successful in recovering infected fish in a short amount of time at laboratory scale. As a future prospective, the efficacy of the *R. mucronata* extract need to be checked against fish infection in open ponds. Besides, the present study focused upon the efficiency of *R. mucronata* extract in treating single bacterial infection but the study can be extended to treatment of multiple bacterial infections. This study proposes that *R. mucronata* extract with its healing properties will be extremely valuable for the fish farmers.

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**Conflict of interest:** Authors state no conflict of interest.

**Compliance with ethical requirements:** The fish fingerlings were collected from local hatchery of Andhra Pradesh. Animal ethical care guidelines were followed for the experimental fishes in the study. As per CPCSEA instruction's protocol, approval for experimentation on fishes is not required.

**Data availability statement:** The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy ethical restrictions.



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## Arduino and UV Sensors Embedded Assistive Hat for Visually Impaired

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### Abstract:

The World Health Organization (WHO) reported that there are 285 million visually-impaired people worldwide. Among these individuals, there are 39 million who are totally blind. There have been several systems designed to support visually-impaired people and to improve the quality of their lives. In this paper, we present an Arduino and UV sensors embedded hat to provide a 3-dimensional guidance for the blind which is wearable and portable in order to show the progress in assistive technology for this group of people. We thus contribute in assisting this population and highlight the improvements, advantages, disadvantages and accuracy. Our aim is to address and present to researchers in this area to design devices that ensure safety and self reliable mobility to visually-impaired people.

**Keywords:** assistive devices, visually-impaired people, obstacles detection, Arduino UNO , Ultrasonic sensor *HC-SR04*, vibrator.

### 1. Introduction

---

The World Health Organization (WHO) fact reported 100,000 students, who are visually impaired according to the American Foundation for the Blind [1,2] and National Federation for the Blind [3]. Over the past years, blindness that is caused by diseases has decreased due to the success of public health actions. However, the number of blind people that are over 60 years old is increasing by 2 million per decade. Unfortunately, all these numbers are estimated to be doubled by 2020 [4].

The need for assistive devices for navigation and orientation has increased. The simplest and the most affordable navigations and available tools are trained dogs and the white cane [5]. Although these tools are very popular, they are not wearable and adds to the discomfort of carrying by engaging one of the hands. The present work involves a wearable hat which is embedded with an Arduino UNO and three ultrasonic sensors to provide a 360 degrees pseudo view to avoid an obstacle hitting the person who is wearing it.

#### 1.1. Assistive Technology

All the systems, services, devices and appliances that are used by disabled people to help in their daily lives, make their activities easier, and provide a safe mobility are included under one umbrella term: assistive technology [6].

In the 1960s, assistive technology was introduced to solve the daily problems which are related to information transmission (such as personal care) [7], navigation and orientation aids which are related to mobility assistance [8,9,10].

Visual assistive technology is divided into three categories: vision enhancement, vision substitution, and vision replacement [10,11]. This assistive technology became available for the blind people through electronic devices which provide the users with detection and localization of the objects in order to offer those people with sense of the external environment using functions of sensors. The sensors also aid the user with the mobility task based on the determination of dimensions, range and height of the objects [6]

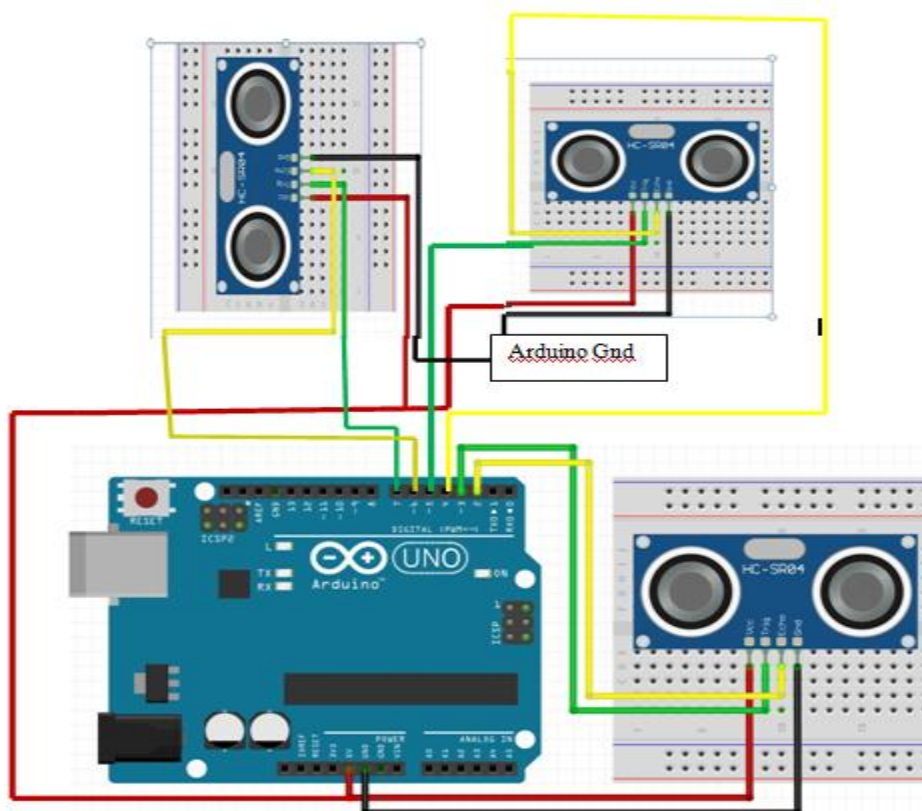
## 2. Components and Specifications

### 2.1. Physical Assembly

Figure 1a shows the schematic of the circuit employed in the assistive hat along with the components namely ultrasonic sensors, Arduino Nano, vibrators and buzzer. It also comprises of the battery holder and a reset button . The circuit design is implemented in Proteus simulator and the Arduino nano is programmed in embedded C using the Arduino IDE 1.8.13

### 2.2. Circuitry Design and Implementation

The Arduino Nano [8] microcontroller, in which the copper wires from the sensor pins of the ultrasonic sensor (Figure 2a), and reset button were soldered with a 9 V battery supplying 3.3 to 5 V to the circuit (Figure 1a).



*Figure 1a Circuit Schematic showing the Arduino UNO microcontroller and UV sensors*





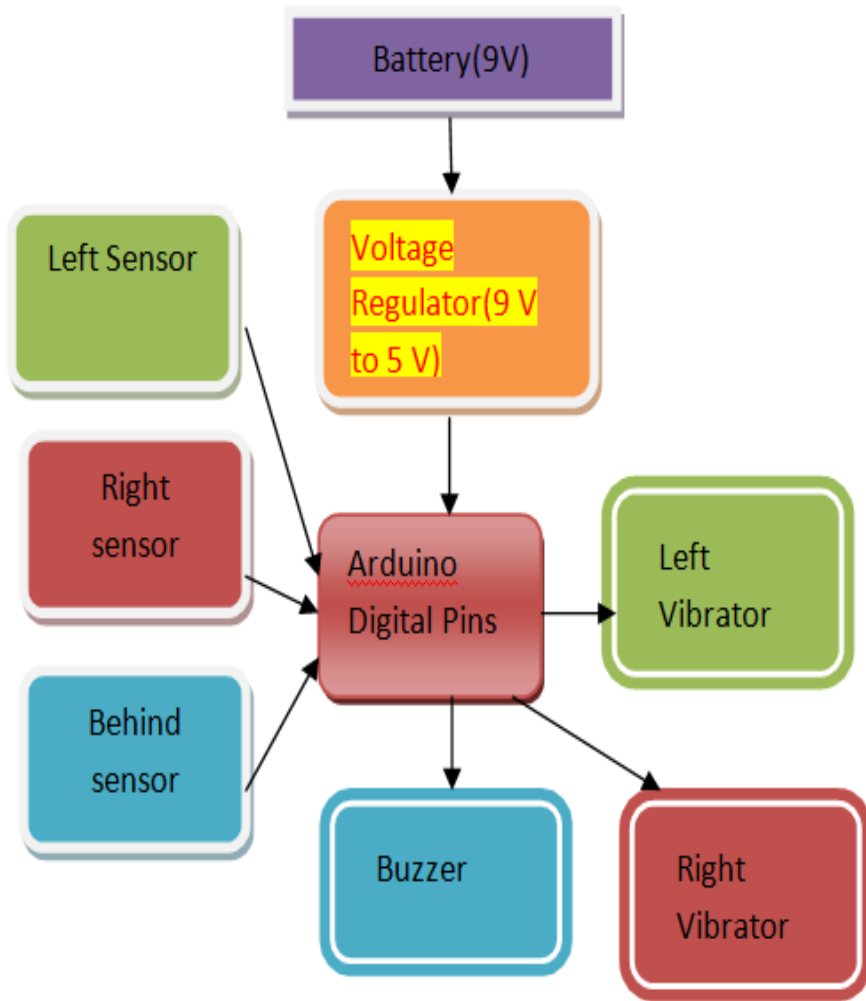
*Figure 1b & 1c The physical picture of the assistive hat and consisting of: Ultrasonic sensor HC-SR04, reset button, Arduino Nano board, vibrators and buzzer.*

### 3 Experiment

To make an assistive hat for the blind we used an Arduino Uno, 3 Ultrasonic sensors, 2 vibrators. An ultrasonic sensor has trigger and echo pins as shown in figure 1c. The first sensor echo pin is connected to Arduino digital pin number 2 and the Trig is connected digital Pin 3. The second sensor echo is connected to Arduino digital pin number 4 and the Trig is connected to digital Pin 5. Similarly, the third sensor echo is connected to Arduino digital pin number 6 and the Trig is connected digital Pin 7. Left vibrator Vcc is connected to digital pin 9 and ground to the Arduino ground. Similarly right vibrator Negative is connected to digital pin 10 and ground to Arduino ground. The third sensor output is given to a buzzer in order to differentiate the direction of the obstacle. After making all these connections our circuit is ready. The code using embedded C language is developed in Arduino IDE 1.8.13 and uploaded using the USB to AB cable in order to dump into the Arduino board.

#### 3.1 Working

Ultrasonic sensors send ultrasonic waves. These waves are absolutely invisible and come back after hitting an obstacle there by activating the Trig pin of the respective sensor. The Trig pin activates (D3 or D5 or D7) to which a left vibrator, right vibrator and a buzzer are connected and thereby gets activated through embedded C program. The obstacle closeness is fixed to 10 cm so that the buzzer or the vibrator gets activated depending on their directional position if the obstacle comes closer than this distance.



*Figure 1d Flowchart of the algorithm fed into the Arduino UNO*



## Arduino Sketch for the assistive Hat

```

HAT_code $
const int trigPin_1=2;
const int echoPin_1=3;
const int trigPin_2=4;
const int echoPin_2=5;
const int trigPin_3=6;
const int echoPin_3=7;
long duration;
int distanceleft;
int distanceright;
int distancebehind;
void setup() {
  // put your setup code here, to run once:
  Serial.begin(9600);
  pinMode(trigPin_1,OUTPUT);
  pinMode(echoPin_1,INPUT);
  pinMode(trigPin_2,OUTPUT);
  pinMode(echoPin_2,INPUT);
  pinMode(trigPin_3,OUTPUT);
  pinMode(echoPin_3,INPUT);
}

void loop() {
  // put your main code here, to run repeatedly:
  digitalWrite(trigPin_1, LOW);
  delayMicroseconds(2);
  digitalWrite(trigPin_1, HIGH);
  delayMicroseconds(10);
  duration =pulseIn(echoPin_1,HIGH);

Done compiling.

Sketch uses 2918 bytes (9%) of program storage space. Maximum is 32256 bytes.
Global variables use 190 bytes (9%) of dynamic memory, leaving 1858 bytes for local variables. Maximum is 2048 bytes.

HAT_code $
if(distanceleft<10)
{
  digitalWrite(2,HIGH);
  digitalWrite(3,HIGH);
}
else
{
  digitalWrite(2,LOW);
  digitalWrite(3,LOW);
}

if(distanceright<10)
{
  digitalWrite(4,HIGH);
  digitalWrite(5,HIGH);
}
else
{
  digitalWrite(4,LOW);
  digitalWrite(5,LOW);
}
if (distancebehind<10)
{
  digitalWrite(6,HIGH);
  digitalWrite(7,HIGH);
}

Done compiling.

Sketch uses 2918 bytes (9%) of program storage space. Maximum is 32256 bytes.
Global variables use 190 bytes (9%) of dynamic memory, leaving 1858 bytes for local variables. Maximum is 2048 bytes.

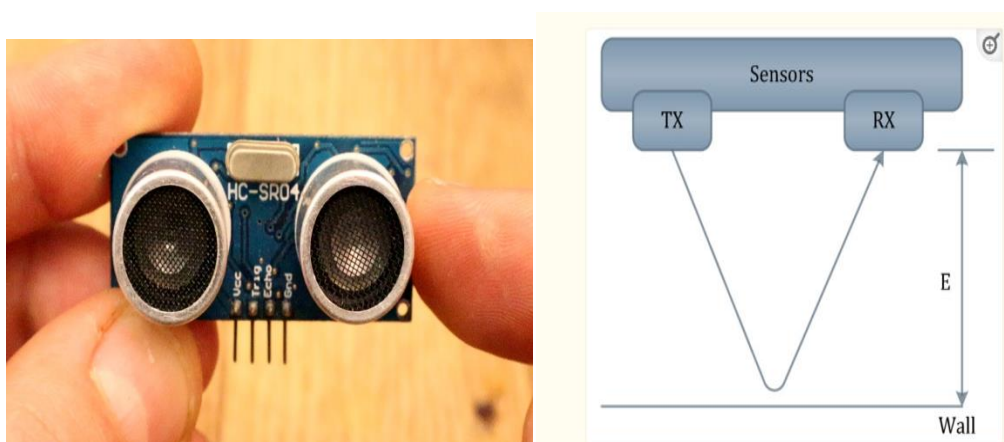
```

## 4 Sensor considerations

### 4.1. Ultrasound Sensor

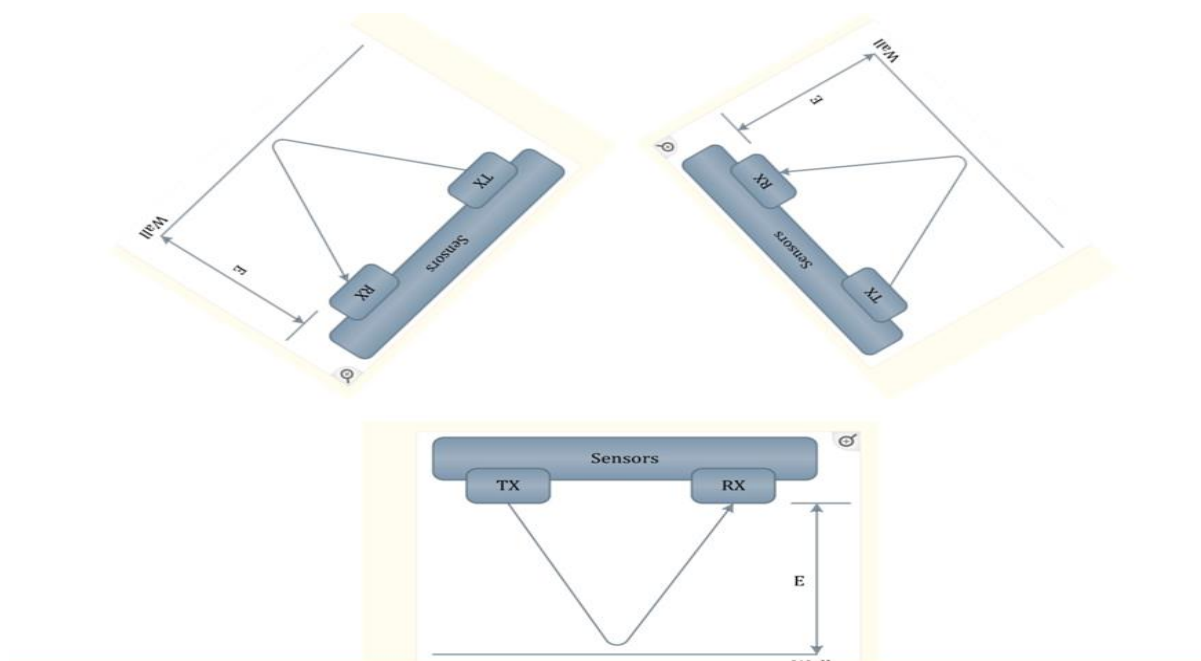
The ultrasound sensor (figure 2a) consists of a transmitter sending an ultrasound wave and a receiver detecting the reflected wave by the targeted physical object. The time taken between the transmission and detected wave is registered for the calculation of the distance from the speed of ultrasound waves at 330 m/s by the programmed Arduino Nano.

The sensors sends a sequence of ultrasonic pulses. If the obstacle is detected, then the sound will be reflected back to the receiver as shown in Figure 2b. The micro-controller processes the readings of the ultrasonic sensors in order to activate the vibrators by sending pulse width modulation. It also provides a low power consumption [15]. Figure 2c shows the arrangement of the three sensors embedded in to the wearable hat in order to have a 360<sup>0</sup> obstacle finding range



*Figure 2a and 2b Ultrasonic sensor HC-SR04 component range of signal*





**Figure 2c Arrangement of the three sensors within the Hat for a 360 degrees view**

## 5 Results and Discussions

The experiment is performed on 10 undergraduate students by covering their eyes in order to study the accuracy of the unit. The observations were recorded by placing an obstacle of 3 to 4 ft height on a concentric circle of radius 12 cm and then of 10 cm. The observations were found to be quite satisfactory where in the buzzer alarmed and the vibrator gave a signal depending on the position of any obstacle for this distance giving a feel of presence of an obstacle for the subject wearing the assistive hat.

## Conclusions

We thus conclude the successful working of a prototype of an assistive hat for visually handicapped people which is economical, long lasting, reliable and portable at the comfort of just wearing as a hat there by enhancing their physical appearance.

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Govt. College Autonomous, Rajahmundry

ప్రభుత్వ కళాశాల స్వయం ప్రతిపత్తి



రాజమహేంద్రవరం, తూర్పు గోదావరి జిల్లా, ఆంధ్రప్రదేశ్.



భాషావిజ్ఞ ప్రత్యేక సంచిక

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రెండు రోజుల  
జాతీయ సదస్సు



21-22 అక్టోబర్ 2022

దాదిత సాహిత్యం - తాత్విక దృక్పథం

Dadit Literature - Philosophical Perspective



నిర్వహణ:

తెలుగు మరియు తత్వశాస్త్ర శాఖల సంయుక్త ఆధ్వర్యంలో

ప్రభుత్వ కళాశాల స్వయం ప్రతిపత్తి

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రెండు రోజుల జాతీయ సదస్సు ప్రత్యేక సంచిక

## దళిత సాహిత్యం - తాత్విక దృక్పథం

21 & 22 అక్టోబర్ 2022

నిర్వహణ

తెలుగు మరియు తత్వశాస్త్ర శాఖల సంయుక్త ఆధ్వర్యంలో  
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# సిని గేయ సాహిత్యంలో దళిత తత్వం

- డా. శ్రీధర్ రిట్టేటియ్య, తెలుగు విభాగాధిపతి, సెంట్ ఆస్సె మహిళా కళాశాల, మెహదీపట్నం, హైదరాబాద్

సామాజిక ప్రతినిధిత్వానికి తార్కాణాలుగా పత్రికలను చలనచిత్రాలను ప్రధాన సాధనాలుగా పేర్కొనవచ్చు. ప్రపంచం నలుమూలల నుంచి ఏ విధమైన సామాజిక దురస్థాయాన్ని అయినా కళ్లకు ఉట్టి పడే విధంగా అవిష్కరించబడే సాధనాలివి. సమాజ చైతన్యానికి, స్పృహకూ చలనచిత్రాలు ఎంతగానో దోహదం చేస్తాయి. ఎంతోమందికినా పాపముదీక్షనా ఎవరికైనా అందుబాటులో ఉంటే సాహిత్యం సిని సాహిత్యమే అనడంలో అతిశయోక్తి లేదు. కవి ఎప్పుడూ సమాజానికి ప్రతిబింబం. మానవుడి పక్షాన నిలబడి ఏ సమస్యనైనా ప్రశ్నించే డిక్కార స్వరమే కవి. ఆలా సిని గేయ సాహిత్యంలో కొన్ని విభిన్న పాఠ్యాలను మీ ముందు ఉంచుతాను. శివాజీ గణేశన్ నటించిన కర్ణ చిత్రంలోని అనువాద గీతాన్ని రాసిన అదార్య డా. సి. నారాయణరెడ్డి గారి పాటలోని పంక్తులను ఒకసారి అవలోకిద్దాం.

గారికి కులమేది? ఏదీ?  
నేలకు కులమేది? ఏదీ?  
నింగికి మరుగేది? ఏదీ?  
కాంతికి నెలవేది? ఏదీ?.. అన్న పంక్తులలో పంచ దూతలు అందరికీ సొంతమనీ, ఏ ఒక్కరి సొత్తు కాదన్న సత్యాన్ని సూటిగా ప్రశ్నిస్తోయిన. ఇందులోనే..

పాలకు ఒకటి తెలి వర్ణం  
ప్రతిదకు కందా స్థల భేదం?  
వీధులకెందుకు కుల భేదం?  
ఆది మనసుల దీర్ఘదు మత భేదం?  
జగమున యశమే మిగులునులే  
ఆది యుగములకైనా చెదరదులే..  
దైవం నీలో నిలుచునులే  
దర్శనం నీలో నడచునులే.. అని సాగే ఈ పాట కర్ణ

పాత్ర కించిత్యాన్ని తెలిపే విధంగా మానవునిగా జన్మనెత్తే ఎవరికైనా రెండే రెండు కులాలు మాత్రమే ఉన్నాయని వెల్లడిస్తుంది. అవి ఒకటి మరొకటి ఒకటి చెడు! మానవులకు మాత్రమే కాదు.. జీవరాశులకు కూడా పైవే ఆధారం!

కొమ్మ చెక్కితే బొమ్మరా  
కాలిచి మొక్కితే అమ్మరా.. అని చాటిన కవి ఆ పాదాలను ఒక్కసారి తలచి చూస్తే ఎవడు నిమ్మ కుర్రాని చెందినవారు? అన్న ప్రశ్నకు సమాధానం లభిస్తుంది. ఒక అగ్ర కులానికి చెందిన యువతి మరొక కులానికి చెందిన యువకునికి మధ్య చిగురించిన ప్రేమకు సాంఘిక కట్టుబాట్లు, వర్ణ వ్యవస్థ వంటివి అడ్డ తగిలినప్పుడు కవి స్పందించిన తీరు చూడండి..

ఏ కులము నీదంటే  
గోకులము నవ్వింది  
మాధవుడూ యాదవుడూ మా కులమే తెమ్మంటే  
అంటూ ఆ పాదాలు సమాజాన్ని నిలదీస్తాయి. ఏడు వర్ణాలు కలసి ఇంద్రధనుస్సువుతాది అన్ని వర్ణాలకూ ఒకటే ఇహమూ పరముంటాది. ఆ రెండే రెండు పాదాల్లో ప్రకృతిని, మానవ జీవితాన్ని అద్భుతంగా పోల్చి సప్తపది చిత్రంలో కవి వేటా సుందరరామమూర్తి గారు దానికి చక్కటి తార్కాణం జోడిస్తారు!

పై వాక్యాల్లో సమతావాదాన్ని, జాతిని జాగృతం చేసే భావాలనీ దర్శిస్తాము. ఇప్పుడు ఆ వర్ణానికి చెందిన ఇతివృత్తాలతో వచ్చిన చిత్రాల్లో సిని కవులు ఏ విధంగా స్పందించారో చూద్దాం!  
ఏటికి ఏతం పెట్టి



ఎయ్యి పుట్టు పుట్టించి  
 గంజిలో మెతుకెరుగరన్నా..  
 కాలైయ్యి కడుక్కొని కట్ట మీద కూర్చుంటే  
 కాకీ తన్ని పోయెరన్నా..

కాకీ పిల్ల తన్ని పోయెరన్నా.. అనే పాదాల్లో బలహీన  
 వర్ణాలుగానూ, జనబలం ఉన్నా మనోబలం  
 లేనివాళ్లుగానూ ఉన్నటువంటి వారి పరిస్థితి సమాజంలో  
 ఎప్పటికీ అణగారే ఉంటుందన్న నిజాన్ని ఇక్కడ కవి  
 వాక్యాలు వెల్లడిస్తాయి. అలాగే మాలపిల్ల సినిమాలోని  
 ఓ పాటలో...

నిజానికి మంచోడే  
 నేలకూలి పోతాడే  
 అన్నచోడే నా యెరిగిల్ల  
 ఇది విన్నావా బర్రె పిల్లా..  
 వినవే బర్రె పిల్లా..  
 నువ్వివినవే బర్రె పిల్లా..  
 విన్నావా బర్రె పిల్లా

విన్నావా బర్రె పిల్లా.. అని సాగే ఓ పాటలో గొడ్డు  
 కాసేవాడు ఓ బర్రె మీద కూర్చొని ఇంకా ఇలా అంటాడు..

గొప్పొళ్ల ఆటలన్నీ  
 గుట్టుమట్టూ లైటకెట్టి  
 చెప్పుకుంటే సిగ్గు సిగ్గు..  
 పచ్చి మోసగాడే  
 పడైమంచం పానుపెక్కి పవ్వళించుతాడటే..  
 ఆడి మాట విననివోడే  
 మాడి మరణించుతాడే..  
 ఈరు మంచి కోరి రోజల్లా  
 పాటు పడే బీదోళ్లు బూడిదవుతారే..

ఎవరెవరో బాగుకుంటారే.. వంటి నిత్యసత్యాలు  
 హృదయాన్ని కలచివేస్తాయి. అవి వారి దీన స్థితిని వారే  
 వల్లై వేసుకుంటూ విధిని నిందించుకుంటూ పలవరించే  
 మాటలు!

బడుగుల బతుకుల్లో ఎప్పటికీ వెలుగులు రానే

రావంటూ కవి బాలాది గారు ప్రాణం బరిదు చిత్రంలో  
 రాసిన ఒక గేయంలో ఆ కటువైన నిజాలేమిటో మనకు  
 కళ్లకు కడతారు!

ఏతమేసి లోడినా యేరు ఎండదు  
 పొగిలి పొగిలి యెడ్చినా పొంత నిండదు  
 దేవుడి గుడిలోదైనా  
 పూరి గుడిసెలోదైనా

గాలి ఇసిరి కొడితే ఆ దీపముండదు.. అనే పల్లవి  
 ఎత్తుగడలో అణగారిన మానవ జీవితాల చీకట్లు ఎన్నటికీ  
 తొలగిపోవనీ, ఒకవేళ గుడ్డి వెలుగులు మిణుకుమన్నా  
 అవి ఒక్క గాలి విసురుకు ఉసూరుమంటాయన్నది  
 ఆయన తాత్విక దృక్కోణం!

పలుపుతాడు మెడకేస్తే పాడియావురా  
 పసుపుతాడు ముదులేస్తే ఆడదాయెరా  
 కుడితి నీళ్లు పోసినా అది  
 పాలు కుదుపుతాది  
 కదుపు కోత కొసినా ఇది

మనిషికే జనమనితాది.. అని పశువునీ పడతినీ పోల్చి  
 చెబుతూ రెండింటినీ ఎలా చూసుకున్నా అవి నిస్వార్థ  
 త్యాగానికే ప్రతీకలంటూ ఆయన చాటుతాడు.

బొడ్డు పేగు తెగిపడ్డ రోజు తలుసుకో  
 గొడ్డు కాదు ఆడదనే నిజం తెలుసుకో.. అంటూ ఈ  
 సమాజాన్ని నిలదీస్తాడు.

అందరూ నడిసాచ్చిన తోవ ఒక్కటే  
 సీము నెత్తురులు పారే తూము ఒక్కటే  
 మేడ మిద్దెలో ఉన్నా  
 సెట్టు నీడ తొంగున్నా  
 నిదర ముదర పడినాక  
 పాడే ఒక్కటే..

వల్లకాడు ఒక్కటే.. అని ఒకరు ఎక్కువ ఒకరు తక్కువ  
 అనే లెక్కలు మనుషులు రాసుకున్న చిట్టా పద్దులని,  
 అవి మన బతుకు పొద్దు గుంకినాక చితికెక్కాల్సినవేనని  
 ఉటంకిస్తాడు.

ఎవరమందారం ఆనే మరో దిశంలో బాలాది గారు  
రావిన మరో గీతంలోని మాత్రవాన్ని గమనిద్దాం!

యోలో యోలో ఉయ్యాల  
యేరేడు జనమోలు మొయ్యాల  
నాలుగు దిక్కుల ఉయ్యాల  
నలుగురు కలిసి మొయ్యాల  
కళ్ళ తెరుసుకుంటే ఉయ్యాల  
కళ్ళ మూసుకుంటే మొయ్యాల.. ఆనే పల్లవిలో మనిషి  
వేటకూ, బావుకూ మధ్య అంతరాన్ని అద్భుతంగా  
అవిభజిస్తాడు. మనుషుల్లో ఎన్ని అసమానతలున్నా  
ఉపిడున్న వంక ఉయ్యాలనీ, మనుగడ చాలించాక అదే  
మనిషిని మొయ్యాలనీ అభివర్ణిస్తాడు!

మాసాలు మొయ్యాల  
మనిషిని చెయ్యాల  
కన్నెక ఉగ్గెట్టి కప్పల బువ్వెట్టి  
కాపాడుకోవాల.. ఆస్తప్పుడు ఉగ్గుపాలల్లో కన్నీళ్లు  
కలిపినా, నోటి ముద్దలో కప్పల గంజి వార్చినా మనిషి  
తనాన్ని కాపాడుకోవాలన్న ఉద్దేశం ఈ వాక్యాల్లో ద్యోతక  
మవుతుంది.

పరిసర్లులాదాల.. అడు  
పట్టుకుంటు రియ్యాల  
రుండాలు మొక్కించి  
గుండాలు రాకుండా గుండెల్లో దాయాల.. అంటూ  
మానవ మనుగడకు చిన్ని చిన్ని ఆనందాలు చేర్చుకుంటూ  
అవడల ఆద్యగోడర్ని దాటుకుని మన ప్రాణాల్ని  
కాపాడుకోవాలంటాడు!

అడుగుడుగున అణగారుతూ బతికుండా  
ఆ బతుకున ఒక మెతుకేసే తోడుండా  
కొడుకైనా గొడుగుయ్యే ఋణముండా  
కరణైనా కరమయ్యే బతికుండా  
పాపాడే వయసేమో పకపక నవ్వుల  
పాపాడే వయసేమో తాతయ్యతో కలిసి  
బోబూదులాదాల..

వెంట బహుశో - కార్తీక చిత్తీకం, ప్రభుత్వ కళాశాల, రాజమహేంద్రవరం - సుమోద

నడికారు పయసేమో నడుమును కట్టాల  
పుట్టిన నేలనూ పెట్టే తల్లిని కాపాడుకోవాల  
ఆనడంలో అణగారి బతుకుతున్నా జీవితాన్ని  
అపురూపంగా చూసుకోవాలనీ, ఆ బతుక్కి వెంకటేశ్వర  
కేసే తోడూ ఉండాని కవి కోరుకుంటాడు. కొడుకైనా  
అవసాన దశలో గొడుగులా నిలబడే ఋణముండా  
శరణంటూ జీవచ్ఛవంలా కొట్టుమిట్టాడుతున్నా ఈ ప్రాణాల్ని  
బతికుండానీ ఆశిస్తాడు!

నడుమొంగే వయసైనా నిలిసుండా  
నలుగురికొకటే న్యాయం కలిగుండా  
సావైనా బతుకైనా పోరాడాల

కడకైనా కలగన్నది నిజమవ్వాల.. అని ఆదములైనా  
అధికులైనా వయోభేదం లేకుండా అంతా కలిసుండా  
అందరికీ ఒకే న్యాయం కలిగుండానీ అంటాడు.  
మరణమైనా, మనుగడైనా పోరాట తత్వాన్ని వదులు  
కోకూడదనీ, చివరకి కన్న కలలన్నీ నిజం చేసుకోవాలనీ  
ఆశావాదంతో ఈ గీతాన్ని ముగిస్తాడు కవి.

దళితుల జీవితాల్ని ఎవరెన్ని కష్టాలకు గురి చేసినా  
కన్నీళ్లలో ముంచినా ఆ కడగండ్ల మాటున కనిపించనీ  
చిన్ని చిన్ని ఆనందాలు, అనుబంధాలూ వారి జీవితంలో  
ఎంతటి ఆశావాదాన్ని రేపుతాయో, ఆ అనురాగాలూ  
ఆప్యాయతలూ ఎలా ఉంటాయో ఈ గద్దర్ గీతంలో  
మన దర్శించవచ్చు.

రికా తోలుకుని బతికే ఓ అన్న తన చెల్లెల్లపై ఎన్ని  
ఆశలు పెట్టుకున్నాడో, ఎంతటి మమకారాన్ని తన  
మాటల్లో రంగరించి పోస్తున్నాడో ఈ పాట వింటే  
తెలుస్తుంది.

చదివినంత నిన్ను సదివిస్తనమ్మా  
ఎదిగినంత నిన్ను ఎదిగిస్తనమ్మా.. అంటూ తన  
సామాజిక వర్గమెలాంటిదైనా, తన స్థాయి ఏదైనా  
అందరితో సమానంగా తన చెల్లెలు కూడా ఎడగాలన్న  
ఆకాంక్ష ఆ అన్నలో కనిపిస్తుంది.



**భావవీణ**

నీకు పెళ్ళిడు వచ్చేనాటికి  
 పువ్వు చొట్టో కూడచెట్టుతా..  
 నచ్చినోదీకే ఇస్తానమ్మా సెలైమ్మా  
 నా కన్నీళ్లతో కాళ్లు కడుగుతా సెలైమ్మా..  
 రిక్త బండీనే మేనా గడతా సెలైమ్మా  
 నిన్ను ఆల్టోరింటికి సాగనంపుతా సెలైమ్మా..  
 అంటూ బతుగు జీవల బతుకుల్లోనూ తొంగి చూసే సున్నితమైన  
 ఆనుబందాన్ని ఒక ఆన్న తన చెల్లెలి కోసం పాడుకునే  
 పాటలో ఆత్యంత రమణీయంగా ఆవిష్కరించడం గద్దర్కే  
 చెల్లింది!

దళిత జీవన చిత్రాల్లో విభిన్న కోణాలను సినీ గేయ  
 సాహిత్యంలో కొంత వరకే మనం నెమరేసుకున్నాము.  
 రచన విస్తృతినీ, సమయాభావాన్నీ దృష్టిలో పెట్టుకుని

కొన్ని గీతాలనే ఇక్కడ ప్రస్తావించడం జరిగింది.  
 అనంతమైన సినీ సాహిత్యంలో ఈ దళిత సాహిత్యానికి  
 అగ్ర తాంబూలం లభించడం మనం గమనించదగింది.  
 ఈ పాటలు రాసిన వారిలో దళిత కవులూ ఉండడం  
 విశేషం! మంచి అన్నది మాల అయితే.. మాల నేనగుదును  
 అని ఉద్ఘాటించిన గుజరాద అప్పారావు నవ సమాజానికి,  
 సమ సమాజానికి ఆనాడే తన సాహిత్యంతో బాటలు  
 వేశారు. సంఘ సంస్కరణ దిశలోనూ, సహపంక్తి భోజనాల  
 మూలంగానూ అన్ని కులాలూ, వర్గాలూ సమానమే  
 అనేటటువంటి దృక్పథాన్ని తన రచనల్లో ప్రోది చేశారు!  
 ప్రధాన సాహిత్య శాఖలకైనా, సినీ గేయ సాహిత్య శాఖకైనా  
 పూర్వ కవుల ప్రస్థానం, వారి రచనలూ శిరోధార్యాలని  
 నా అభిప్రాయం!

**ఉపయుక్త గ్రంథాలు :**

1. యూ ట్యూబ్ లింకు ద్వారా సేకరించిన సమాచారం  
 సినిమాలు :  
 మాలపిల్ల  
 కర్ణ  
 ప్రాణం ఖరీదు  
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# Quantitative Estimation of Indole-3-acetic Acid in Shoot Tips and Young Leaves of *Tecoma stans* and *Ixora coccinea* from Polluted and Control Regions

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## ABSTRACT

Plants are sensitive to pollution and especially air pollution can alter the physiological processes of plants affecting growth patterns. Study was carried out to determine the IAA production and response of plants exposed to vehicular emission. Two plant species namely *Tecoma stans* and *Ixora coccinea* which were exposed to roadside vehicular pollution were taken and control plants were collected from the college campus (unpolluted zone) and Estimation of IAA is carried out using Salkowski reagent, which upon reaction with IAA, yields pink color, due to AA complex formation and reduction of Fe<sup>+3</sup>. A decrease and increase in IAA concentration was observed in *Tecoma stans* and *Ixora cocenia* respectively growing in pollution zones as compared to control zones clearly demonstration air pollution stress.

**Key words:** Air pollution, IAA, Tecomastans, Ixoracoccinea, Stress, Salkowski reagent.

## Introduction

Air pollution comes from natural and anthropogenic sources and has a negative impact on plant growth and development. Plants show damage in a variety of ways. All the different types of air pollutants directly or indirectly affect plant growth and development. Plant growth and development is also dependent on plant hormones traditionally, five major hormone classes are auxins, cytokinins, gibberellin, abscisic acid and ethylene and a newly added Brassinosteroids have been reported to help growth and development in plants. Plant growth regulators are molecules other than nutrients which in minute concentration effect the physiological processes of the plants. Auxins represent one of the most important group of plant hormones because of their pleotropic effect on the plant growth and develop-

ment. Auxin is the most versatile phytohormone, which can coordinate plant growth even under stressed conditions.

Air pollution is one of the devastating abiotic stress than cause substantial crop loss. The frequency and magnitude of pollution stress is intensified due to global climate change. Plants have evolved various sophisticated mechanisms to sense pollution and activate different defense responses rapidly, to protect its vital cellular structures. Phytohormones are the endogenous messenger molecules that mediate plant growth and development and responses to various biotic and abiotic stresses. All major hormones have been reported to play critical roles in response of plants to air pollution (Elizabeth *et al.*, 2020).

In the present investigation was carried out to understand the changes in the concentration of IAA in

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plants subjected to air pollution stress.

### Materials and Methods

Estimation of IAA is carried out using Salkowski reagent. The reagent is a mixture of 0.5M ferric chloride (Fe Cl<sub>3</sub>) and 35% Perchloric acid (HClO<sub>4</sub>) which upon reaction with IAA, yields pink color, due to AA complex formation and reduction of Fe<sup>+3</sup> (Kamner *et al.*, 2001). The OD of colour developed is read at 540 nm.

Two plants *Tecoma stans* and *Ixora coccinea* were selected for the experiment. The plant samples were collected from both the polluted and control region in the early hours of the day and brought to the lab in an ice box. The sample were surface sterilized with sodium hypochlorite and thoroughly rinsed two to three times with distilled water. The tips and young leaves were separated and weighted. One g of the material was macerated with extraction solution and filtered using Whattman's filter paper 1.

To 1 ml of the filtrate, 4 ml distilled water and 4ml of reagent was added and incubated for 25 mins in dark and the OD was recorded at 540 nm. Using a standard graph the quantity of IAA in the samples was calculated separately in shoot tips and young leaves of all plant samples.

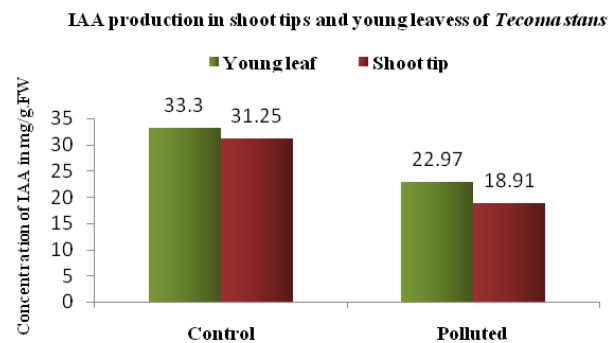


Fig. 1. Concentration of IAA in *Tecomastans* shoot tip and young leaves

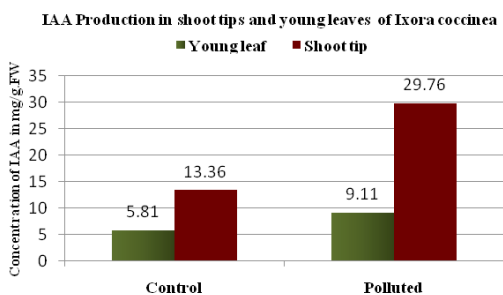


Fig. 2. Concentration of IAA in *Ixora coccinea* shoot tip and young leaves

### Results and Discussion

Plants of *Tecoma stans* and *Ixora coccinea* from control and polluted area were selected for the experiment. The amount of IAA was estimated separately, in the shoot tips and young leaves.

In *Tecomastans*, the concentration of IAA in the shoot tips was 31.25 mg/g FW and 33.3 mg/g fresh weight in the young leaves in the plants growing in the control (unpolluted) zones. Whereas the amount of IAA in the shoot tips was 18.91 mg/g FW and 22.97mg/g. FW in the young leaves in the plants growing in polluted region. A decrease of 29% is seen in shoot tip and 36% decrease was observed in young leaf as compared to the control plant. This decrease in the activity of auxin indicates stress-induced auxin signaling attenuation (Blomster *et al.*, 2011).

In the shoot tips of *Ixora coccinea* 13.3 mg/g F.W of IAA was noted and 5.81 mg/g FW was noted in the young leaves of the plant growing in the control (unpolluted) zone. The plants growing in the polluted region showed 29.76 mg/g FW. in the shoot tips and 9.11 mg/g FW in the young leaf was observed. An increase of 22% in the shoot tips and 57% in the young leaves was observed in plants collected from polluted region as compared to the control plants. Auxin transport has received much attention and the role of polar auxin transport (PAT) by auxin carrier proteins during unstressed conditions and gravitropism has been well established. By contrast, the changes in PAT during abiotic stresses remain largely unknown (Ruud *et al.*, 2018).

### Conclusion

It is clear that plant responses to abiotic stress are complex and involve multiple signaling pathways of growth and physiology. In the present investigation altered production of IAA has been noted in plants growing in the polluted zone as compared to the plants growing in the control zone. In *Tecoma stans*, plants growing in polluted zones showed decreased amount of IAA as compared to the control plants. Whereas *Ixora coccinea* plants collected from polluted zones showed an increase in IAA in both the shoot tips and young leaves.

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**NICE: NETWORK INTRUSION DETECTION AND COUNTERMEASURE SELECTION  
IN VIRTUAL NETWORK SYSTEMS**

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**Abstract**

Basically every industry and even a few sections of the general population part are tackling distributed computing today, either as a supplier or as a buyer. Regardless of being young it's not been unbroken untouched by programmers, hackers and other "criminals" to break into the web servers. Once debilitated these internet servers will function a place to begin for leading any assaults against purchasers within the cloud. One such assault is the Denial of Service (DoS) [1] or its form Distributed Denial of Service (DDoS) attack. Particularly, aggressors can investigate vulnerabilities of a cloud framework and trade off virtual machines to facilitate substantial scale Distributed Denial-of- Service (DDoS). DDoS assaults more often than not include early stage activities, for example, multi- step misuse, low recurrence vulnerability examining, and compromising recognized defenseless virtual machines as zombies, lastly DDoS assaults through the compromised zombies. Inside of the cloud framework, particularly the Infrastructure-as-Service (IaaS) mists, the discovery of zombie investigation assaults is to a great degree troublesome. To keep vulnerable virtual machines from being traded off in the cloud, a multi-stage disseminated vulnerability identification, estimation, and countermeasure determination system called NICE has been proposed, which is based on assault diagram based logical models and reconfigurable virtual system based countermeasures is proposed. The framework and security assessments show the proficiency and adequacy of the proposed arrangement. In our, we will attempt and execute NICE.

**KEYWORDS:** Security in virtual systems, Cloud Computing, Intrusion Detection and mitigation.

**I. Introduction:**

Nowadays usage of cloud has become extremely common amongst technological users as well as corporate users. This extensive usage of cloud has led to an increasing concern in security. Improper use of cloud and cloud resources to access private data and also to deploy attacks on systems is the top trending security issue. Hackers and illegal traders employ vulnerable applications and the reservoirs in cloud to create loopholes in the systems and to the data stored in the cloud. This makes the detection of the hackers difficult in cloud and on the other hand an easy passage for them to escape successfully and unnoticed. In the conventional data centre, where data is stored and monitored in a centralized fashion by a system administrator, the security holes arising due to illegal measures can be patched. However, patching of these holes in cloud, where the users have personalized control over their virtual machines, can lead to the violation of Service Level Agreements (SLAs). Again, the cloud users may themselves install and use vulnerable applications in their data centre which can lead to nefarious use of cloud.

NICE (Network Intrusion detection and Countermeasure selection in virtual network systems)[2] has been proposed to set up a protection inside and out interruption identification structure. For better assault recognition, NICE consolidates obstructing of the specific system address into the interruption identification forms. We must note that the outline of NICE does not plan to enhance any of the current interruption discovery calculations; for sure, NICE utilizes a reconfigurable virtual systems administration way to deal with distinguish and counter the endeavors to trade off VMs, consequently anticipating zombie VMs. In view of the aggregate conduct of VMs, NICE can choose suitable activities. Utilizing this methodology, NICE does not have to piece

activity streams of a suspicious VM in its initial assault stage. The commitments of NICE are exhibited as takes after:

- A new multi-stage circulated system interruption identification and anticipation structure in a virtual systems administration environment that catches and reviews suspicious cloud movement without intruding on clients' applications and cloud administrations.
- NICE consolidates a product changing answer for isolate and review suspicious VMs for further examination and insurance. Through programmable system approaches, NICE can enhance the assault discovery likelihood and enhance the strength to VM misuse assault without interfering with existing ordinary cloud administrations.
- NICE enhances the usage on cloud servers to minimize asset utilization. Our study demonstrates that NICE devours less computational overhead contrasted with intermediary based system interruption recognition arrange.

Additionally, NICE occasionally checks the virtual framework vulnerabilities inside of a cloud server, and after that in light of the seriousness of distinguished vulnerability towards the shared assault objectives, NICE will choose whether or not to put a zombie in system assessment state. Once a VM enters assessment state, Deep Packet Investigation (DPI) is connected, and/or virtual system reconfigurations can be conveyed to the assessing VM to make the potential assault practices unmistakable. NICE is supposed to identify and alleviate collective assaults in the cloud virtual systems administration environment. The arrangement researches how to utilize the programmability of programming changes based answers for enhance the discovery exactness and annihilation casualty abuse periods of synergistic assaults. The framework execution assessment exhibits the attainability of NICE and demonstrates that the proposed arrangement can altogether lessen the danger of the cloud framework from being abused and mishandled by inside and outer assailants. NICE just examines the system IDS way to deal with counter zombie explorative assaults. So as to enhance the discovery precision, host-based IDS arrangements are expected to be consolidated and to cover the entire range of IDS in the cloud framework. We will explore the versatility of the NICE arrangement by examining the decentralized system control and assault investigation model.

We develop a virtualized environment and install Xen server in it to create a cloud environment. Then we deploy two or three virtual machines in them. Each virtual can either run an application or not. These virtual machines are Xen clients. They access the operating system and all the software of the Xen server by sharing their ip address with the server. The basic objective is that at a particular time we will trigger all the virtual machines to launch an attack on a target whose position is irrespective. The target can be in a different server.

The main goal is to synchronize the virtual machines and to make them act as DDOS agents so that they can launch an attack together on the target. The attack needs to be triggered so that the virtual machines need be told when to start sending DDOS attacks. The increased count of attacks coming from the IP addresses within a stipulated time period will lead to the automatic blocking of that IP address. However, if that time span is over, that particular IP address will be able to send requests. Similarly if the load of requests increases, it will be blocked and denied access. At the same time when one or two IP addresses are blocked due to their large number of requests or attacks, other IP addresses can freely access the target. There is also a monitor which will run in the background keeping a record and also displaying the requests come from which IP addresses along with the time of the current and last incoming request.

### **Distributed Denial Of Service (DDoS)**

A DDoS assault is a dangerous attempt to make a server or a system asset distracted to clients, for the most part by briefly intruding on or suspending the administrations of a host joined with the Internet. Distributed Denial of Service (DDoS) attack, in which one PC and one web association is utilized to surge focused on asset with bundles, a DDoS assault utilizes numerous PCs and numerous Internet associations, regularly disseminated internationally in what is alluded to as a botnet.



DDoS attacks are broadly speaking divided into 3 types:

- **Volume Based Attacks:** As cited by Tao Peng, Christopher Leckie, and Kotagiri Rama mohana rao Department of Computer Science and Software Engineering, The University of Melbourne, Australia, volume based attacks incorporates UDP surges, ICMP surges, and other spoofed packet surges. The assault's objective is to immerse the data transfer capacity of the assaulted site.
- **Convention Attacks:** As referred by Jelena Mirkovic, Computer and Information Sciences Department University of Delaware Newark, Peter Reiher Computer Science Department UCLA Los Angeles, CA, convention attacks incorporates SYN surges, divides bundle assaults, Ping of Death, Smurf DDoS. This kind of assault devours genuine server assets, or those of moderate correspondence hardware, for example, firewalls and burden balancers, and is measured in Packets every second.
- **Application Layer Attacks :** As referred by Yonghua You, Queen's University, Kingston and Zulkernine M, application layer attacks incorporates Slowloris, Zero-day DDoS assaults, DDoS assaults that objective Apache, Windows or OpenBSD vulnerabilities. Contained apparently true blue and honest solicitations, the objective of these assaults is to crash the web server, and the size is measured in Requests every second.

### **Specific DDoS Attacks Types**

Some specific and notably in style and dangerous styles of DDoS attacks include:

#### **UDP Flood**

This DDoS assault influences the User Datagram Protocol (UDP), a session less systems administration convention. This kind of assault surges arbitrary ports on a remote host with various UDP parcels, creating the host to over and over check for the application listening at that port, and (when no application is discovered) answer with an ICMP Destination Unreachable parcel. These procedures saps host assets, and can eventually prompt distance.

#### **ICMP (Ping) Flood**

Comparable on a fundamental level to the UDP surge assault, an ICMP surge overpowers the objective asset with ICMP Echo Request (ping) bundles, for the most part sending pings or requests as quick as could be expected. This sort of assault can expend both active and approaching transmission capacity, since the casualty's servers will frequently endeavor to react with ICMP Echo Reply bundles, coming about a noteworthy general framework stoppage.

#### **SYN Flood**

A SYN surge DDoS assault misuses a known shortcoming in the TCP association arrangement (the "three-way hand shake"), whereby a SYN solicitation to start out a protocol association with a bunch should be replied by a SYN-ACK reaction from that hosts, and afterward affirmed by an ACK reaction from the requester. In a SYN surge situation, the requester sends different SYN asks for, yet either does not react to the host's SYN-ACK reaction, or sends the SYN asks for from a satirize IP address. In any case, the host framework keeps on sitting tight for affirmation for each of the solicitations, tying assets until no new associations can be made, and at last bringing about dissent of administration.

#### **Ping of Death**

A ping of death ("POD") assault includes the assailant sending numerous distorted or vindictive pings to a PC. The greatest bundle length of an IP parcel (header) is 65,535 bytes. Nonetheless, the Data Link Layer typically stances points of confinement to the greatest casing size - for instance 1500 bytes more than an Ethernet system. For this situation, a vast IP bundle is part over numerous IP bundles (known as parts), and the beneficiary host reassembles the IP pieces into the complete parcel. In a Ping of Death situation, taking after vindictive control of section substance, the beneficiary winds up with an IP parcel which is bigger than 65,535 bytes when reassembled. This can flood memory cushions designated for the bundle, bringing on disavowal of administration for genuine parcels.

### **Slowloris**

Slowloris is a profoundly focused on assault, empowering one web server to bring down another server, without influencing different administrations or ports on the objective system. Slowloris does this by holding whatever number associations with the objective web server open for whatever length of time that could reasonably be expected. It finishes this by making associations with the objective server, yet sending just a fractional solicitation. Slowloris always sends more HTTP headers, yet never finishes a solicitation. The focused on server keeps each of these false associations open. This in the end floods the greatest simultaneous association pool, and prompts disavowal of extra associations from honest to goodness customers.

### **NTP Amplification**

In NTP Amplification assaults the culprit misuses publically-available Network Time Protocol (NTP) servers to overpower the focused on server with User Datagram Protocol (UDP) activity. In a NTP enhancement assault, the question to-reaction proportion is anyplace somewhere around 1:20 and 1:200 or more. This implies that any aggressor that acquires a rundown of open NTP servers (e.g., by utilizing apparatus like Metasploit or information from the Open NTP Project) can without much of a stretch create an overwhelming high-transfer speed, high-volume DDoS assault.

### **HTTP Flood**

In HTTP surge DDoS assaults the aggressor abuses apparently genuine HTTP GET or POST solicitations to assault a web server or application. HTTP surges don't utilize twisted parcels, ridiculing or reflection strategies, and require less data transfer capacity than different assaults to cut down the focused on location or server. The assault is best when it constrains the server or application to designate the greatest assets conceivable because of every single solicitation.

### **Zero-day DDoS Attacks**

"Zero-day" is simply obscure or new assaults, abusing vulnerabilities that no patch has however been discharged. The term is surely understood amongst the individuals from the programmer group, where the act of exchanging Zero-day vulnerabilities has turn into a prevalent action.

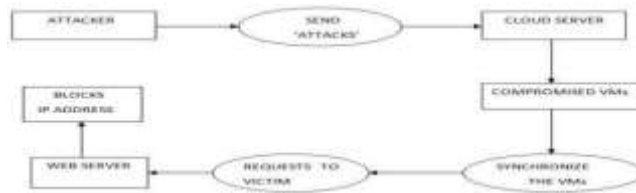
## **II. Literature Survey:**

This segment manages a percentage of the current systems, a review of the work did via analysts in the space of security difficulties and unwanted distributed denial of service attacks crosswise over decentralized systems. The overview of papers is done to know the current methods being utilized for security difficulties like mitigation against such attacks and strategies to prevent them in future. This extensive usage of cloud has led to an increasing concern in security. Improper use of cloud and cloud resources to access private data and also to deploy attacks on systems is the top trending security issue. Hackers and illegal traders employ vulnerable applications and the reservoirs in cloud to create loopholes in the systems and to the data stored in the cloud. This makes the detection of the hackers difficult in cloud and on the other hand an easy passage for them to escape successfully and unnoticed. In the conventional data centre, where data is stored and monitored in a centralized fashion by a system administrator, the security holes arising due to illegal measures can be patched. However, patching of these holes in cloud, where the users have personalized control over their virtual machines, can lead to the violation of Service Level Agreements (SLAs). Again, the cloud users may themselves install and use vulnerable applications in their data centre which can lead to nefarious use of cloud.

As proposed by Duan et al[3] of Florida State University in OpenFlow-Based Intrusion Prevention System in Cloud Environment methods Volume 9 Issue 2, March 2012, ISSN: 1545-5971, techniques were proposed to identify the compromised VM or VMs or the spam zombies. Care was taken to identify the IP addresses and put them in blocked lists. After that, no requests were tended to from those blocked IP addresses. At the same time when requests came from other sources they were allowed easy access. This blocking of IP address temporarily ensured that the target site does not crash and also that the resources are not consumed unnecessarily by attackers



and hackers. The strategies are so devised that the server is not throttled by unending requests, this helps prevent distributed denial of service [4]attacks and helps the server to tend to actual authentic requests.



**Figure1: Architectural Diagram of the Proposed System**

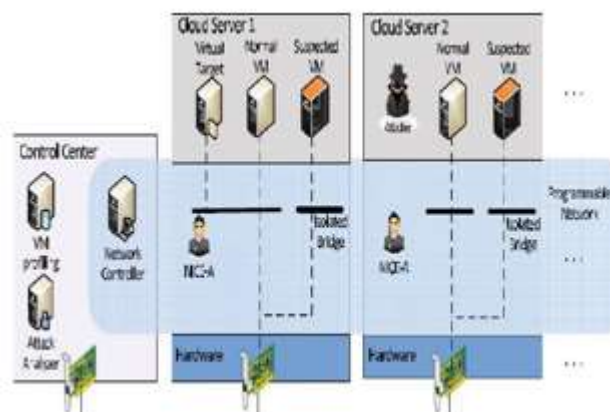
In this system, a stream of requests or attacks is sent one after the other to the target site. The target site is residing on a different server; rather the location of the target site is not important. The main attacker uses compromised virtual machines to coordinate, synchronize and launch an attack on the victim site. After the attack command is issued, the compromised VMs launch an attack. Once the target site notices the innumerable attacks using up all the resources, it blocks those particular IP addresses for a dedicated time span. There are many other techniques to prevent DDoS attacks. After that time span those IP addresses are free to send requests (attacks) again. However, if the same continues, they will be blocked again. During that time when a particular IP address is blocked, other IP addresses are free to send requests. All these steps are taken to ensure that a particular server or site is not blocked by innumerable requests or attacks coming from compromised VMs or zombie machines.

NICE aims to implement a thorough in built defense framework which aims to mitigate DDoS attacks and also take measures to prevent them in the future. It consolidates a product changing answer for isolate and review suspicious VMs for further examination and insurance. Through programmable system approaches, NICE can enhance the assault discovery likelihood and enhance the strength to VM misuse assault without interfering with existing ordinary cloud administrations. It enhances the usage on cloud servers to minimize asset utilization. Our study demonstrates that NICE devours less computational overhead contrasted with intermediary based system interruption recognition arrangement.

**III. System Design: Architectural Design**

The architectural design is concerned with establishing a basic framework of a system. It involves identifying the major modules or sections of the system and communications between these components. In the following sub-sections we delve into the design aspects and the sub systems involved in this architecture.

**Block Diagram**



The block diagram consists of three modules and their functions are described below:

**Attacker module**

The most significant job of the attacker is to synchronize the compromised VMs to launch an

attack on the target. It also sets the time duration for the attack. Also, it receives notifications whether the attack was successful or not.

### Compromised VM module

The compromised VMs or the zombie machines wait for the attack command from the attacker. The command also contains other parameters, namely, which site to attack and the duration of the attack. These VMs wait for response from the target site implying whether the attack was successful or not. This response is then forwarded to the main attacker.

### Web server or target site module

The target site after receiving the request from any sender checks the IP address of the sender. Target server checks the IP address is present in the database or not. If the IP is already present, it checks the last visit time and increment the visit count by one for the IP. If the IP is not present, it stores the IP details and its current time as the last visit time. The target server finds the number of visit count from the IP within five minutes, and if the count is more or equal to the pre-defined threshold value, the target server puts the IP address in the block list and suspends the IP for 10 minutes. For the next ten minutes any request from the compromised VMs IP will be blocked. This disables redundant loss of information and resources since the IP address from where the pings were coming are now blocked [5].

## IV. Implementation:

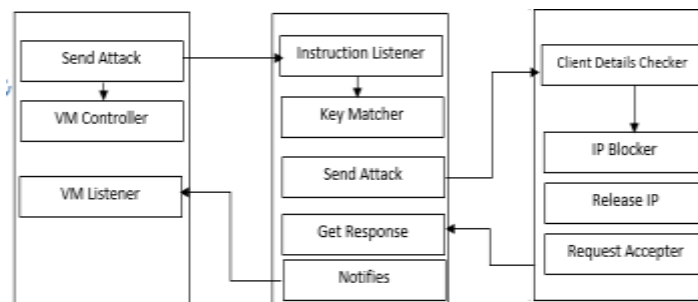


Figure2: Implementation of the Proposed System

Algorithms between the Attacker, Compromised VMs and the Target

#### Algorithm to authenticate the Attacker

- **Step1:** The Attacker launches an attack command to the compromised VMs.
- **Step2:** The compromised VMs or the zombie machines listens to the main Attacker and receives the URL to be attacked and the time duration and the key.
- **Step3:** The compromised VMs verify the key with the Key Server.
- **Step4:** If the key is verified then the authentication is confirmed and the zombies start sending the request to the URL
- **Step5:** The VMs wait for the notification or response from the target server and updates to the attacker.
  - **Step5:** If the key is not matched the compromised VMs does not attack the target server.

Algorithm for communication between attacker, compromised VMs and Victim

- **Step1:** Attacker begins the procedure in the traded off VMs and the procedure begins listening at port number 5001, 5002 and 5003 separately.
- **Step2:** Attacker sets the objective URL, time span of assault and sends the direction to the traded off VMs utilizing TCP/IP convention.
- **Step3:** The traded off VMs gets the direction through the port number.
- **Step4:** The traded off VMs subsequent to accepting the objective URL and term of assault begins sending solicitation to the Victim server utilizing HTTP convention.
- **Step5:** Victim begins getting the solicitations and sends reaction to the traded off VMs utilizing HTTP convention.
- **Step6:** If the quantity of solicitations sent by the zombies surpasses a specific edge, the casualty puts the arrangement of those IP addresses in the blocked rundown for a specific time of time.



- **Step7:** After that time farthest point is over, the traded off VMs or zombies are allowed to send asks for once more. However in the event that they again begin throttling the objective and superfluously devour discriminating assets, they are blocked once more.  
Algorithm for setting the time, duration and URL

- **Step1:** Attacker sets the time of attack as t sec.
- **Step2:** Attacker sets the time duration of attack as d sec.
- **Step3:** Attacker sets the target server URL as url as http request. Step4: Attacker sets the port number 2000 that it is listening to.
- **Step4:** If the time of the compromised VMs are different from the attacker then they set the time difference accordingly so that all of them have the same time

Attacker waits for compromised VMs response and starts listening at port number 2000. Compromised VMs sends response to the attacker through the port number 2000 using TCP/IP protocol.

The above mentioned are the algorithms which have been used to develop the proposed system. The first algorithm was for authentication purpose. We use a key to determine whether the attack command has actually come from the main attacker or not. The compromised VMs check for the key on receiving the attack command, if the key matches, the attack is synchronized and launched, however if the match is unsuccessful, the VMs sit tight.

The second algorithm deals with the communication between the main attacker, the zombie machines and the victim or the target server. We have used socket programming, java and TCP/IP connection to establish a communication between the above mentioned counterparts. The compromised VMs are at port numbers 5001, 5002 and 5003 respectively. They all have different IP addresses too. These attackers keep listening through their port numbers for the attack command. The main attacker sends the target URL or site, the duration of attack to the zombie machines with the help of TCP/IP convention. Upon receiving the attack command, the VMs synchronize and launch an attack on the target URL with the help of HTTP request/response service. That the target site is successfully hit by innumerable pings, the main attacker gets notifications or responses that the attack was successful, this communication is done via HTTP. The target site continuously monitors all the requests it receives. If it discovers that there are innumerable requests coming from one or more than a single set of IP addresses, it then puts those IP addresses in the blocked list for a particular time period. After that time span expires, those IP addresses are free to send requests again. However if they again start throttling the server, and redundantly using all the critical resources so that the target cannot tend to authentic requests, those IP addresses are again blocked for a designated time span. While those IP addresses are blocked, other users can easily access and avail the resources of the target site without any hindrance.

The main attacker which monitors over all the compromised VMs, synchronizes them, makes sure that they are all up and running, listens to port number 2000 for the notifications received if the attack was successful or not.

## **V. Future Work:**

In future we will try to provide better authentication schemes in the target server. This authentication scheme is for the zombie machines to know that the attack command is actually coming from the authentic source. One better scheme will be if we try to implement a priority based customer ID based blocked scheme. The priority based scheme can be achieved based on the premium server. We can spot a premium server over time and by its behavior. This will enable scalability and efficient working since all the data and software will be stored in the premium server.

## **VI. Conclusion:**

NICE (Network Intrusion detection and Countermeasure selection in virtual network systems) has been proposed to set up a protection inside and out interruption identification structure.

For better assault recognition, NICE consolidates obstructing of the specific system address into the interruption identification forms. NICE utilizes a reconfigurable virtual systems administration way to deal with distinguish and counter the endeavors to trade off VMs, consequently anticipating zombie VMs. Additionally, NICE occasionally checks the virtual framework vulnerabilities inside of a cloud server, and after that in light of the seriousness of distinguished vulnerability towards the shared assault objectives, it is supposed to identify and alleviate collective assaults in the cloud virtual systems administration environment. Our study demonstrates that NICE devours less computational overhead contrasted with intermediary based system interruption recognition arrange

NICE just examines the system IDS way to deal with counter zombie explorative assaults. So as to enhance the discovery precision, host-based IDS arrangements are expected to be consolidated and to cover the entire range of IDS in the cloud framework. We will explore the versatility of the NICE arrangement by examining the decentralized system control and assault investigation model.

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## **MALARIA DETECTION USING DIFFERENT DEEP LEARNING MODELS**

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**Abstract** - Malaria is a female Anopheles mosquito-borne disease that transmits a motile infective form to the host body like humans, which reproduce asexually within the blood cells of the host. The standard symptoms of malaria are fever, headache, tiredness, and vomiting. In severe cases may cause coma and death. During this research, we used deep neural networks to detect the malaria virus in human blood cells. Traditional malaria detection techniques require experts to check blood cells under a microscope. The proposed method during this research shows a system with end-to-end automated models employing a deep neural network that performs both feature extraction and classification using blood smear cell images. During this research, we got used VGG-19, ResNet-50, DenseNet, MobileNet, and a base model to suit the information to find the most effective performing model.

**Keywords** – Malaria, Deep Neural Networks, Feature Extraction, Classification

### **I. INTRODUCTION**

Malaria is classified as a contagion contamination this is due to a unmarried-celled microorganism it in truth is belonging to the genus protozoan parasite of the plasmodium business enterprise in which 5 of their species can infect human beings. the disease is specifically spread via the imply of chew-inflamed girl anopheles' mosquitoes. based totally on latest information the malaria disorder puts around 40% of the planet population to danger with nearly 240 million instances pronounced yearly, Africa and specifically the sub-Saharan- Africa nations are the foremost way to malaria. typically, there are two fundamental medical strategies commonly accustomed diagnose malaria microscopy of skinny blood cells and an antigen diagnostic exam. the preceding can be a completely time ingesting operation, generally with doctors not less than 5000 cells have to be diagnosed manually to validate, the situation, although the latter is some distance quicker than the previous one, the antigen-based speedy diagnostic assessments are much less powerful.

This research focuses on designing an accurate malaria diagnosis model that may be implemented without any dependencies on skilled technicians and testing the model accuracy to induce high-quality results. Automated image analysis software could remove the foremost serious limitation of the worldwide accepted microscopy method in normal, dependency on human experts for diagnostic accuracy of the results. Automating the detection process means using the knowledge, the practice of conventional methods, and implementing it to get fast and efficient results.

Machine learning is a field of computer science where decisions are made by analyzing data and reading the info to urge the specified output. Within the past decade, the health sector has seen a big growth, lots of research is ongoing to form healthcare automated to create the method easily available to everyone.

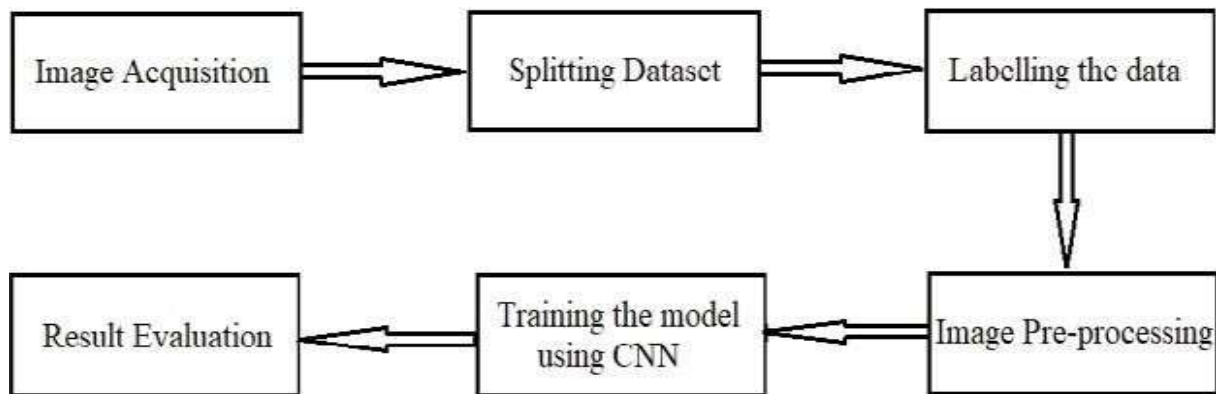
To automate the diagnosis process, much researches are conducted on various machine learning

models. Machine learning models require plenty of tuning, correlation analysis, and feature engineering. The machine learning method isn't scalable with more data provided. Machine learning requires feature engineering and have training which isn't a handy tool. Deep learning models are reliable and simply scalable with a better accuracy rate.

Currently, with the event of AI-primarily based systems which are called laptop-aided analysis or selection guide systems, malaria detection the use of blood films became extra efficient one in all of the maximum present day AI techniques is Deep learning which might be accustomed classify cellular photographs and assist to forestall incorrect diagnostic selections.

CNN is a type of neural network which is mainly used for images and a lot of improvement is going on inside the mission of DL, and it is used considerably within the situation of CV for the prognosis of clinical ailments and especially for statistics which can be supported pics.

## II. WORKFLOW



**Fig. 1. Block Diagram**

In the Image acquisition phase, we acquire the malaria dataset from NIH website. The data consists of microscopic blood cell images which consists of parasitized and un-infected images.

In the splitting dataset phase, we split the dataset into training and testing so we will fit the algorithm on training data and evaluate the model using testing data.

In the pre-processing phase, we pre-process the image so as to suit that input image to the model.

In the Training phase we fit different models on our data like ResNet, DenseNet, MobileNet, VGG-19 on our malaria dataset.

In the Result Evaluation phase, we evaluate the results using confusion matrix.

## III. METHODOLOGY

### Setting Up the Cloud

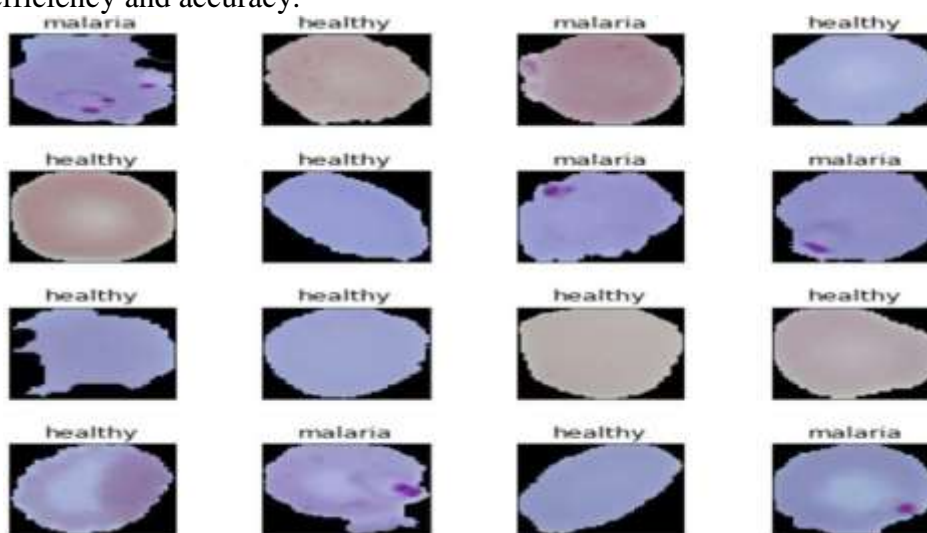
This research focuses on comparing performances of various deep Convolutional Neural Networks that need high computational power for execution. The Graphics Processing Unit (GPU) or multiple CPU computation is required to run deep neural networks. during this research, we used to google colab to perform operations on the malaria dataset.

### Pre-processing

This section will help understand all the pre-processing techniques utilized in the research to record the model performance difference. The malaria dataset consists of 27,557 images of which 13,778 are parasitized images and 13,779 are non-infected images. We merged files of both non-infected images and parasitized images and labelled them as healthy and malaria for the respective files. The dataset is split into three sets for training, validation, and testing.



The ratio of a train to validation to check is 63:7:30. we decide to require only 63% of dataset as training to avoid high computational intensity because of the big dataset. The training dataset consists of 63% of the dataset with 8707 healthy images and 8653 malaria images, the validation dataset consists of seven with 1001 healthy and 928 malaria images, and therefore the testing dataset is 30% of the full dataset having 4144 and 4124 healthy and malaria images, respectively. After analyzing the dataset, images within the dataset have different dimensions varying over the dataset. Scaling images up gives a bonus in performance but also requires lots of computation time and memory space. it's better to keep up this trade-off of accuracy and computation. during this research, we decided to resize all the photographs. This resulted during a better classification score with a good processing speed. Due to the fact neural networks get maintain of inputs of the same period, all snap shots need to be resized to a hard and speedy length in advance than inputting them to the CNN. The bigger the fixed size, the much less shrinking required. Less shrinking means much less deformation of features and styles within the picture. This will mitigate the class accuracy degradation way to deformations. But, huge pictures not handiest occupy greater area within the memory however additionally cause a larger neural community. Therefore, developing each the space and time complexity. It's far obvious now that deciding on this regular size for images can be a depend of change-off amongst computational efficiency and accuracy.



**Fig.2: Malaria Dataset**

### **Normalization**

Normalization is a vital pre-processing task that minimizes the colour and variation intensity present in stained input images from different laboratories. in line with past 20 research, stain normalization has proven to significantly increase the accuracy of the unseen dataset by approximately eight percent. during this research, the pictures are collected from the pre- existing dataset of human blood cells which is ready from laboratory examination. The smear slides are prepared within the laboratory using various chemical stains which ends in colour variation thanks to the utilization of various chemicals and marking procedures. This staining leads to model learning and handling more complex models with a various set of images results in maximizing the error rate. an answer to standardize this is often normalization.

Stain Normalization could be a common pre-processing technique that attempts to cut back colour variability and improve the generalization of algorithms by transforming the input file into a standard space. In stain normalized digital pathology samples, regions of virtual tissue specimens are mapped to comparable colour characteristics irrespective of the scanning device, stain supplier, and coaching protocols. Way to the reduced variability in colour characteristics of tissues, stain normalization has established development in pc-assisted diagnostic tools.

In this research, we've got implemented stain normalization within the training and validation dataset

while leaving the test dataset untouched. After implementation as shown in plots the range of images is transformed into a narrower range and it's evident that the semantic meaning of the pictures is preserved.

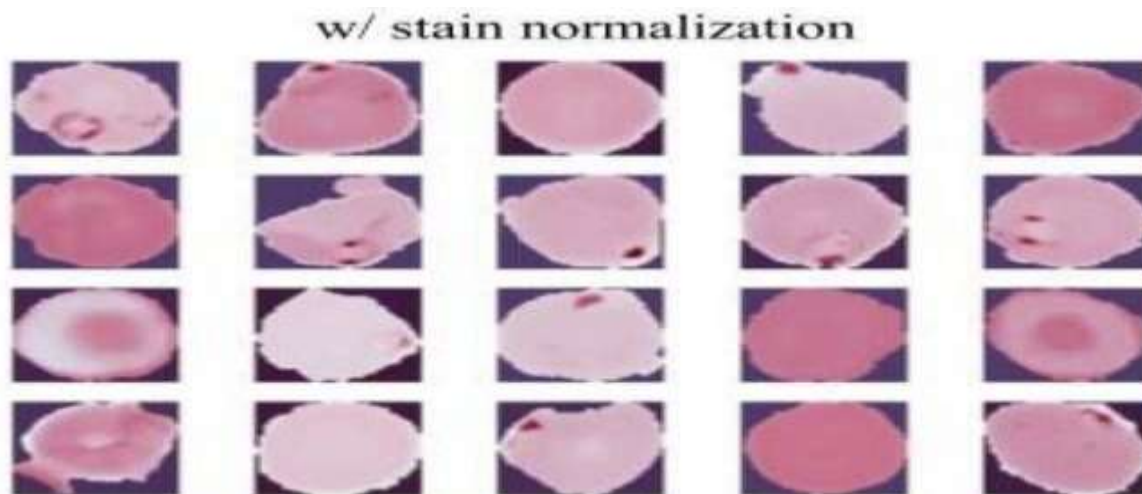


Fig.3: Cells with Stain Normalization

#### IV. Model Architecture and Implementation

##### Transfer Learning

Transfer Learning could be a feature that allows users to transfer the knowledge of pre-trained models and use it in their own problem set. rather than creating a model from scratch during this research, we used the models that are trained on large datasets like ImageNet with 100,000 data points and explored the ability of transfer learning which is proven to be significant in many image classification sorts of research.

##### VGG-19

vgg-19 can be a convolutional neural network it is 19 layers deep. VGG-19 is one variant of the VGG model. there are different variations of vgg like VGG-eleven, VGG- 16, and others. vgg was created by using the visual geometry group at oxford and as a result the name VGG. It uses deep convolutional neural layers to beautify accuracy. VGG-19 Architecture consists of 16 convolutional layers and three fully connected layers with five max-pool layers.

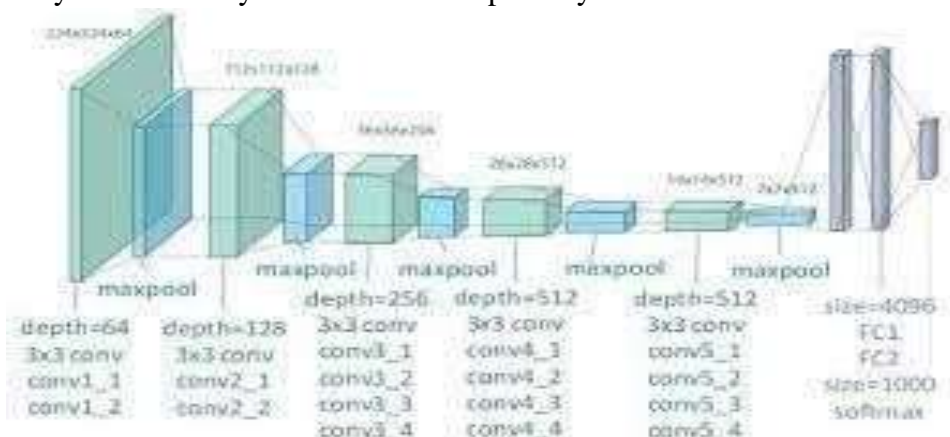
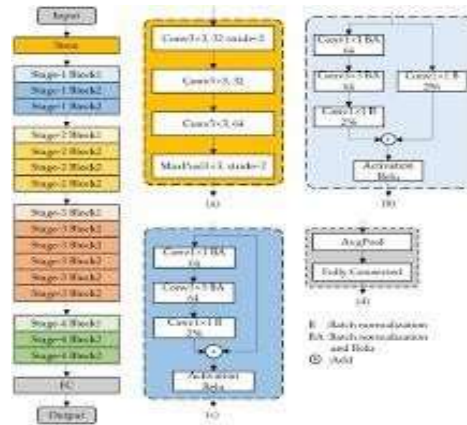


Fig 4: VGG – 19 Architecture



**ResNet**

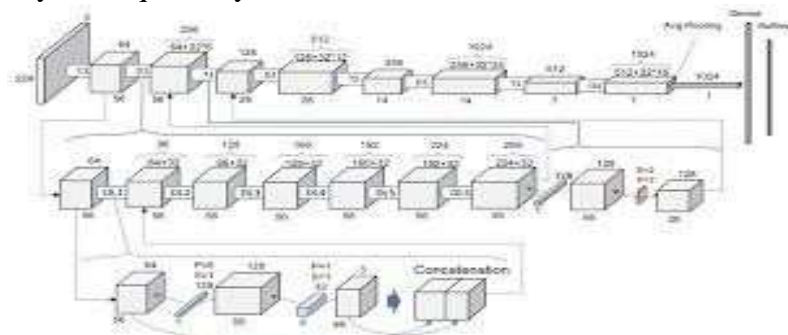
ResNet-50 is a convolutional neural network which is 50 layers deep. You can load a pretrained model of the network trained on quite 1,000,000 images from the ImageNet database. The pretrained network can classify images into 1000 object categories, like keyboard, mouse, pencil, and many animals. As a result, the network has discovered rich function representations for a large variety of pics. It has an photograph length of 224 by 224.



**Fig 5: ResNet Architecture**

**DenseNet**

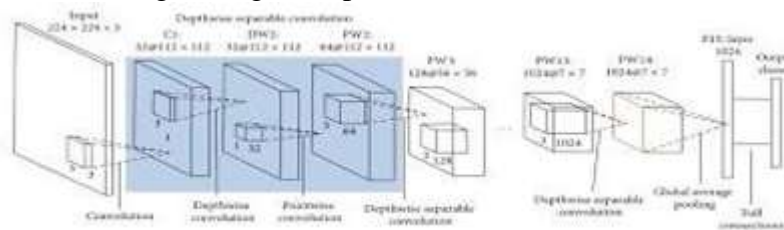
DenseNet might be a style of network that utilises dense connections between layers, through dense blocks, wherein we connect all layers directly with each other. To hold the feed-ahead nature, every layer obtains additional inputs from all preceding layers and passes on its personal characteristic-maps to all or any subsequent layers.



**Fig 6: DenseNet Architecture**

**MobileNet**

it is designed to be accomplished in mobile programs, and its tensor waft's first cellular imaginative and prescient version. It makes use of intensity sensible separable convolutions. it notably reduces the quantity of parameters as compared to the network with ordinary convolutions with same depth inside the nets. This leads to light weight deep neural networks.



**Fig 6: Mobile Architecture**

**V. RESULTS**

Research is completed on human red corpuscle smear images to classify as infected or healthy. The research uses different pre-trained Convolutional Neural Networks with transfer learning and fine-tuning them on the malaria dataset and recording their performances.

Research reveals that after pre-processing input images and fitting the various pre-trained models on that, different models gave different results.

The ResNet model gave accuracy of 92.72% with 87% precision and 75 recall. The VGG model gave accuracy of 90.43% with 84% precision and 67% recall. The MobileNet model gave accuracy of 93.06% with 90% precision and 74% recall. The DenseNet model gave accuracy of 92.85% with 90% precision and 76% recall. The base model gave accuracy of 94.38% with 94% precision and 63% recall.

The following table shows the results of the analysis with respect to different models:

ModelName	Accuracy	Precision	Recall	F!- Score
ResNet	92.72	87	75	81
VGG	90.43	84	67	75
MobileNet	93.06	90	74	81
DenseNet	92.85	90	76	82
Proposed	94.38	94	63	85

**Fig 7: Model Evaluation Results**

The following image is the front-end of the malaria detection web application



**Fig 8: Front End**

Then we provide the positive data sample to the web application and then the following is the output of that data sample



**Fig 9: Predicting Parasitized Image**



Then we provide the negative data sample to the web application and then the following is the output of that data sample



**Fig 10: Predicting Un-Infected Image**

## **VI. CONCLUSION**

In this research, we experimented with end-to-end deep learning neural networks to boost malaria diagnosis classification performance. We showed that pre-processing techniques like normalization, standardization, and marking don't contribute much to improving performance. Instead, methods like data augmentation showed positive results by increasing the performance of the models. We also compared different models like VGG-19 and ResNet-50 and their performances. We established VGG-19 and ResNet-50 models from scratch and used transfer learning and hyper tuning the parameters. Transfer learning could be a great technique and will be wont to gain satisfactory performance compared to machine learning models that need plenty of feature scaling and engineering. In future works, we attempt to specialize in the specification of the models employed in the research to seek out the driving performance factors. We plan on finding ways to enhance the performance by manipulating the specification and hyper tuning the features to realize an excellent better-performing model.

## **VII. FUTURE ENHANCEMENTS**

To develop a system satisfying the user needs isn't possible at one go. we want to upgrade the applying. a number of the long run enhancements of this method are:

- Optimizing the pre-trained models so as to extend its accuracy and precision.
- Deploying the online application to the 000 world where it may be utilized by anyone within the real time situation after optimizing the model.

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