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Blanching: A sustainable and effective treatment for extending shelf life of bamboo shoots

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ABSTRACT

Bamboo shoots are widely consumed in fresh, fermented, canned and dried forms in various parts of the world. They are not just a good source of antioxidants and phytonutrients but also possess medicinal properties. However, as they are highly perishable, physiological changes such as browning and lignification lead to deterioration affecting the commercial value of the product. Therefore, in this study, bamboo shoots were subjected to water and steam blanching, as a heat treatment for 2, 4, 6, 8 and 10 min at 100 °C and then immediately cooled in ice water for 2 min. The treated and control samples were analysed for the presence of enzymes PAL, POD, PPO, phenolic content and MDA and see the changes in the color of the bamboo shoot. The results of the study showed there is a reduction in enzymes like Peroxidase (POD), phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO) and phenolic content which inhibit lignifications process. There was an increase in malondialdehyde (MDA) content suggesting lipid peroxidation with both types of blanching. Steam blanching was found to have a more significant ($p < 0.05$) effect to preserve the freshness of the bamboo shoots and prolongs the shelf life of the product by inactivating enzyme activity.

1. Introduction

Bamboo is one such long-lived, woody-stemmed perennial grasses which is strong, versatile and highly renewable species. It is found in moist deciduous, semi-evergreen, tropical, subtropical and temperate areas of forest (Tewari et al., 2019; Diver, 2001). More than 1250 species belonging to 75 genera have been reported to be distributed worldwide. Bamboo shoots exhibit a great potential as a food resource and is one of the commonly collected, consumed and sold nutritious vegetable amongst the tribal and rural communities of the North Eastern India. They are consumed in fresh, fermented, canned and dried forms (Singhal et al., 2017). They have been reported to be a good source of nutrition being high in fiber and low in fat. They are not only a storehouse of nutritional elements but also contain some important antioxidants and medicinal components which can help prevent the onset of metabolic disorders (Singhal et al., 2013).

Women of developing and tribal societies play significant role in cultivating and accessing food resources from diverse ecosystems to achieve food and nutritional security (Singh et al., 2007). Sustainable Development Goal (SDG) 2 emphasizes significance of providing the food and nutrition security to the citizens in order to end hunger. Bamboo shoot is one such food which is a part in women's development through livelihood and entrepreneurship and provides sustainable solution to food security from diverse ecosystems and thereby establishes a strong coherence with Sustainable Development Goal (SDG) 2 to end hunger, SDG 5 to gender equality, SDG 13 to climate change and SDG 15 to life on land.

Bamboo shoots being highly perishable are edible only for few days. Physiological changes such as browning and lignification leads to deterioration which affects the commercial value of bamboo shoots. Chemical changes such as enzymatic browning is a complex process involving the action of several enzymes like Polyphenol oxidase (PPO), Phenylalanine ammonia-lyase (PAL) and Peroxidase (POD) **which** facilitate the synthesis of by participating in a chain of oxidation process of the phenolics (Shen et al., 2006; Luo et al., 2007).

The quality of the shoots can be maintained and the shelf life can be extended by delaying the enzymatic browning and lignification process (Badwaik et al., 2015).This can be done by inhibiting the enzymatic activity by applying heat treatment. Blanching is a cooking process which inactivates various enzymes, helps in softening the tissues and also

Abbreviations: POD, Peroxidase; PAL, Phenylalanine ammonia-lyase; PPO, Polyphenol oxidase; MDA, Malondialdehyde; SDG, Sustainable Development Goal; TERI, Tata Energy Research Institute; TPC, Total phenolic content; ΔE, Total color change; GAE, Gallic Acid Equivalent; CA, Cinnamic Acid; FW, Fresh Weight.

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increases the shelf life by reducing the microbial attack (Ruiz-Ojeda & Peñas, 2013). Therefore it becomes important to investigate the activity of enzymes like PAL, POD, PPO and MDA to determine the effectiveness of the blanching process. Blanching may cause undesirable changes on the physical properties such as color and texture because of the heat induced (Sanjuan et al., 2005). Therefore in the present study comparison of two types of blanching methods is done to see the effect on the enzymes PAL, POD, PPO, phenolic content and MDA and see the changes in the color of the bamboo shoot.

2. Material and methods

2.1. Bamboo shoot procurement and sample preparation

Bamboo shoots of *B. vulgaris* species was procured from TERI Gram, Haryana. Outer sheath of the shoots was removed and the soft, white, young and tender portion was washed and air-dried. Bamboo shoots were subjected to water and steam blanching. The untreated bamboo shoot sample was taken as control. The treatment was done in triplicates.

2.1.1. Water blanching

Hundred grams of shoots were blanched in 300 mL of boiled water at 100 ˚C containing 0.5% NaCl for 2, 4, 6, 8 and 10 min in case of water blanching. It is then immediately cooled in ice-cold water for 2 min. Excess water was drained and the sample was analysed

2.1.2. Steam blanching

It was performed on a double boiler. Hundred grams of shoots were kept on a perforated plate through which steam can pass. Three hundred mL of water was boiled at 100 ˚C containing 0.5% NaCl for 2, 4, 6, 8 and 10 min. It is then immediately cooled in ice-cold water for 2 min. Excess water was drained and the sample was analysed for the enzyme activity, phenol content and color.

2.2. Enzyme extraction

All steps of enzyme extraction were carried out at 4 ˚C. Five grams of the homogenized bamboo shoot was extracted with 0.1 M phosphate buffer pH 7 containing 5 g of polyvinylpyrrolidone (PVP) using a magnetic stirrer for 15 min. The homogenate was filtered through filter paper and then centrifuged at 10,000 g for 20 min at 4 ˚C. The supernatant was filtered and collected as an enzyme extract.

2.3. Enzyme assays

2.3.1. PPO

To measure PPO activity phosphate buffer solution pH 7 (0.1 M, 1.95 mL), 1 mL of 0.1 M catechol as a substrate and 50 μL of the enzyme extract were pipetted into a test tube and mixed thoroughly. Then the mixture was rapidly transferred to cuvette. The absorbance was taken at 410 nm continuously at 25 ˚C for 5 min. One unit of PPO acidity corresponded to the change of 0.1 absorbance units in 1 min.

2.3.2. POD

For POD activity, the reaction mixture contained 0.15 mL of 4% (v/v) guaiacol, 0.15 mL of 1% (v/v) H_2O_2, 2.66 mL of 0.1 M phosphate buffer pH 7 and 40 μL of the enzyme extract. The blank sample contained the same mixture solution without the enzyme extract. The absorbance was measured at 470 nm. POD activity was defined as a change of 0.01 absorbance units in 1 min.

2.3.3. PAL

For PAL activity, the bamboo shoot tissue (5 g) was homogenized in 10 ml 5 mM chilled sodium borate buffer (pH 8.8), containing 5 mM b-mercaptoethanol and 0.5 g polyvinylpyrrolidone (PVP). After centrifugation at 10,000 *g* for 20 min at 4 ˚C, the supernatant was assayed for PAL activity (Ouyang & ying, 1985). PAL activity was determined by the production of cinnamate for 30 min at 30 ˚C with continuous shaking, measured by the absorbance change at 290 nm. One unit of PAL activity corresponded to the formation of 1 lM cinnamic acid in 1 h.

2.4. Detremination of MDA content

Tissue samples (1.0 g) were homogenised in 4.0 ml of 5% (w/v) trichloroacetic acid (TCA) and centrifuged at 10,000 g for 20 min. The supernatant was mixed with 2.0 ml of 0.67% TBA, heated at 100 ˚C for 30 min and then immediately cooled on ice. After centrifugation at 5000 g for 10 min, absorbance of the supernatant was measured in a spectrophotometer at 450, 532 and 600 nm. The MDA concentration was calculated according to the formula: $6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$.

2.5. Total phenolic content (TPC)

The samples were homogenised in 80% ethanol. The extract was centrifuged at 10,000 g for 20 min at 4 ˚C. The residue was re-extracted under the same conditions. The supernatant was pooled together and the methanolic extract was used for estimation of TPC. One-hundred microlitres of the methanolic extract was diluted to 3 mL with distilled water and 0.5 mL of Folin–Ciocalteau reagent was added. After 3 min, 2 mL of 20% of sodium carbonate was added and the contents were mixed thoroughly. The color was developed and absorbance measured at 765 nm after 30 min using gallic acid as a standard. The results were expressed as mg gallic acid (GAE) /100 g of dry weight material.

2.6. Color

Color of dried samples was evaluated with a Hunter Lab colorimeter (Model No. Miniscan XE plus 4500 L, Hunter Associates Laboratory, Inc., VA, USA). The instrument (45˚/0˚geometry, D 65 optical sensor, 10˚ observer) was standardized with black and white reference plates (No. LX-17,760, $X = 79.30$; $Y = 84.11$; $Z = 88.94$). The results were expressed as Hunter L (whiteness/darkness), a (red/green), and b (yellow/blue) values. In addition, the total color change (ΔE) was calculated from the L, a and b values and was used to describe the color change.

2.7. Data analysis

Data is presented as mean ± standard deviation. The differences between treatments were analyzed using ANOVA. Significant differences between mean values were determined using Duncan's Multiple Range Test ($p < 0.05$). All statistical analysis was performed using Microsoft Excel 2013.

3. Results and discussion

Blanching is one tool which inactivates enzymes and thus delays browning and lignification process. Bamboo shoots were subjected to water and steam blanching for 2, 4, 6, 8 and 10 min and the effect on the activity of PAL, POD, PPO, phenolic content and MDA was studied. The effect of blanching on the color attribute of bamboo shoot was also observed.

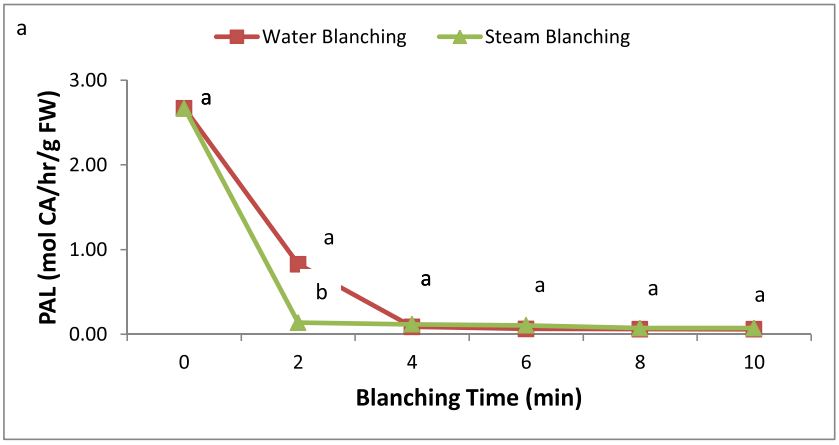

Fig. 1. Effect of blanching treatments on PAL. Results are means of triplicate ± standard deviation. Different letters on top indicate significant differences ($p < 0.05$).

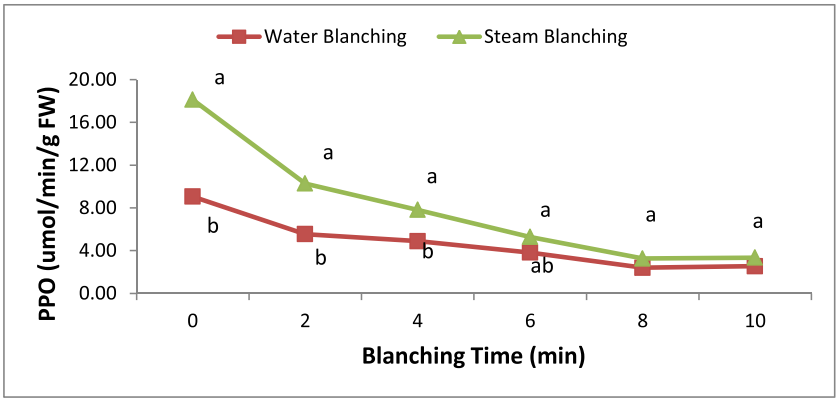

Fig. 2. Effect of blanching treatments on PPO. Results are means of triplicate ± standard deviation. Different letters on top indicate significant differences ($p < 0.05$).

3.1. Effect of blanching on PAL, POD, PPO

PAL is the key enzyme of lignin biosynthesis and phenolic components (Assis et al., 2001; Luo et al., 2012). Increase in its activity leads to the synthesis of phenols and thus blanching treatment to bamboo shoots inactivates this enzyme leading to the reduction of phenolic components. In the present study its activity decreased both with water and steam blanching as compared to the fresh bamboo shoots which had the maximum PAL activity of 2.67 mmoles CA/hr/g fresh wt. Water and steam blanching reduced the PAL activity in 2 min by 69% and 95% respectively. It is clear from Fig. 1 that steam blanching had a more significant effect ($p < 0.05$) on the PAL activity as compared to water blanching. The results of the study are in alignment with a recent study by Zheng et al., 2014 in which the blanching treatment decreased the PAL activity dramatically within 9 min. Heat treatment at 45 °C for 5 h significantly delayed the rise in the activities of PAL and POD (Luo et al., 2012)

In the present study effect of blanching time was studied in fresh bamboo shoots and observed that 12 min of blanching at 95 °C inactivated the PPO enzyme (Fig. 2). In the present study the fresh shoots exhibited a PPO activity of 9.06 umol/min/g FW. Steam and water blanching reduced the PPO activity by 91.2% and 72.2% respectively at 10 min. Therefore, steam blanching was found to have a more inhibitory effect than water blanching on PPO activity. Similar results were reported by Zheng et al., 2014 where PPO activity of fresh bamboo shoots decreased by 77.98% after 9 min and it completely inactive at 12-min blanching treatments. Effect of blanching on PPO has been studied in potato (Sotome et al., 2009), brussel sprouts (Olivera et al., 2008a, 2008b), peas (Lin & Brewer, 2005) and many other vegetables.

POD enzyme is conventionally used evaluate the extent and accuracy of heat treatment extent (Goncalves et al., 2007). POD activity decreased in both types of blanching although steam blanching was found to be much more significant ($p < 0.05$) and effective in reducing the POD activity (Fig. 3). Two minutes of steam blanching could decrease the enzyme activity by 67.2% where as water blanching could decrease only by 15.7%. Results were found similar to study by Zheng et al., 2014 where no POD activity was detected at 6 min suggesting that it is a heat sensitive enzyme.

3.2. Effect of blanching on MDA

Lipid peroxidation takes place when the plant cells are wounded by external environment, which would cause accumulation of MDA. So MDA content is associated with the degree of lipid peroxidation. In the present study, MDA content increased with blanching time in both water and steam blanching, with steam blanching showing a significant ($p < 0.05$) increase (Fig. 4).

3.3. Effect of blanching on the total phenolic content

Phenolic compounds commonly found in different parts of plants have been reported to possess antioxidant properties (Udayakumar et al., 2010). Browning of fruit and vegetables was related to increasing PAL activity and increased levels of phenolic compounds (Nguyen et al., 2003). In the present study, the phenolic content in the fresh bamboo shoot sample was found to be 1.21 mg/g FW (Fig. 5). The content decreased with both steam and water blanching, although steam blanching showed a significant reduction ($p < 0.05$) in a lesser time. Similar results were reported by Cano et al., 1997 where a significant reduction in the phenol content was observed upon steam and microwave blanching of banana slices. Another report by Zheng et al., 2011 found out that total phenolic contents in both boiled

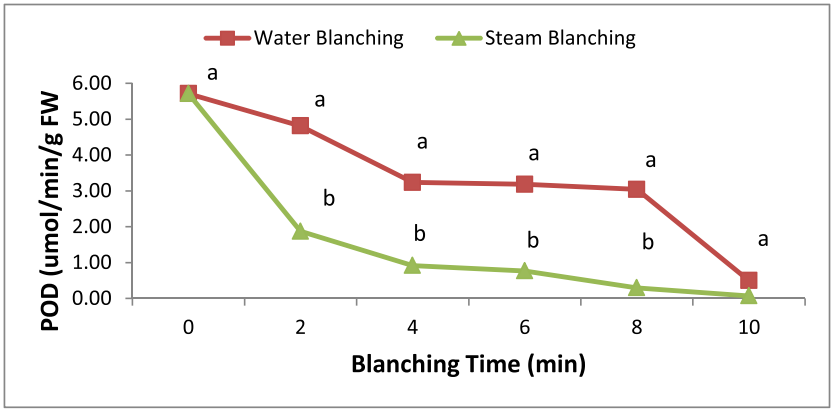

Fig. 3. Effect of blanching treatments on POD. Results are means of triplicate ± standard deviation. Different letters on top indicate significant differences ($p < 0.05$).

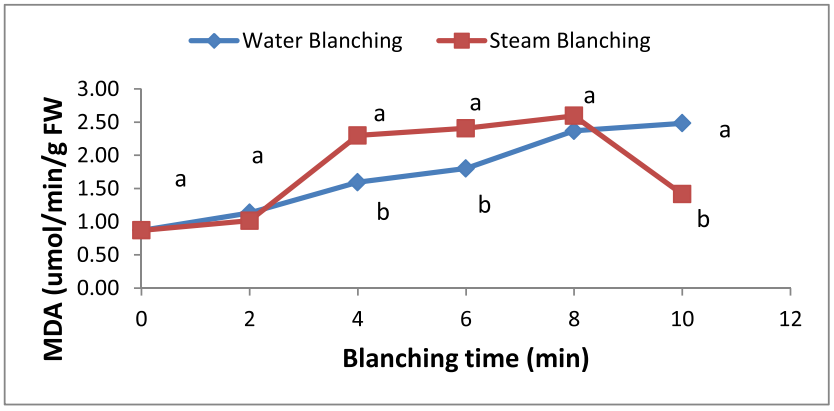

Fig. 4. Effect of blanching treatments on MDA. Results are means of triplicate ± standard deviation. Different letters on top indicate significant differences ($p < 0.05$).

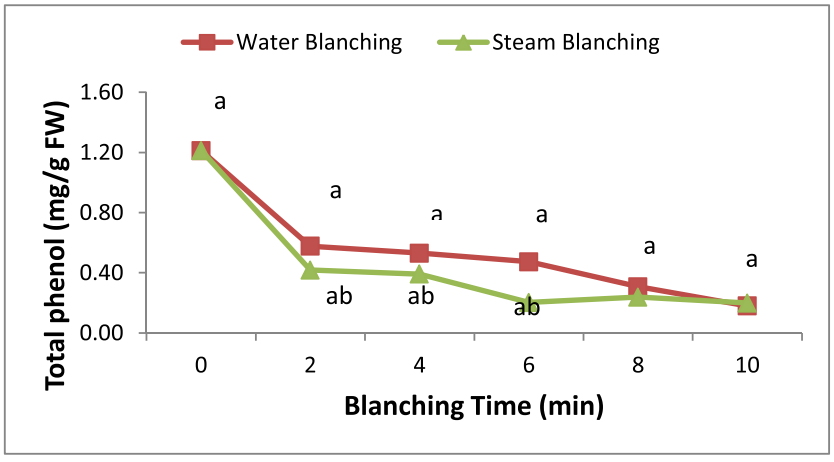

Fig. 5. Effect of blanching treatments on the total phenol content. Results are means of triplicate ± standard deviation. Different letters on top indicate significant differences ($p < 0.05$).

and stir-fried samples decreased slightly. Fresh bamboo shoot contained 101.65 mg/100 g of the total phenolic content which degraded when it was blanched at 75, 85 and 95 °C for 10, 20 and 30 min (Badwaik et al., (2015)).

3.4. Effect of blanching on color

In the present study, significant changes ($p < 0.05$) were observed in the *L*, *a* and *b* values indicating that the color of bamboo shoots is affected by blanching treatment (Table 1). *L* value was found to decrease suggesting reduced lightness with both water (69.7) and steam (68.2) blanching. The *a* value was found to increase with blanching time suggesting increased redness. The *b* value decreased with blanching time reaching to a value of 16.7 and 17.1 at 10 min of water and steam blanching respectively as compared to 25.8 of the control sample. The total color difference (ΔE) is an index of color change during blanching treatment. The color change of greater than 2 is visible. According to our study ΔE was found to increase with blanching time and reached to a value of 18.8 and 18.7 in case of water blanching and steam blanching respectively. In a report by Cano et al., 1997 steam-blanched banana slices showed the greatest total color difference compared to the untreated samples. According to Zheng et al., 2014, the *L* and *a* values

Table 1
Effect of blanching treatments on the color of bamboo shoots.

Treatments	Blanching Time (min)	*L*	*a*	*b*	Δ*E*
Control		84.40±1.62^{a}	−3.56±0.08^{f}	25.84±0.88^{a}	0
Water Blanching	2	81.30±6.01^{ab}	−2.42±0.03^{e}	19.36±0.94^{bc}	10.10±3.52^{b}
	4	77.00±4.67^{ab}	−2.01±0.12^{d}	19.62±1.05^{bc}	10.84±4.22^{b}
	6	76.49±2.12^{ab}	−2.05±0.03^{d}	21.71±3.38^{ab}	9.92±1.39^{b}
	8	74.29±3.06^{ab}	−1.38±0.01^{c}	16.56±0.47^{bc}	13.95±0.76^{a}
	10	69.70±8.27^{ab}	0.33±0.09^{a}	16.68±1.21^{bc}	18.85±7.58^{a}
Steam Blanching	2	80.33±7.67^{ab}	−2.49±0.07^{e}	20.54±1.20^{bc}	10.76±4.54^{b}
	4	76.49±2.94^{ab}	−2.05±0.04^{d}	21.71±1.15^{ab}	9.37±0.21^{b}
	6	73.68±1.12^{ab}	−1.22±0.11^{c}	16.14±1.95^{c}	14.88±1.27^{a}
	8	71.77±1.59^{ab}	−0.66±0.10^{b}	17.76±0.5b^{c}	15.29±3.37^{a}
	10	68.23±3.91^{b}	−2.01±0.15^{d}	17.07±1.26^{c}	18.71±4.59^{a}

Results are means of triplicate ± standard deviation, expressed on fw basis. Different alphabets on the bar heads denote significant differences ($p < 0.05$).

of bamboo shoot decreased, while value of *b* increased and ΔE greater than 2.

4. Conclusion and recommendations

Bamboo shoots is a local vegetable eaten mostly in the North Eastern region amongst the tribal communities. It is a nutritious vegetable possessing antioxidant properties and is often known to be a functional food by most of the researcher's view. As we all are aware that consumption of bamboo shoots in various forms is getting globally popular not just because of its nutritional profile, but also because of its important role in providing food security, combating malnutrition and micronutrient deficiencies. But because of its perishable nature, it deteriorates very fast showing browining and lignification owing to the physiological changes due to enzymatic activity. Therefore the present study focuses on using a simple and cost-effective technique of using blanching as a tool to delay the post-harvest changes in bamboo shoot. It became clear from the study that blanching can be an effective treatment for preserving the vegetable for long and retain freshness, though steam blanching showed greater reduction in the enzymes with time as compared to the water blanching treatment.

Other vegetables like peas, carrots, corns are blanched and available commercially throughout the year and are not just limited to the seasonal availability. Likewise bamboo shoots with all the nutritional and medicinal benefits can be preserved with industrial inputs and processing technology and should be commercialised in view of the research attempts. Blanching is a very simple and cost-effective technique which can be used both at the domestic and industrial level for producing commercial bamboo shoot products which have a great potential in the global market. Government of India is already promoting bamboo and its products on all the platforms and under the MNREGA, employment and livelihood schemes the rural women can be trained to produce and sell bamboo shoots using proper processing techniques. Also the use of bamboo shoots contributes to achieving the SDG on zero hunger and building healthy nation.

Ethical approval and consent to participate

Not Applicable

Consent for publication

All the authors have given the consent to publish the study.

Availability of data and materials

Not applicable

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.

CRediT authorship contribution statement

Poonam Singhal: Conceptualization, Methodology, Writing – original draft, Visualization, Investigation. **Santosh Satya:** Writing – review & editing. **S.N Naik:** Writing – review & editing.

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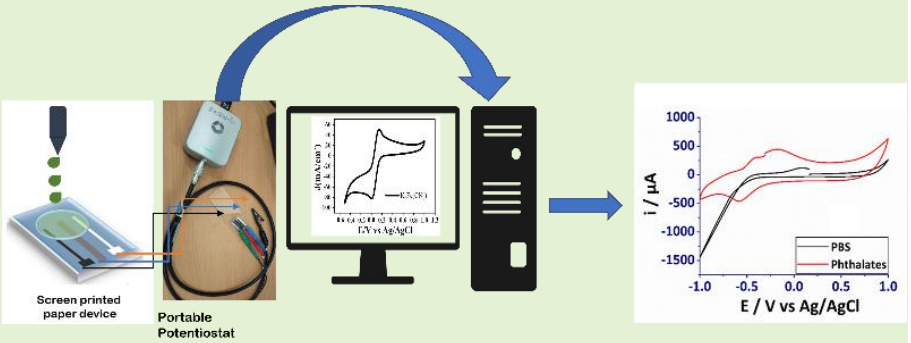
Disposable Paper Based Miniaturized Device for Sensing of Phthalates

Jaligam Murali Mohan[1], Sanjeet Kumar[1], Khairunnisa Amreen, Arshad Javed, Satish Kumar Dubey and Sanket Goel, *Senior Member, IEEE*

***Abstract*—One of the most frequent plasticizers that can cause endocrine disruption is phthalates. The phthalates are utilized in the production of consumer plastic items, leading to increase the risk of exposure of the items stored in water bottles, beverage drinks and food. Further, bioaccumulation and harmful consequences amongst animal life is also prevalent. Therefore, detection of phthalates over a period of time is crucial in food safety and quality. The present work aims to develop a point-of-care, screen-printed, disposable paper device, for detection of phthalates. The device was integrated with buckypaper for efficient detection of phthalates. Primarily, phthalates response was analyzed in the standard three-electrode system where the buckypaper was used as a working electrode. Further, a paper device was fabricated using screen-printing approach and the device was tested for phthalates sensing. The traditional approach and the screen-printed device exhibited a very similar electrocatalytic oxidation of phthalates at E = -0.25 V using the miniaturized portable Potentiostat. Hence, can be used for point-of-care analysis. Subsequently, various electrochemical parameters were also examined. The device provided a linear range of 70 ppm to 15 ppm with a detection limit of 12.64 ppm and quantification limit as 42.03 ppm. Finally, the device was validated for repeatability and stability. As the analysis for real time sensing, local tap water was used with the standard addition method and the recovery percentages were remarkable. Based on the promising results and benchmarked form-factor, this device has the potential to be used as point-of-care testing platform.**



***Index Terms*— Bucky paper, Phthalates, Paper based device, Screen printing.**

I. Introduction

Phthalates are used worldwide as a significant industrial chemical with varied commercial applications, like, used as additives, solvents and plasticizers [1][2] . Phthalates of higher molecular weight, such as Diisononyl phthalate and di (2-ethylhexyl) phthalate, account for roughly 80 percentage of the phthalate occurred due to plasticizers in almost every plastic items, including beverage, food packaging and medical applications [3]. In contrast, lesser molecular weight phthalates, such as dimethyl and diethyl phthalates, account for the remaining 20% of phthalate production in insecticides, cosmetics, commercial paints, and even pharmaceutical applications [4][5] . Phthalates has high chance of exposure to the environment because they are not covalently bonded with the molecular structure of the resulting compound [6][7]. They can percolate easily, dissolve as they are soluble in nature and sssfextensive industrial use, the exposure of phthalates to living beings has significant health effects like development of deficiencies, reproductive disorders, hampered growth rate of children and pregnant women etc. [9][10].

Some significant studies also reported that a high concentration of phthalates had led to damage to the sperm count and a decline in the anogenital distance in the children. Critical illnesses, like breast cancer, disorders, like premature breast development and shortened pregnancy with early deliveries, are some of the health hazards faced by women upon exposure to Phthalates [11]. Interestingly, common daily used products, like packaged drinking water, soft drinks and other beverages bottled in plastic containers, have proven to be a significant source of phthalates across the globe. Therefore, the world health organization (WHO) has given a tolerable limit of the phthalates in the drinking and freshwater as 36 nM [12] which is almost a trace amount. With the adverse effects of phthalates in the daily life, it is essential to detect the phthalates as a safety parameter. Several research works have been developed to determine phthalates[1] [13].

The purely laboratory-based techniques, like high-performance liquid chromatography (HPLC), solid-phase

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extraction (SPE), gas chromatography (GC) and electrochemical techniques, like electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV), square wave voltammetry (SWV) and differential pulse voltammetry (DPV) have been reported for the determination of phthalates [14][15][16][2]. Other modern approaches, like molecularly imprinted polymer (MIP) based method proposed using the solid phase extraction, has also proven as efficient in phthalates detection. Zhang et. al. developed a sensor for the sensing of dibutylphthalates and its performance was investigated by DPV and CV. The lower limit of detection of 0.0052 ppm was achieved [8]. In another work, a similar approach of MIP was used for the detection of diisononyl phthalates (DINP). The DINP imprinted polymers were used to modify the glassy carbon electrode (GCE). The proposed system has the capability for the detection of phthalates in the food samples. The sensor has high sensitivity and does not require any prior sample treatment for real-time sample detection [11].

Zi et al. developed an interdigitated electrode (IDE) with gold surface-modified electrodes on a silicon substrate. Later, the IDE was functionalized with polymer as well as phthalate template. Different concentrations of phthalates were prepared in Milli Q water and analyzed. Finally, the obtained results in IDE were validated with HPLC [2][3]. Li et al. synthesized a compound of magnetic graphene oxide with gold nanoparticle-based imprinted polymer for dibutyl phthalates (DBP) sensor. The synthesized materials showed excellent repeatability with a standard deviation of 2.5 % [17].

Medina et al. improved an electrodeposition method for the adhesion of gold nanoparticles on the boron-doped diamond electrodes. This modified electrode was employed for the electrochemical degradation of phthalates [18]. In another work, Xiong et.al used diaminodecance (DAD) with the composition of graphene and cyclodextrin for the detection of diethyl phthalates. The electrodes were characterized by the CV and EIS studies. A linear range of 0.2-1.2 µM with the limit of detection of 0.001 µM was achieved on the improved electrode [15]. Liu et. al. detected the dimethyl phthalates (DMP) by using electrochemical immunosensor. The DMP antibodies with the combination of platinum, lead and bovine serum albumin were used to check the response of DMP. The developed immunosensor used differential pulse voltammetry to find a linear range of 0.001 ppm to 1 ppm with limit of detection of 0.033 ppm [14]. Annamalai et.al. proposed enzyme based electrochemical biosensor was developed. The enzyme-based sensor uses esterase as enzyme with modification of Polyaniline (PANI) and copper nanoparticles with carbon naotubes. This developed enzyme-based sensor has capability to find the phthalates esters in long term stored PET(Polyethylene Terephthalate) bottles as well as industrial wastes which discharged into the lakes [19]. Table 1 gives a comparative study of these approaches with the developed method. From the literature, it was observed that phthalates could be determined with the complex reaction mechanisms with the immunosensors, enzymes and complex nanoparticles modified in the standard analytical systems.

Thus, there is a need to create a simple, portable and miniature electrochemical sensor for efficient detection of phthalates. Herein, a plain buckypaper, consisting of enriched carbon fibers was used to detect phthalates. The current study integrated a screen-printed paper-based device with buckypaper working electrode to detect phthalates. Phthalates response was initially studied using a standard three-electrode method. Later, a paper device was fabricated with screen-printed technology and tested with phthalates using a concentration of 1 mM. The oxidation potential for phthalates was obtained at E = -0.25 V using the standard and a screen-printed device. The screen-printed device was then tested for various electrochemical properties such as scan rate, pH, concentration, and interference effects. Finally, a real sample analysis was conducted in the device with locally obtained tap water and determined that the recovery percentages were obtained in the screen-printed paper device.

TABLE 1
COMPARISON TABLE WITH THE PREVIOUSLY REPORTED WORKS

Method	System	Limit of detection	Ref.
MGO@AuNPs@MIP	Standard three electrode system	0.0008 µM	[4]
BDD@gold particles	Standard three electrode system	-----	[5]
B-CD/G-DAD@GCE	Standard three electrode system	0.01 µM	[6]
Pt@Pb	Standard three electrode system	0.0014 µM	[7]
MIP@Agarose	Standard three electrode system	0.027 µM	[8]
CuNP/CNT/PANI/esterase	Standard three electrode system	8×10^{-5} µM	[9]
Bucky paper	Screen printed method	12.64 µM	This Work

II. MATERIALS AND METHODS

Carbon conductive ink (CI 2001) was used to screen print counter electrode was purchased from Snab Graphix India private limited, India. Ag / AgCl ink (011464) used to screen print reference electrode was procured from ALS Co., Ltd, Japan. Multi-walled carbon nanotube (MWCNT) based buckypaper was obtained from Nano Tech Labs Inc., United States. Whatman filter paper Grade 1 (thickness 180 µM) was used as a substrate for a three-electrode system base was purchased from Sigma Aldrich, India. Different concentrations of phthalates were prepared using Diethyl phthalate. PBS (Phosphate Buffered saline) with pH 7 of 0.1 M was prepared using monobasic sodium phosphate anhydrous and dibasic sodium phosphate dehydrate. For interference study analysis, Hydrazine sulphate, Ammonium fluoride, Sodium nitrite, Orthophosphate and Potassium nitrate were purchased from Sigma, India. For Screen printing, squeezer and mesh are purchased from the local store. All the electrochemical analysis like cyclic voltammetry (CV) and chronoamperometry (CA) analysis were carried out using EmStat4S from Palmsens, Netherlands.

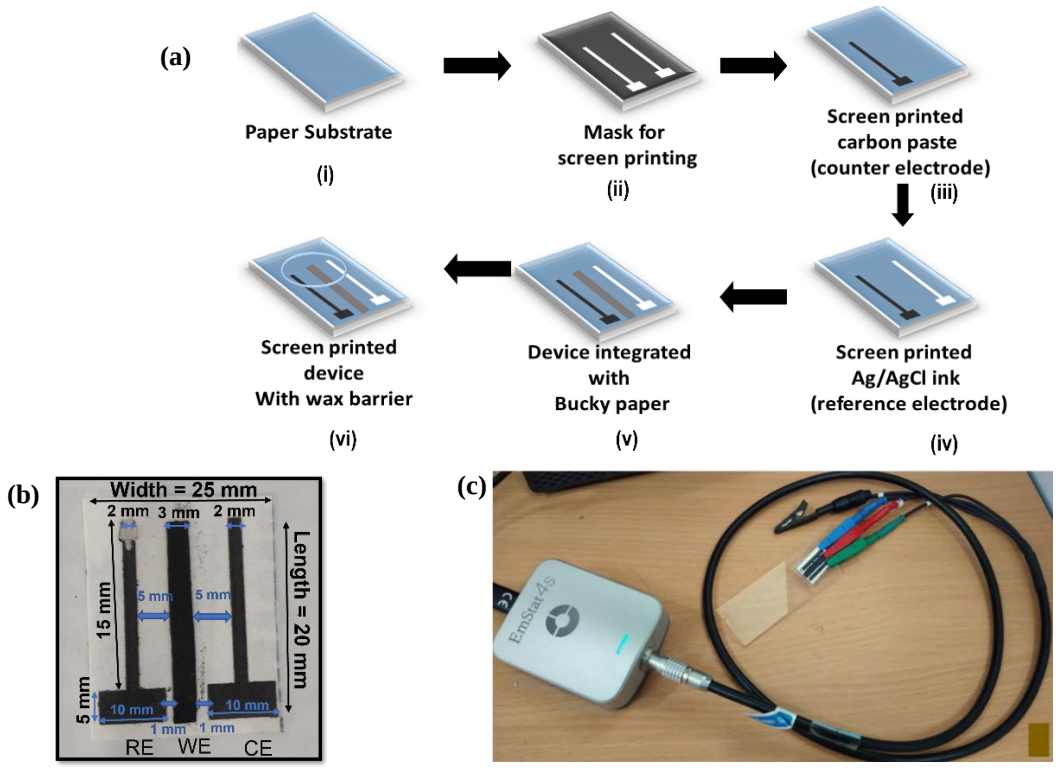

Fig.1 (a). Step wise screen-printed electrode fabrication process, (b) Real device with all dimensions, (c) Experimental set-up of the Real device with portable Potentiostat.

III. EXPERIMENTAL ANALYSIS

A. Fabrication Procedure

The sensing of DEP (Diethyl phthalates) was carried out using the carbon-based electrode printed on the Whatman filter paper (Fig. 1a). The paper substrate was cleaned in ethanol (70%) to remove any paper residues in the pores and left to dry in the oven for around 15 minutes at 500 °C. Polyvinyl Chloride (PVC) sheet (thickness = 250 µM) was used to create a mask to print for the auxiliary electrode (CE) and the reference electrode (RE). The mask was created with the help of a CO_2 laser by using the constraints of speed = 5.5% (375 mm/s), Power = 15 % (4.5 Watts). After completing the mask with PVC, the mask was aligned on the screen-printing mesh with the wooden frame with the bottom as paper substrate. Then, the carbon paste was squeezed on the mask to create the counter electrode on the paper [20] [21]. Paper with the carbon electrode was again placed in the oven for around 700 °C for 30 minutes to dry the electrodes. After drying the carbon counter electrode, the Ag /AgCl was squeezed on the other side of the mask to make the reference electrode. Finally, the paper substrate with carbon and Ag/AgCl was air-dried for around 12 hours.

Finally, a piece of buckypaper was precisely cut with 20 mm × 2 mm and affixed in the middle of both the CE and RE electrode with a gap of 1 mm on both sides with two-sided tape. Lastly, the wax barrier (thickness is around 100 µM) was created using the procedure followed in the paper [22]. The thickness of the RE, CE and WE is maintained around the 30 µM, and while the fabrication the tolerance is maintained around the ≤ 5%. The all dimensions of electrode shown in (Fig. 1b) and the Real device testing on the electrodes with portable Emstat Potentiostat (Fig. 1c).

B. Electrochemical activity of phthalates

CV analysis was carried out to analyze the electrochemical behavior of Diethyl phthalates (DEP). The buckypaper, consisting of carbon nanotubes (MWCNT), was used as a working electrode to analyze the DEP. Hence, no tedious electrode modifications and reagent or redox mediators are used here making the fabrication simpler and cost-effective. A screen-printed and buckypaper integrated device was tested with 1 mM concentration of DEP in various pH solutions using buckypaper as a working electrode (WE), conductive paste of carbon as a counter electrode (CE) and carbon altered Ag/AgCl ink as a reference electrode (RE).

Phthalates are commonly found in stored food and water containers, alcohol, oil, and water, among other things in different pH behaviors, it was vital to test the platform at varied pH levels. Hence the phthalates are analyzed in different pH, As can be seen in Fig. 2 (a), (b) and (c), it was observed that for pH 2, 3 and 4, DEP did not show any redox behaviour, whereas for pH 5 and 6 shown in Fig. 2 (d) and (e), slight oxidation and reduction peaks were observed at -0.25 V and -0.42 V, respectively. A clear redox process was followed for the DEP in pH 7. Further, pH 8-9 failed to show any redox process. This analysis concluded that the DEP electrocatalytic oxidation is a pH dependent reaction with the buckypaper. However, it is tricky to predict the exact reason for this behavior[10]. Subsequently, as can be seen from Fig.1 (f), it was observed that paper device undergoes redox process in 0.1 M pH 7 phosphate buffer solution (PBS).

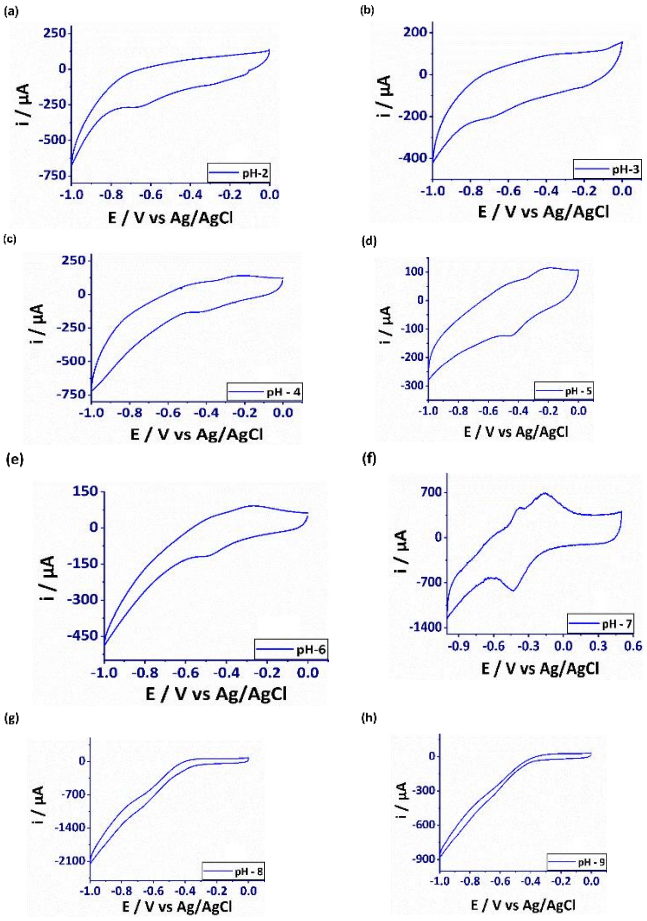

Fig. 2. Behavior of DEP on the developed paper device towards the various pH solutions (a) CV response for pH 2, where no redox behavior for DEP, (b) CV response for pH 3, shows no redox behavior for DEP, (c) CV response for pH 4, shows no redox behavior for DEP, (d) CV response for pH 5,shows the redox behavior for DEP (e) CV response for pH 6, shows redox behavior for DEP (f) CV response for pH 7, (g) CV response for pH 8 and (h) CV response for pH 9 in 0.1 M PBS at 50 mV/s scan rate.

To confirm and validate the developed paper device, a buckypaper, with dimensions 20 mm × 2 mm (L × W), was used as working electrode to develop a full paper-based device for the phthalate's detection. The platinum was used as a counter electrode (CE) and commercially available standard Ag / AgCl was used as a reference electrode (RE). The cyclic voltammetry analysis was used to test the DEP in bulk system. Fig. 3 (a and b) represents the behavior of buckypaper towards the DEP in the developed paper device and the standard 3-electrode system. From the graph Fig. 3 (a) and (b), an oxidation peak was observed at a lower potential of -0.25V in both the paper device and standard system. With this, we concluded that the developed device shows the same reproducibility as that of the standard system. This comparison between the standard system and the paper device, there was no change in the behavior of DEP towards the buckypaper. So, further analyses were carried out with screen printed paper device.

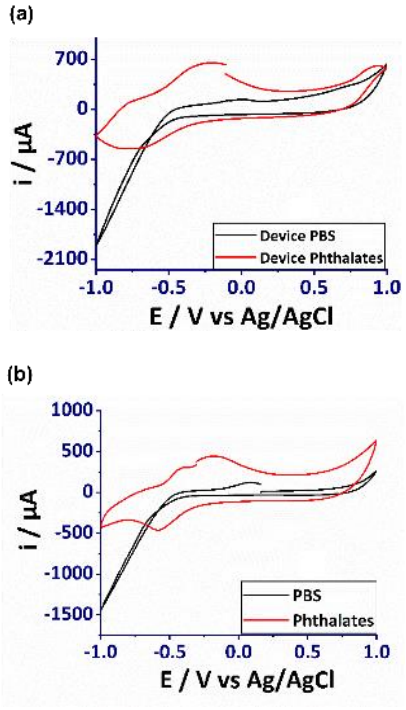

Fig. 3. (a) Cyclic voltammetry response of developed paper device in 1 mM DEP in pH7 PBS at 50 mV/s scan rate (b) Cyclic voltammetry response of bucky paper in 1 mM DEP in pH 7 PBS at 50 mV/s scan rate in the standard system.

C. Effect of variable scan rate

The scan rate effect on peak current was examined as presented in Fig. 4(a). It was observed that the oxidation peak currents had a linear relationship with scan rates ranging from 20 mVs^{-1} to 100 mVs^{-1}, confirming a typical diffusion-controlled electrode technique. The surface area of the working electrode was calculated as 9 mm^2. The no of electron participates in the reaction (assumed to be 2), the anodic peak current was calculated as 610 µA and the concentration was taken as 70 µM. The diffusion coefficient ($D = 1.175 \times 10^{-3} cm^2 s^{-1}$) was calculated using the Randles equation (Equation 1) [11]

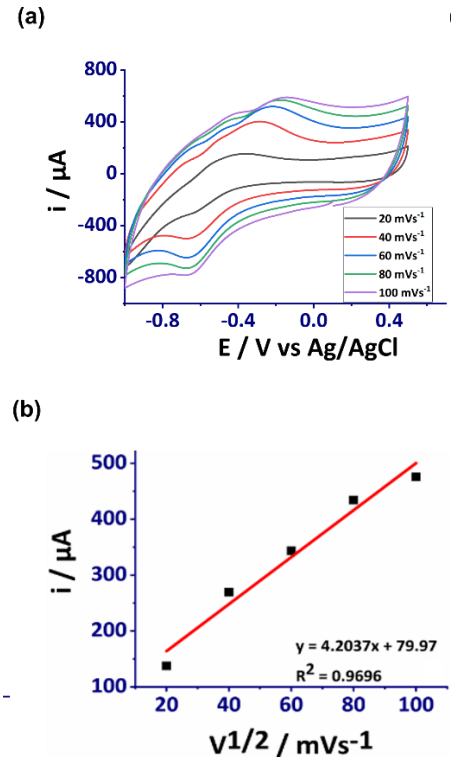

Fig. 4. (a) Scan rate effect of phthalates in the various scan rates form 20 – 100 mVs^{-1} (b) Scan rate calibration plot.

$$I_{pa} = (2.69 \times 10^5)\, n^{3/2}\, ACD^{1/2}V^{1/2} \quad (1)$$

where A= surface area of the electrode, I_{pa} = anodic peak current, n = no of electron, C = concentration, D = Diffusion coefficient, V = scan rate.

D. Effect of concentration

Chronoamperometry was used to study the effect of various concentrations of DEP on the developed device. The chronoamperometry experiments were carried out by applying the constant oxidation potential of -0.25 V in 0.1 M pH 7 PBS solutions for various DEP concentrations. The current versus time plot shown in Fig. 5(a) for different DEP concentrations ranging from 15 µM to 70 µM. From the plot, it was seen that increasing the concentration from 15 µM to 70 µM, the current increased linearly. Fig. 5(b) shows the baseline calibrated plot of the linear concentration range. The LOD (Limit of Detection) and LOQ (Limit of Quantification) were calculated using the following formulas:

LoD = 3.3 *Standard deviation/ Slope (2)

LoQ = 10*Standard deviation/ Slope (3) [12]

The theoretical LoD and LoQ were computed as 12.64 µM and 42.03 µM.

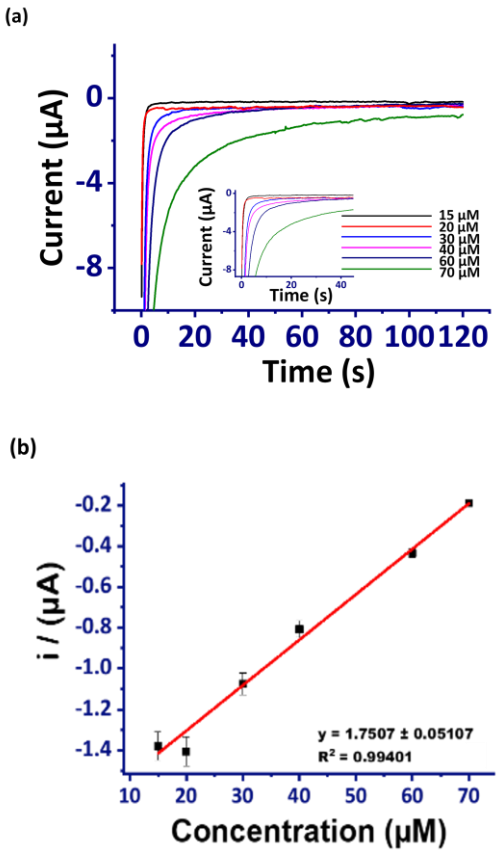

Fig. 5. (a) Chronoamperometry responses of phthalates towards the device (fig: Inset CA) (b) Base line (green) corrected corresponding calibration plot.

E. Interference effect on phthalate detection

Water bodies are especially vulnerable to phthalate pollution because up to 1% of the phthalate component of plastic goods may be discharged into the aquatic environment. Excessive phthalate exposure, through drinking water, can lead to various health problems, including endocrine system disruption, cancer and developmental defects. Therefore, it is essential to check the device interference study with the other co-existing chemicals present in the water bodies for the real time applicability. The interferants chosen to test the device were ammonium fluoride, hydrazine sulphate, orthophosphate, potassium nitrite, and sodium nitrate which are commonly present in water either as nutrient, mineral or pollutant. The CV analysis was opted to study the interference effect with the same potential as that of the phthalates. Phthalates of 1 mM concentration showed an oxidation peak potential at -0.25 V. To check the interference study of interferants same 1 mM concentration within the potential window of phthalates, i.e., -1 V to + 0.5 V, was opted. From this analysis (Fig.6), the other interferants did not give any characteristic oxidation and the reduction peak suggesting that there was zero interference among these chemicals on the screen-printed paper device.

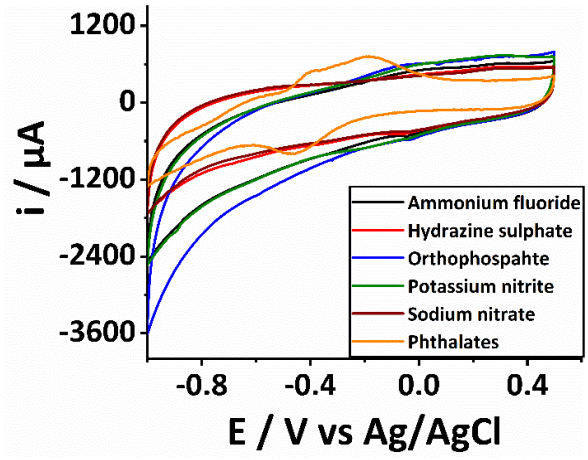

Fig. 6. Cyclic voltammetry responses of phthalates with other common interferants with the concentration of 1 mM.

F. Real sample study

Real samples acquired from crude tap water were utilized to demonstrate the sensitivity and selectivity of the designed screen-printed device. The analysis was carried out using a typical standard addition method. Fig. 7 represents the CA response of concentrations, i.e., 30 µM, 20 µM and 15 µM to tap water samples. The performance results of this investigation are shown in Table 2, where considerable recovery values are seen from the device.

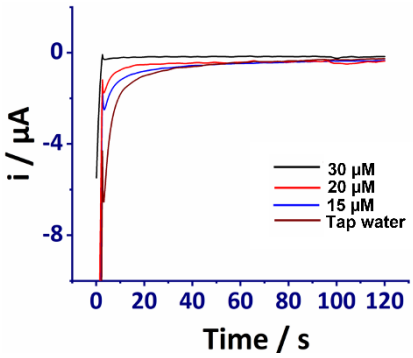

Fig. 7. Chronoamperometry responses of phthalates in tap water by spiking concentrations of 15, 20 and 30 µM by standard addition approach.

TABLE II
TAP WATER ANALYSIS USING STANDARD ADDITION METHOD

Sample	Added (µM)	Found (µM)	Recovery (%)
Phthalates (Tap water)	30	28.89	96.30
	20	18.21	91.05
	15	14.34	95.60

G. Repeatability and Stability Analysis

The repeatability analysis was carried out by using the chronoamperometry technique. These studies were performed on the same single paper device. The experiment was triplicated. A linear concentration range of 15 µM and 70 µM was selected for this study. The same optimized parameters were used as that of the concentration and the real sample analysis. From Fig. 8 (a) and 8 (b), it can be observed that negligible amount of current was altered, hence, we can conclude the device was repeatable.

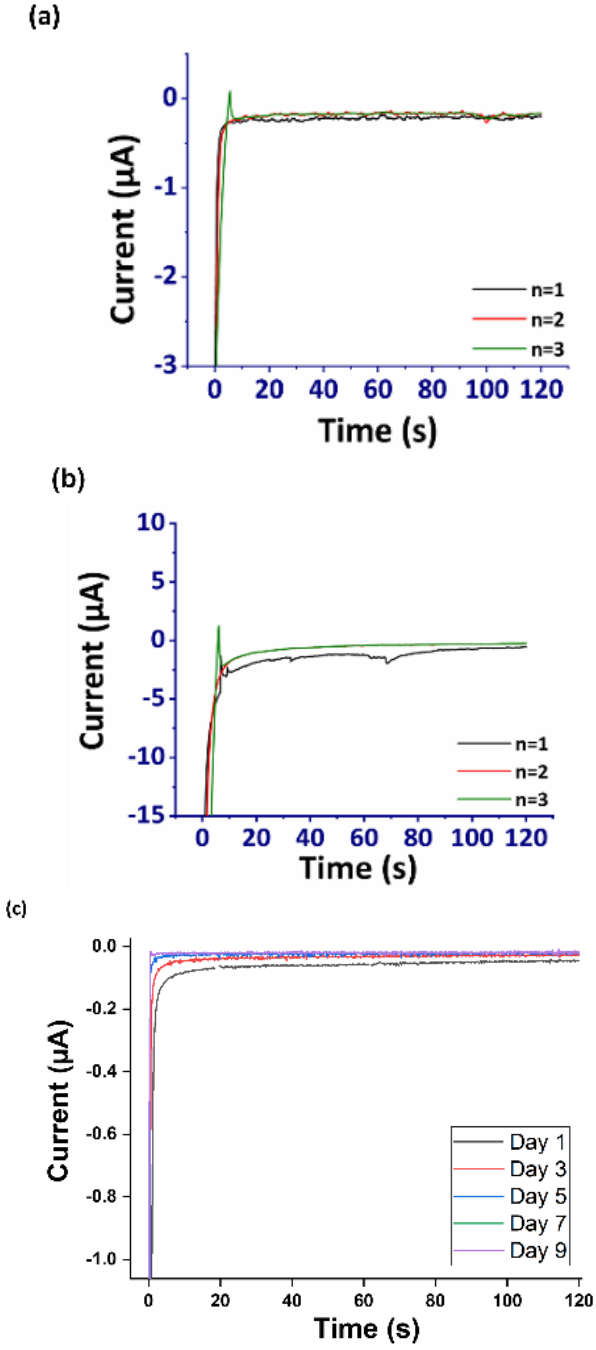

Fig. 8 Repeatability Analysis using chronoamperometry (a) 70µM and (b) 15 µM (c) Stability analysis of the device.

Further, the stability study was carried out on the developed paper device using the chronoamperometry for 10 days. Fig. 8 (c), shows the chronoamperometry results for five alternate days (Day 1, Day 3, Day 5, Day 7 and Day 9). From the graph it can be clearly observed that there was no change in the peak current. The maximum deviation of all five days was < ±5%. The lower deviation of ±5% clearly shows that the developed device showed great stability.

IV. Conclusion

The present study combines a screen-printed paper-based device with buck paper to electrocatalytically monitor phthalates. A Bucky paper was utilized as a working electrode to test the electrochemistry performance of phthalates towards the device. The phthalates response was initially studied using a standard three-electrode method. Later, a paper device was created utilizing screen-printed technology and examined with phthalates at a concentration of 1000 ppm with miniaturized potentiostat. The redox potential for phthalates was measured to be -0.25 V using both the standard method and a screen-printed device. The screen-printed device was then tested for various electrochemical properties such as concentration, interference, scan rate and pH effects. A linear range of 70 ppm to 15 ppm was found with a limit of detection (LoD) of 12.64 ppm and limit of quantification (LoQ) as 42.03 ppm. Finally, the tap water analysis on the developed device concluded the impressive recovery percentages. Finally, the paper device was evaluated for repeatability and stability, and it successfully demonstrated higher reproducibility and stability. In the present study plain bucky paper was used as working electrode for the detection of phthalates. Since the bucky paper consists of the multi walled carbon nanotubes which have a larger surface area because of this characteristic it can be easily modified with the nanocomposites and further can be used to test the various bio and chemical analytes. Hence the present work can be further extended with the improved limit of detection as a low-cost device by enhancing the catalytic reaction of buck paper with various nanocomposites.

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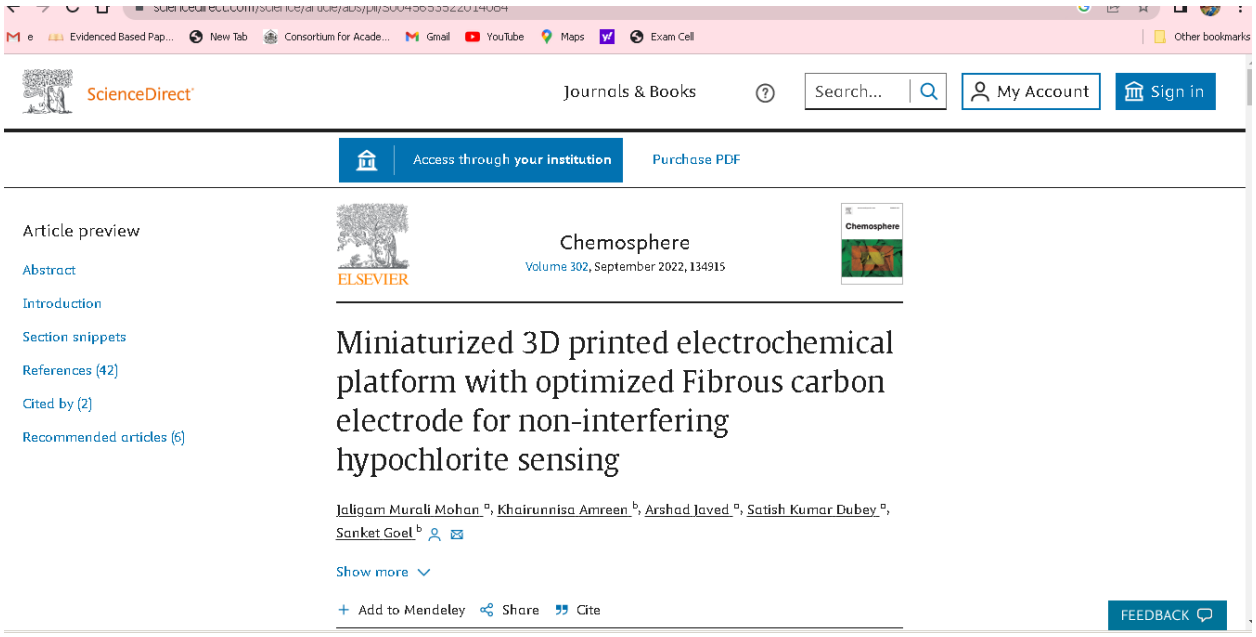
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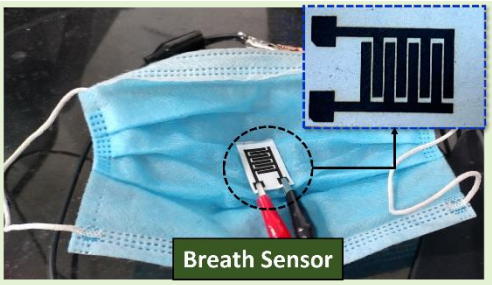
Miniaturized Paper based Sensor with reduced Graphene Oxide as Interdigitated Electrodes for Multiple Applications

Ritesh Kumar Singh, Pavar Sai Kumar, Khairunnisa Amreen, Satish Kumar Dubey and Sanket Goel

***Abstract*— A paper-based miniaturized sensor, with reduced graphene oxide (rGO) as an active electrode, has been realized. Using this device, multiple applications, like temperature, breath, blow and touch sensing, were demonstrated. The sensor showed good sensitivity as well as accuracy. Whatman filter paper of grade -1 was used as a substrate for laser ablation, to form reduced graphene oxide (rGO) on it in interdigitated electrode (IDE) format. Subsequently, physico-chemical characterizations of the material with various characterization techniques were carried out. The sensor gives the sensitivity of 1.46 % ℃$^{-1}$ and the linearity (R^2 = 0.993) with resolution of 1℃ between the temperature range of 25℃ to 60℃ showing the negative temperature coefficient of 0.0016℃$^{-1}$. Further the sensor has been for the breath, blow and touch sensing application.**



***Index Terms*— rGO, temperature sensor, breath, blow, touch sensor**

I. Introduction

RECENTLY, flexible electronics has attracted researchers in both industrial and academia sectors in the domains of healthcare management, environmental parameters monitoring, energy harvesting etc. One such application of flexible electronics is temperature sensing. There are different types of temperature sensors like thermometers, thermocouples, and infrared thermometer, etc. Out of these sensors, resistive type temperature sensor is considered better than the others owing to its fast response and easy fabrication process [1]–[3]. Hence, a temperature sensor made with flexible substrates could have a futuristic application. Presently, the common substrates used in flexible electronics are polyethylene naphthalate, polyimide and polyethylene terephthalate [4]–[6] . However, among them the polyimide is mostly chosen for the flexible device because of its properties such as high thermal stability as well as the good mechanical property. Nevertheless, using these polymers, lesser cost affectivity, especially for scaling up the production, is challenging. Hence, a simple origami paper substrate can be an alternative. The rGO based sensor can also be used for the measuring the humidity as the value the permittivity is effected by changing the humidity [7]

Paper or origami- based substrates have continually been in demand for the fabrication of paper electronics. Ease of fabrication and disposal make them advantageous over other polymer-based substrates. Several healthcare applications wherein paper devices were fabricated for sensing have been reported [8]–[10]. These paper substrates are used as either base supporting material or for generation of other carbon derivatives via laser ablation, chemical treatment etc. It was observed that exposure of paper substrate to a specified laser with speed, power and intensity, a carbon derivative, graphene oxide or reduced graphene oxide (rGO) can be produced. As such materials shows good conductivity and electron-shuttling activity, formation of carbon-based material over a paper with single-step laser ablation is of huge significance. In the present work, fabrication of rGO on a paper-substrate and its use for temperature sensing, sensor stability and repetition were carried out. The prepared material was tested for various other sensing applications like breath, blow and touch [7][10]–[12].

The demand for monitoring the temperature based on the flexible substrate shows the potential application in the healthcare industry as the temperature monitoring of the human body is important because its variation indicate the fever or other medical issue. Thus user can take the necessary precaution as early as possible [14]–[16]. In future the interdigitated electrode (IDE) based temperature sensor can be connected to the circuit containing the signal processing unit for conversion of resistance to temperature.

Herein, a miniaturized paper-based interdigitated electrode-based sensor, with laser-induced reduced graphene oxide (LIrGO) as an active electrode, has been demonstrated. Using this device, multiple applications, like temperature, breath, blow and touch sensing, were demonstrated. Table 1 summarizes various temperature sensors with the LIrGO based electrodes.

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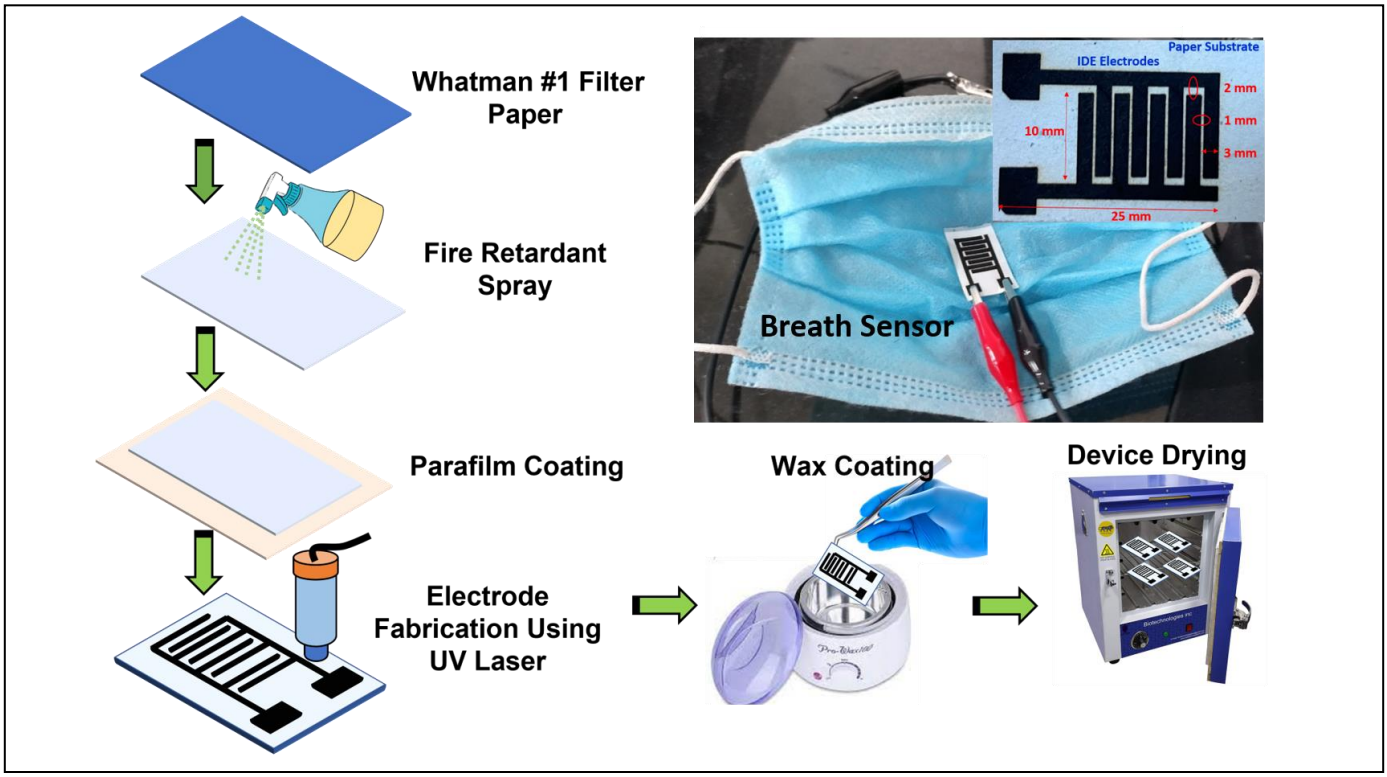


Fig. 1. Schematic for interdigitated electrode for temperature sensing application, all the dimensions are in mm unit.

TABLE I
COMPARISON OF THE DIFFERENT TEMPERATURE SENSOR WITH THE FABRICATED LIRGO BASED SENSOR

Sensing material	Range [^{o}C]	Linearity	Accuracy [^{o}C]	Sensitivity % $^{o}C^{-1}$	Ref.
rGO	25-45	0.99	0.1	1.30	[17]
rGO	25-43	0.99	10	0.62	[18]
Gr woven fabric	20-50	yes	5	1.34	[19]
Gr/PDMS	25-75	yes	0.5	0.8	[20]
rGO	25-60	0.993	1	1.46	This work

rGO (reduced graphene oxide), Gr- graphene. SEM- Scanning electron microscopy, XRD (X-ray diffraction), XPS (X-ray Photon spectroscopy), UV-Vis (Ultra violet Visual spectroscopy).

II. EXPERIMENTAL SECTION

2.1 Material and equipment

Whatman filter paper of grade-1 has been used as a substrate for fabricating the sensor was procured from Sigma Aldrich, India. A blue diode multitool head with Laser (450 nm, 2W) was used for laser ablation (obtained from Zmorph Wroclaw, Poland). LCR meter (Keysight, U1700 series) was purchased from amazon, the hot plate was used for providing the temperature. A source meter (B2192A from Keithley) has been used to measure the current vs time.

2.2 Sensor fabrication:

The temperature sensor was fabricated on a Whatman filter paper of grade 1. For that the first step involves making the paper fire retard, for that the fire retard solution is prepared using the boric acid and borax by dissolving in the milli Q 18.2 MΩ.cm DI water. Then after drying it in oven a parafilm coating is done on the done side of the surface paper for providing the support to the substrate, then after the blue diode laser is used for the laser ablation on the surface on the paper for carbonization and printing the interdigitated electrode pattern. The dimensions of the IDE structure were 25 mm X 15 mm. The pattern was first designed in the CAD software solidswork and it is converted into .dxf file format which is further converted to the g-code using the voxelizer software which will be printed by the blue diode laser. After fabricating the sensor, wax printing was done for making the device stable as rGO will not leach out of the surface and it protects the sensing layer form the external environment factor.

The wax printing was done by the heating the wax so much that it turns in the liquid state and then dropping the sensor in it and taking it out so that all the wax will go inside the pore of the paper substrate as well as the rGO so after this the sensor is kept in the oven at 60^{o}C for 15 min so that the wax settle properly. The gap between the 1 mm, the length of the finger is 10 mm, the width of the electrode is 2 mm, the number of finger in fabricated sensor is 8.

2.3 Equipment

For the characterization of the material the various technique is used such as SEM (Apreo LoVac, FEI), XRD (Ultima- IV, Rigaku), XPS (XPS, k-Alpha, Thermo Fisher, UK), UV-Vis (UV-Vis, V-650, Jasco, Easton, MD, USA).

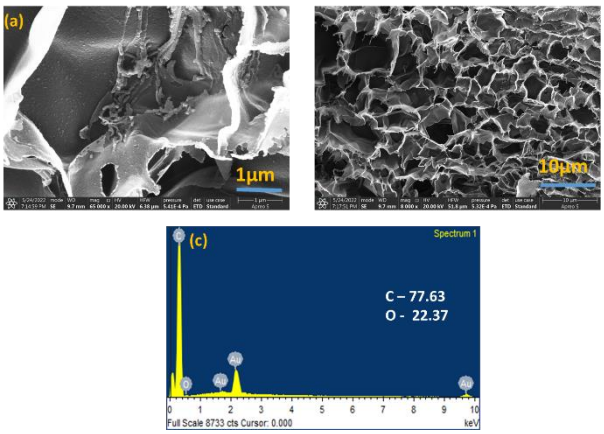


Fig. 2. Surface morphology analysis using the SEM technique (a) SEM image at the different resolution i.e 1 µm and (b) at 10 µm which indicate the surface carbonization (c) the EDX analysis indicating the percentage of the carbon and the oxygen on the surface the Au is coming due to the sputter coating

III. 3. Results and Discussion

3.1 Physico-chemical characterization:

For the physico-chemical characterization of the material the technique used are scanning electron microscopy (SEM), EDX, X-ray Photon spectroscopy (XPS), X-ray Diffraction, Ultra-violet- visible spectroscopy.

3.1.1 SEM characterization

For understanding the surface behavior of the Laser Induced reduced Graphene Oxide (LIrGO) the SEM analysis was done. The SEM analysis of the different magnification was taken, and it was observed that there was uniformity in the porous network which helps in the carbonization of the surface which can be seen in the Fig. 2 (a-b). This feature helps in achieving the high electron transfer mechanism due to increase in the surface area which is useful in temperature sensing application [17]. This increased surface are have the good thermal conductivity.

3.1.2 Energy dispersive X-ray Spectroscopy (EDX)

For checking the composition information and their percentage on the surface of the electrode EDX analysis was performed as shown in Fig. 2 (c). Here the percentage of the carbon was more than that of the oxygen which indicates that the surface was sufficiently carbonized for the sensing application [21].

3.1.3 X-ray Photon Spectroscopy (XPS)

XPS was performed for the analysis of the material for checking the presence of carbon (C) and oxygen (O). Fig. 3 (a) show the intensity peaks of the carbon which was more when it was compared with the oxygen which indicates that when the laser was ablated on the paper substrate, the oxygen containing functional group was eliminated [22].

3.1.4 X-ray Diffraction (XRD)

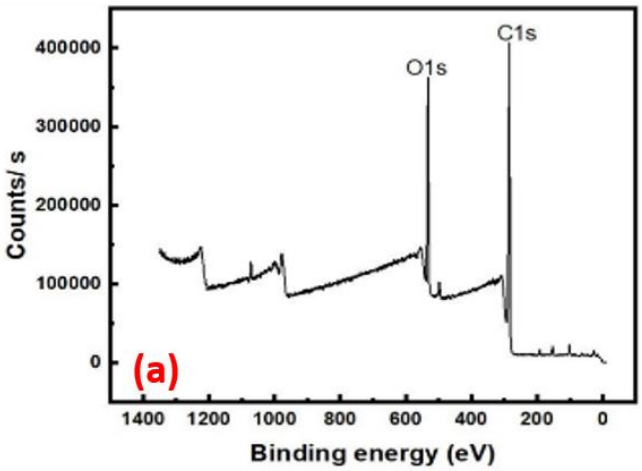
XRD was performed for verifying the crystal nature of LIrGO (Ultima –IV, Rigaku). Rate of scanning was taken 1 per minute and the position of the angle was taken between 10 to 70 by using Cu-kα (λ = 6.15 nm) for the radiation source. While analyzing the XRD peak which can be seen in Fig. 3 (b). The diffraction peak was observed at 24.3 which has the plane value (002) in JCPDS card no – 75–20478 which indicated the presence of the paper substrate. The peak at 14.3^{O} indicates the presence of the oxygen functional group [23].

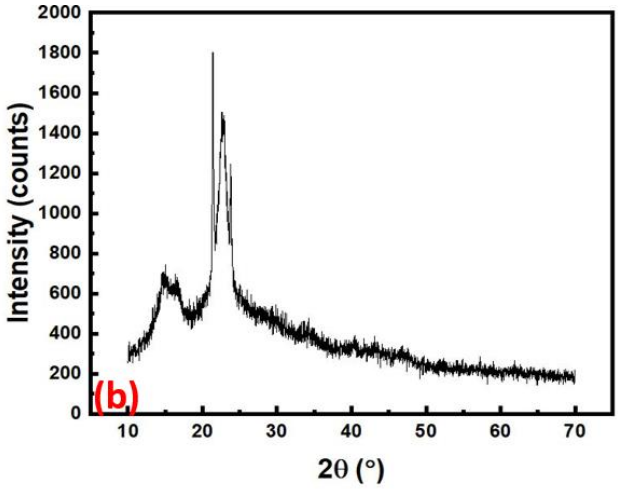
3.1.5 UV-Vis

The UV-Vis was performed for the rGO. The sample is prepared in the ethanol by scrubbing the rGO from the paper substrate and sonicating it in ethanol for 1 hr. It can be seen in Fig. 3 (e) the peak at the 280 nm observed which indicates the π-π* transition present in the aromatic ring of carbon-carbon (C-C) bonding which can be seen as a red shift at 280 nm [24].

The frequency of the LCR meter was 500 Hz for the measurement [25].While heating the unhybridized electron become sp2 hybridization as well as the phonon is generated [23][24]. For determining the performance analysis of the fabricated sensor, the response obtained was analyzed for the sensitive behavior, which were calculated as follows:

$$\Delta R/R_0 \times 100$$





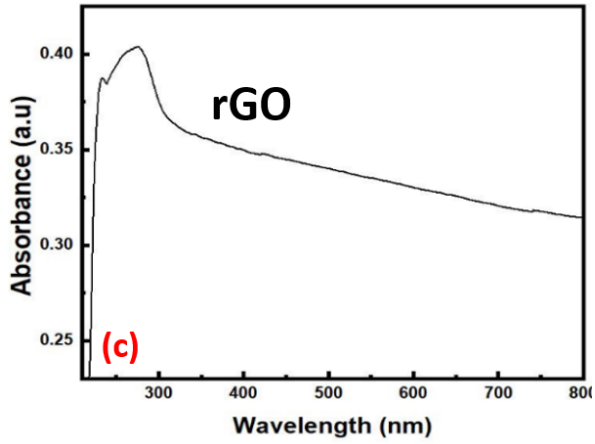
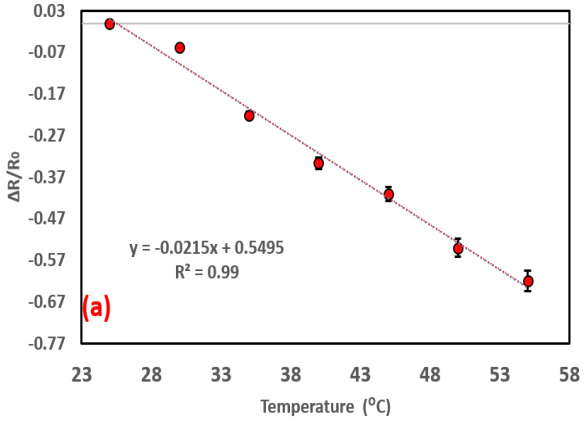


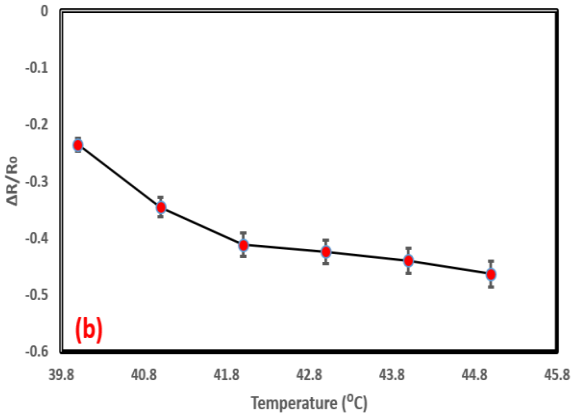
Fig. 3. Various characterization of the rGO for material study (a-c) XPS of the rGO indicating the carbon and oxygen (d) XRD analysis (e) UV-Vis analysis.

here ΔR = change in the resistance which is given by $\Delta R = R_T - R_0$ in which the R_T is the resistance at the measurement temperature other than room temperature, R_0 is the resistance at the room temperature. Thus, the sensitivity of the sensor is defined as:

$$S = \frac{\Delta R}{R0} \times \left(\frac{1}{\Delta T} \right) \times 100$$

here ΔT corresponds to the change in the temperature [18], [26].





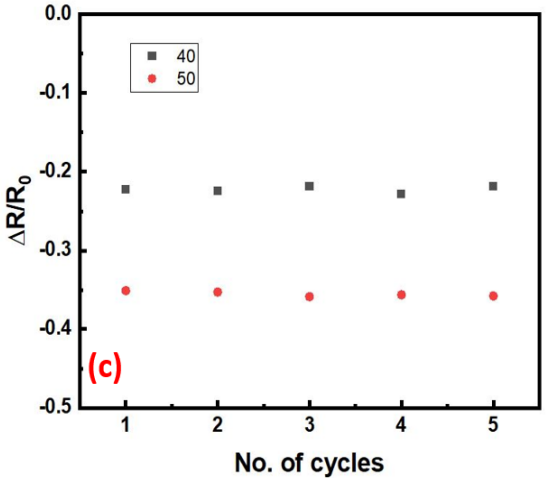


Fig. 4. Electrical characterization of the proposed temperature sensor (a) Temperature sensor response over the range of 25 to 60°C with the temperature interval of 5°C, (b) Resistance variation of the sensor between the temperature 40 to 45°C with the interval of 1°C (c) Repeated cycle test of the sensor between the temperature 40 and 50°C (d) Experiment setup for the sensor performance.

The range of the temperature for the experiment were 25-60°C selected because after 60°C the wax coating on the surface of the electrode start to evaporate and the external hindrance start to degrade the performance of the fabricated sensor. The temperature of the human body also lies between 37-38 °C thus this temperature range is suitable, thus sensor is able to measure the body temperature as well as the protection from the external environment is also there due to the wax printing, as shown in Fig. 4 (a). The sensor showed the negative temperature coefficient (NTC) behavior. The sensitivity of the fabricated sensor had a sensitivity of 1.46 $\%C^{-1}$ with an excellent linearity (R^2 = 0.993). The SD for the fabricated sensor were 0.31%.

In Fig. 4 (b), the resolution of the temperature sensor was 1°C which can be used to measure temperature on human skin. Additionally, the repeatability and stability test for the sensor was performed. The repeatability test was performed between 40 °C and 50 °C which as shown in Fig. 4 (c). With the help of wax printing, the durability of the device has been enhanced. Based on the mechanism explained above the sensor exhibits the negative temperature coefficient. The Arrhenius equation is used for the sensor study when the temperature is above 300 K. The relation is as below:

$$R(T) = R0 \exp\left(\frac{Eg}{kT} \right)$$

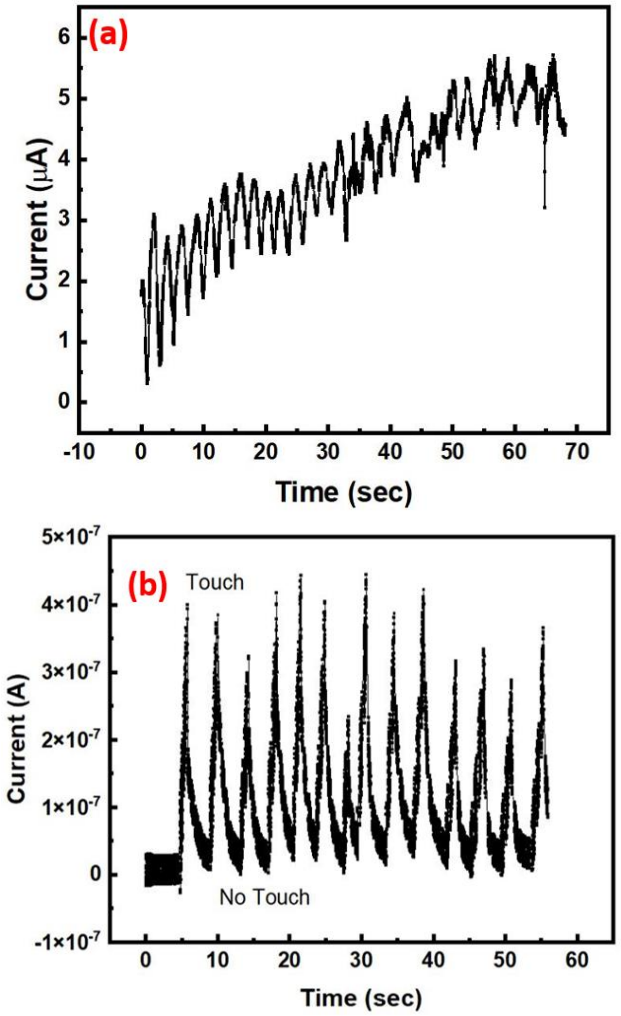
where the symbol has their usual meaning such as R(T) is showing the dependency of the resistance on the temperature, R_0 shows the value of resistance measured at the room temperature, Eg is the activation energy of the material, k is the Boltzmann constant, the activation energy can be calculated from the graph plotted between the ln(R) and 1000/T and the calculated value comes to be 11 meV. Due to the electron-phonon interaction by applying the temperature the activation energy obtained has the low value i.e 11 meV [19].

3.3 Other Potential Application

Besides the temperature sensing the other potential application of the fabricated sensor was explored such as breath sensing, touch sensing and blow sensing.

3.3.1 Breath Monitoring and touch sensing

Breath monitoring is an essential diagnostic approach in health management especially pertaining to respiratory ailments. Hence, there are certain reports describing the detailed study about it [27], [28]. The result of the breath monitoring can be seen in Fig. 5 (a), fabricated sensor was placed inside the mask The result of the breath monitoring can be seen in Fig. 5 (a), fabricated sensor was placed inside the mask, fixed using a double-sided tape. As the person wearing the mask started inhaling and exhaling the breath there was a variation in the resistance due to the variation in the temperature which can be seen in Fig. 5 (a). This process corresponds to the change in current with respect to time. The effect of the humidity can be ignored as the sensor was encapsulated with wax [20]. Additionally, the touch application by using the fabricated sensor has been explored in which the other side of the sensor is touched and the change in the corresponding resistance can be observed in Fig. 5 (b). It is because of variation of the impedance while touch which results in variation in the current [29], [30].



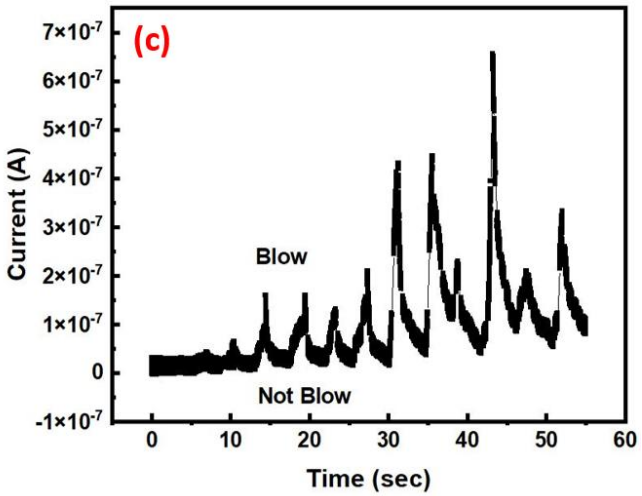


Fig. 5. Application of the fabricated sensor (a) result for the breath monitoring using the sensor (b) touch sensor application (c) blow sensing application using the fabricated sensor.

3.3.2 Blow Sensing

The blowing application was performed in which it was observed that there was an increase in the temperature while blowing on the backside of the sensor, whose response has been plotted between current and time, which can be seen in Fig. 5 (c) [22]. Further in the future, the circuit for the data acquisition system, which consists of the microcontroller, ADC, Wheatstone bridge, and many more, for the sensor can be designed so that the complete device can be used in the real-time application for the healthcare sector.

Fig. 4. Electrical characterization of the proposed temperature sensor (a) Temperature sensor response over the range of 25 to 60ºC with the temperature interval of 5ºC, (b) Resistance variation of the sensor between the temperature 40 to 45ºC with the interval of 1ºC (c) Repeated cycle test of the sensor between the temperature 40 and 50ºC (d) Experiment setup for the sensor performance.

IV. CONCLUSION

The paper-based sensor is fabricated using the LIrGO as the sensing material in the interdigitated electrode pattern which is encapsulated using the wax printing. The sensor shows the good sensitivity and have good negative temperature coefficient value. The sensitivity of the sensor was calculated to 1.46 % C^{-}

^{1} along with the value is $0.0016C^{-1}$. Other potential application for the fabricated sensor also explored such as breath, blow and touch sensing. For understanding the material characteristic various material characterization were performed. In future the microcontroller-based circuit can be designed for converting the resistance value to the respective temperature.

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Three Different Rapidly Prototyped Polymeric Substrates With Interdigitated Electrodes for *Escherichia coli* Sensing: A Comparative Study

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***Abstract*— This work delves upon the development of different types of miniaturized and 3D printed devices having interdigitated electrodes (IDEs) for the detection of Escherichia coli (E. coli) bacteria. The IDEs were fabricated using different approaches including laser-induced graphene (LIG) on polyamide, direct laser writing on glass, and polymeric 3D printing technique, and their suitability for bacteria detection has been compared. The electrochemical impedance spectroscopy (EIS) technique was employed to detect the E. coli bacteria in the prepared miniaturized devices, and the sensory response was compared. EIS was performed in the frequency range between 1 Hz to 1 MHz to record the bacterial growth and activities as a function of change in electrical impedance, and detection performance of the different miniaturized devices with IDEs were compared. It was observed that the LIG-based IDE sensor provided better sensitivity compared to that of the other two approaches. The obtained results indicate that the magnitude of impedance changes by around 2.5 mΩ per doubling of E.coli cells. With fast and flexible fabrication process capabilities, such microdevices may be used as suitable IDE sensors for microscale pathogenic detection for biomedical and clinical analysis.**

***Index Terms*— Escherichia coli (E. coli), electrochemical impedance spectroscopy (EIS), interdigitated electrodes (IDEs), direct laser writing (DLW), laser-induced graphene (LIG), 3D printing.**

I. Introduction

GLOBALLY, illness due to the advent of bacterial infections has become a major source of health concern owing to the fast spread of resistant bacteria [1]. In the rigorous efforts to battle multidrug-resistant infections and promote antimicrobial control, rapid, creative, and cost-effective tests that identify and describe drug resistance bacteria remain an urgent need [2], [3]. Bio-micro-devices with target specific pathogenic detections for biomedical analysis can be highly advantages. In this context, Impedance based sensing has been explored off lately. The impedance systems measure the absolute or relative differences in impedance, capacitance, or conductance, at regular intervals during bacteria growth at a fixed temperature [4].The other techniques include the use of biosensors such as optical biosensors, piezoelectric-based biosensors, and electrochemical biosensors for determining the presence of bacteria [5], [6]. Common acute illnesses, like asymptomatic carriage, diarrhoea and the haemolytic uremic syndrome, were caused by Escherichia coli (E. coli) bacteria. Even a tiny amount of biological contamination in drinking water may cause dangerous and harmful water-borne diseases [7]–[9].

Out of several electroanalytical methods, Electrochemical Impedance Spectroscopy (EIS) is a technique for quantitative sensing that typically uses interdigitated electrodes (IDEs) [5]. In last two decades, IDEs has attracted significant interest among researchers of industry and academia as electrochemical transducers for various biosensing applications [10]. Owing to the advantages like easy miniaturization approach, need of no additional reference electrode and increasd signal-to-noise ratio makes them superior over the conventional three-electrode systems. Further, IDEs do not require any specific markers for detection of bioanalytes and hence a label free sensing approach can be done in biosensing [11]. As the interaction of the bioanalyte with the IDE surface gives electrical impedance changes, no additional capturing markers are needed. Therefore, detection of microorganism with this method could be cost-effective and portable in comparison to the bulk three electrode based sensing [12]. In this technique, the study of impedance behaviour is analysed for a system at a given potential with variable frequencies [13]. EIS is applied in a variety of analysis, including chemical analysis, fuel cells, battery research, corrosion studies etc.[14] To understand the aquatic systems, total impedance (Z) is an important measure for analysing electrode-electrolyte interactions. In this context, IDEs have been realized and used as the capacitive chemical sensor as well as the gas sensor. Such IDEs are conventionally

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fabricated in a clean room which requires complex procedures and skilled hands. Further, the surface of the electrode is also required to be modified for enhancement of the signal to increase the sensibility [4].

EIS technique has been already employed to know the change in impedance with the change in the concentration of E. coli bacteria [15]. Here, impedance variation is commonly monitored with the two electrodes, working electrode and counter electrode, when exposed to various bacterial concentration solutions [16]. The electrochemical impedance changes when microbial cells are present and developed in the medium [17]. The ionic metabolites were released in two ways by the cells [18]. The principal source was mainly the emancipation of the ions from the bacteria cells to the surrounding environment so that it can absorb oxygen and produce CO_2 with other organic acids. In both circumstances, an increase in ionic concentration causes the cell suspension to become more conductive, resulting in a change in the impedance which can be monitored, when the electrodes are dipped in the bacterial solution [19].

IDEs have recently been used in microfabrication technologies as a major approach in microdevice realization, also [20], [21]. To conduct EIS based sensing, two electrodes were connected wherein, one of the electrode systems serves as the working electrode, while the other serves as the reference electrode. The real-time change in impedance was monitored with the change in the applied frequencies [22]. Wu *et al.*, discovered that during EIS studies, the screen-printed IDEs allow a large number of E. coli cells to attach to the electrode surface, resulting in a change in the large impedance with the frequency of 12 Hz [23]. This is observed because of the contribution to the impedance by the electrode part dominates at low frequencies (less than 100 Hz), yet the impedance displays a higher response at frequencies greater than 10 KHz [24]. This phenomenon is explained by Equation (1) [4].

$$|z| = \sqrt{R_s^2 + (\frac{1}{\pi f C_{dl}})^2} \tag{1}$$

where, Z denotes impedance, Rs is the resistance of the given solution, f is the frequency of input signal and C_{dl} is the double-layer capacitance of IDE. The resistance of the solution changes with the change in the concentration of the bacteria when the cells were added to the buffer and produces the change in ionic concentration [25]. Furthermore, adding cells to the buffer can modify the effective ionic mobility, resulting the second term of the equation (1) i.e. (1/(πfC_dl)), in resistance to be varied. The impedance of the capacitive component given in equation (1) is responsible for the creation of a double-layer structure of IDE. The term Rs (i.e. solution impedance) dominates at high frequencies, whereas the second term of the equation (1) influences at lower frequencies [4].

By using several complementary approaches, the concentration of the cell can be increased to withstand or overcome adverse conditions [26]. The conventional methods, like spectra plotting of impedance and modelling of circuits using the given data, are no longer valid because of the voltage divider error and machine limitations. It has also been reported that at low frequencies impedance spectroscopy is not only suitable for the detecting of living cell concentrations, but also useful to find physiological states of the cells [27]. The dielectric behavior of the E. coli bacteria can easily be understood at only low frequencies (usually f < 104Hz), as the E. coli bacteria particles are conductive at higher frequencies [28]. As a result, the impedance of the given bacteria changes mainly at higher frequencies. The resistive and capacitive components of the impedance get altered, when the characterization was done at lower frequencies [29]. Small amplitude (10 mV) was applied to diminish the artefacts caused by the motion of fluids about electric fields. To analyse the impedance measurement as a function of cell growth, the cell culture was traditionally done on the electrode surface and measurements were taken. These are time intensive and the results obtained were electrode surface modification dependent.

In the present work, EIS has been harnessed to detect pathogens (E. coliDH5α), and the specificities of microbial cell development were investigated. As the present method was dependent on the EIS technique rather than imaging, it was prone to be scalable. Bacteria were detected with this technique either by impedance change caused by substances produced by the micro-organisms, or by the bacteria attaching to the electrode. One of the primary gaps in bacterial sensing applications is developing novel ways for detecting susceptible bacteria in an easy, economical, and receptive manner [30].

This work describes a single-step, rapid prototyping process of miniaturized IDE sensor that uses laser carbonization (LIG), Direct laser writing (DLW), and 3D printed device-based approaches to prepare interdigitated electrodes (IDEs). Three different substrate materials were used here. For DLW based IDE fabrication, copper cladded board was used. For laser induced graphene IDE, polyimide sheet was used. For developing 3D printed IDE, conductive graphene filament was used. Graphene filament and copper board are highly conductive in nature. However, upon exposure of the polyimide to the laser, exceedingly conductive laser induced graphene was obtained which had better conductivity than the other two. Further, the active surface of LIG was more with additional functional groups which plausibly gave enhanced sensing and selectivity than the bare graphene filament and copper clad. In addition to this, LIG IDE was flexible whereas, the limitation of the other IDEs was rigidity [31]. The performance of these sensors towards E. Coli detection was compared and it was revealed that the IDE sensor based on LIG provided a higher sensitivity than the other two. Additionally, Table I gives the summary of some of the IDEs reported for E.Coli detection in the literature. Furthermore, when compared to the conventional techniques used, the given technique offers a wider range of applications and can perform real-time quantification.

II. Experimental Section

A. Materials and Equipment

The polyimide films and negative dry film photoresist, with a thickness of 40 μm and 35 μm, respectively, were procured from India Mart, India. Isopropyl alcohol (IPA) was purchased from Sigma-Aldrich, UK. Kapton tape and FR4

TABLE I
COMPARATIVE SUMMARY OF REPORTED LITERATURES WITH IDES BASED E.COLI SENSING

IDE substrate	Bioreceptor/ matrix	LoD (CFU/mL)	Ref
Indium Tin Oxide	Antibodies	10^6	[32]
Gold/Silicon dioxide	Antibodies	10^5	[33]
Chrome over glass	Antibodies	10^4	[34]
Gold over glass	Antibodies	10^4	[35]
Conductive polysilicone	Antibodies	3×10^4	[36]
Gold over quartz glass	Antibodies	10^3-10^6	[37]
Gold on ceramic substrate	Lectins and Antibodies	10^2	[38]
Graphene with gold	Antibodies	10-100 cells/mL	[39]
Gold	Aptamer	267 cells/mL	[40]
3D-Au-Ni microelectrode	Au-Ni nanoparticles	104 cells/ml	[41]
3D- Au-Agar microelectrodes	Au nanoparticles	104	[42]
LIG, Copper clad, Graphene filament	substrate	50	This work

Copper Cladded Board were purchased from DiT Lab of Technology, Osmanabad, India. CO2 laser system (VLS 3.60 from Universal Lasers, AZ, USA), direct laser writing system (HOLWS-PUV, Holmarc), and Flash forge creator pro (Flash forge, USA) were used for fabrication. A potentiostat (SP-150 from Biologic, France) was used to conduct the EIS study in a two-electrode configuration. E.Coli: PTA-1977, Shewanella oneidensis: 700550 were the strains used here.

B. Polyimide Laser Carbonized IDE Device

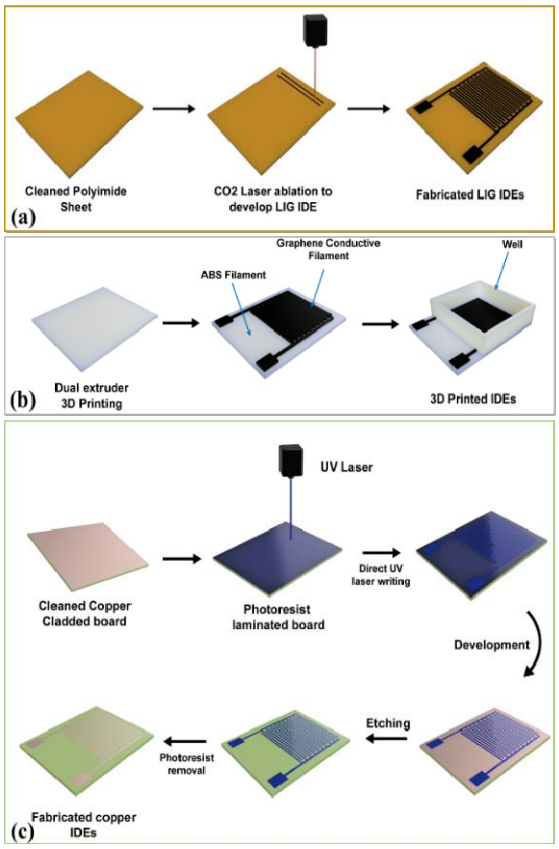
A CO_2 laser engraver was used to fabricate the LIG devices. Initially, the films were cleaned with IPA. To adhere the polyimide film with the acryl sheet substrate, Kapton tape was used. Corel Draw V.7 software was used to design the device. The parameter used to fabricate the IDEs using a CO2 Laser system were 4.5% power and 6.5% speed with 1000 PPI. Scheme 1 (a) shows the schematic representation of the LIG-based IDE sensor.

C. 3D Printed IDE Device

For 3D printing, the electrodes of the sensor patches were developed using conductive materials such as graphene filament and PLA (Polylactic acid) filament. In terms of electro-mechanical qualities, conductive materials are available in different moulds and widths. Graphene, one of the carbon allotropes, has been used as an electrode. Flexible prototypes have also been created using 3D printing and other methods. The electrodes were designed using SolidWorksTM, which was linked to the printing system. It took about 15 minutes to make a 3D-printed mould. Before using the moulds for casting, they were completely cleaned with isopropanol. Scheme 1 (b) shows the schematic representation of the 3D printed IDE sensor.

D. Direct Laser Written IDE Device

A DLW equipment was used to fabricate IDEs on glass by maskless photolithography. The design was made with



Scheme 1. Schematic representation of the IDE fabrication for (a) LIG based IDE fabrication on polyimide sheet (b) 3D printed based IDE fabrication using graphene filament (c) DLW based IDE fabrication on a copper –cladding FR4 PCR board.

Layout Editor, then loaded into the given software system. The DLW system has a GaN laser diode with a wavelength of 405 nm and maximum power intensity of 65,000 W/m2. Herein, first, the Dry film photoresist was placed to the FR-4 copper-clad board using a hot roll laminator. The spacing and width were optimized and thus the width of 917 μm has obtained. Then the unwanted photoresist was removed using 0.85 % of sodium carbonate. The exposed copper was etched using Ferric chloride solution. Finally, the resist was removed and rinsed with PBS, and, thus dried with air. Scheme 1 (c) shows the schematic representation of the DLW-based IDE sensor.

E. Interdigitated Electrode Design and Modelling (IDEs)

Designing and optimization of sensing impedance using IDE were performed in our previous work [43], [44]. This paper investigated all of the design parameters, as well as the limiting variables for parameter selection and their optimal values. The length of each finger was determined by the breadth of all of the selected substrates. The bottom limit value for setting the IDE width was determined by the nozzle thickness of 3D printers, DLWs, and CO2 Laser. Because it was the most reproducible single-width that could be printed by all of the devices, the width of each electrode was decided to be 917 μm.

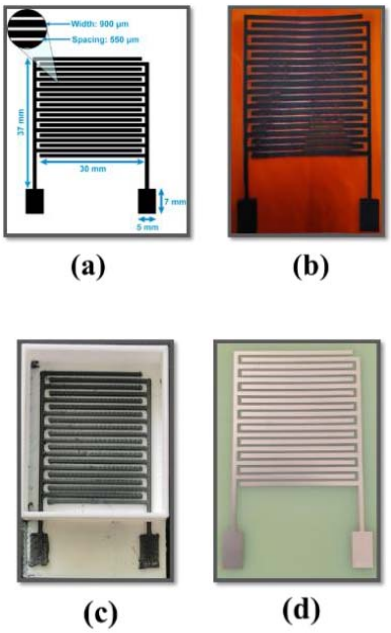


Fig. 1. (a) Optimized Design (b) LIG-based IDE on polyimide sheet (spacing = 553μm, and width = 917μm) (c) 3D printed based IDE using graphene filament (spacing = 553μm, and width = 917μm) (d) DLW based IDE on a copper –cladding FR4 PCR board (spacing = 553μm, and width = 917μm).

The method to determine the sensitivity of sensors using IDE was performed by measuring the surface of conduct between the bacterial solution and the given electrodes. The most important factor to calculate the spacing and width is the metallization ratio (a), which plays an important role to calculate the sensitivity of the sensor. Herein, the metallization ratio was computed with the optimized value of0.66. Based on the selected width of the electrode, a distance of 553μm was computed between each finger. The number of fingers, which was critical in defining the cell constant, was another criterion that needed to be chosen. Analytically, it was observed that no obvious change in cell constant beyond 20 fingers was observed. In addition, the copper cladded board, polyamide sheet, and 3D printed substrate could easily support 20 fingers. After the geometric parameters were chosen, the IDE arrays were built-in Layout Editor. By optimizing the design with AutoCAD software, the electrode width and spacing were obtained as 553 μm and 917 μm, respectively. The design additionally included 5 mm $\times$ 7 mm, contact pads to allow for alligator clip clipping during EIS analysis. Fig. 1 shows the fabricated IDE sensors on various substrates. To eliminate the problem of short circuits, the optimization of all the three given designs was done. The average finger width was 917 μm, with a gap of 553 μm was finally used as the optimized design.

F. Setup for Experiments

To commence, separate stock solutions of various analytes were created. E. coli bacteria was used to make a stock solution. To obtain solutions of various concentrations, the E. coli bacterium solution was serially diluted. The given solution was used to conduct the EIS study with all three types of fabricated Interdigitated electrodes. To avoid temperature-induced effects, all studies were carried out at room temperature (24°C). The frequency range was optimized and thus range chosen was 1 Hz to 1 MHz at 20mV. To perform various studies and to minimize the effect of interference with different bacteria freshly prepared sensors were taken. A water-ethanol cleaning protocol was used for different concentrations of the same bacteria. Alligator clips were used to establish strong and effective contact. The IDE was dipped in the analyte solution using a 50 mL beaker, and responses were recorded as a result.

G. Experimental

Initially, the EIS study was solely focused on tap water, with no Luria Bertani (LB) Broth medium or no bacteria added. Thereafter, in tap water, LB media was added to study the change in impedance by performing the EIS analysis. This experiment was performed with all three IDE-based sensors developed. However, this was done to understand the impact of background change in impedance with the changes in frequency under the given range. Also, to eliminate or minimize the probable variabilities in accurate microbial cell count prediction caused by these various sample background conditions. Finally, E. coli (DH5α) bacteria were mixed with LB medium in tap water, and impedance changes were measured about frequency changes. Subsequently, EIS investigations were carried out on all three substrates. Bacteria concentrations in the range of 2.52 $\times$ 10^4 CFU/mL to 25.2 $\times$ 10^4 CFU/mL were added to the given solution and impedance study was monitored.

The data was processed to find direct and derived impedance parameters that can be used to determine bacterial cell concentration. Specificity tests, which try to detect a specific bacterial cell, were not included in this set of investigations. These tests provide an easy and economical way to sense the bacteria. All of the trials were carried out three times. To determine the varied impedance values about the provided substrates, a certain frequency (40KHz) was used optimized after scanning various frequencies. For calibration purposes, all the data were examined at 1 Hz frequency and further examination was done for the given range of frequencies. All the experiments were performed three times, to confirm the reproducibility. The examinations were carried out in two stages, whereby in the first stage, as a control, LB media was used and thus impedance was examined. In the second stage, the impedance of the EIS electrode of cultured bacteria was used for all the given substrates.

H. Bacteria Detection

To detect the bacteria, EIS analysis was performed from 1 Hz to 1 MHz frequency. With all three types of fabricated sensors, each experiment was repeated three times using three separate electrodes with same bacterial concentration to know the reproducibility of the given sensors. The change in impedance for all three types of IDE sensors was measured at a set frequency. Changes in impedance for LIG-based IDE sensor, DLW-photolithography-based IDE sensor, and 3D printer-based IDE sensor were monitored as 20 Ω, 4KΩ, and 1KΩ for (40 kHz) frequency, respectively.

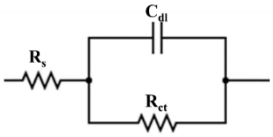


Fig. 2. Randle equivalent circuit.

I. Electrical Impedance Estimation and Comparable Circuit

EIS estimations were done with a potentiostat with frequency response analysis. The basic concept was to provide the AC voltage to the given biological sample and get back the differential voltage as a signal. A voltage signal of 20 mV was provided and the impedance was estimated in the frequency range of 1 Hz to 1 MHz. To carry out this estimation, a comparable circuit given by Randles (Fig. 2) was used. The given notations used were Rs = resistance of the solution, Rct = resistance of the charge transfer, and Cdl=double-layer capacitance.

J. Bacteria Preparation and Spike Tests

Bacterial strains were collected from Department of Biology, BITS Pilani, Hyderabad. (DH5α) E. coli strains were cultured on LB agar plates. Fresh isolate colonies (streaked from 80 °C stocks) were placed into nutrient-rich broth on the day of the experiment, cultured at 37 °C at 180 rpm, overnight. At 620 nm, the optical density was observed. After that, the cells were diluted to desirable various concentrations to perform the impedance effect on all of the substrates. Using a modified McFarland standard solution, cell stock concentration was calculated using OD620 nm turbidity in Equation 2.

$$y = 29.857x^3 + 9.8803x^2 + 10.35x + 0.5238 \qquad (2)$$

where y is the cell number (in 108CFU/mL units) and x is the Optical Density at 620 wavelengths. Viability plating and ocular microscopic enumeration of cells were used to test the accuracy of these cell counts.

III. Results and Discussions

A. Impedance Response of the E. Coli Bacteria on Different Substrates

It is also worth noting that the recorded spectra were less noisy at higher frequencies. This is because the total impedance has decreased. Fig. 3 depicts the Impedance response of various controls like tap water lone, LB media alone, Tap water+ LB media and the optimal solution of Tap water+LB media+E.coli over various substrates. As can be seen, with addition of standard E. Coli, Impedance response increased.

B. Impedance Response of the Various Concentration of the E. Coli Bacteria on Different Substrates

Fig.4 illustrates the results of an EIS performed for various concentrations of E. coli bacteria using all of the mentioned IDEs at frequencies in the range of 1 Hz to 1 MHz. The given spectrum shows the relation of impedance with the excitation frequency. For higher frequencies (>1000 Hz), there was a noticeable divergence in impedance values for different substrates, which was related to the resistance of bacteria solution. Herein, the maximum impedance was noticed using the DLW

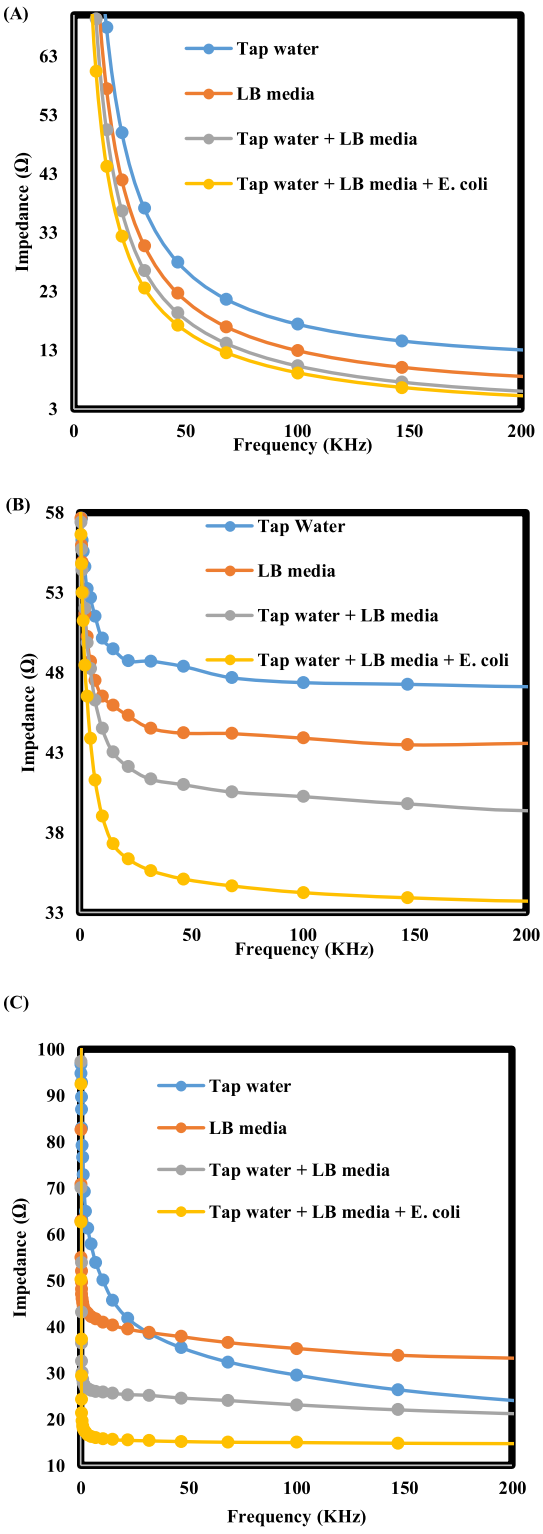


Fig. 3. (A) Impedance response of the E. coli bacteria using Direct Laser Writing (DLW) technique. (B) Impedance response of the E. coli bacteria using Laser-Induced Graphene (LIG) technique. (C) Impedance response of the E. coli bacteria on 3D printed substrate.

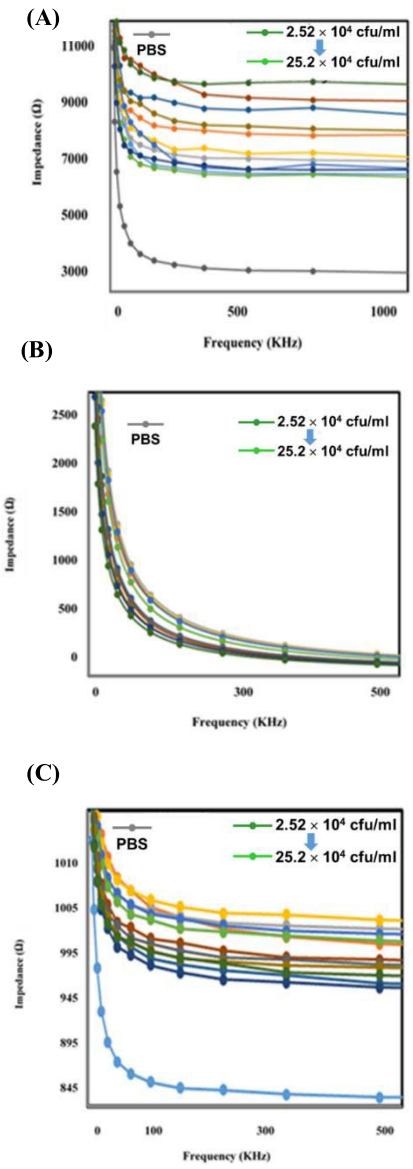


Fig. 4. (A) Impedance response of the various concentration of the E. coli bacteria using various concentration of E. coli bacteria using LIG-based IDE.(B) Impedance response of the Direct Laser Writing (DLW) technique (C) Impedance response of the Different concentrations of E. coli bacteria using 3D printed device IDE.

based IDE sensor. At lower frequencies, there was very little difference in impedance between the three substrates. As a result, only at higher frequencies, various IDE sensors can be distinguished based on their impedance characteristics.

Thus, the response of the impedance was influenced only at higher frequencies and also impact the bacteria cells. When cells were incubated in LB medium, impedance diminishes over time. However, a semipermeable cell membrane was present in the live bacteria, which allows the movement of essential solutes like ions. The cells get metabolized in a nutrient-rich environment like LB broth, the doubling rate of E.coli cells was found to be 15 minutes. Furthermore, it was noted that E. coli bacteria used of very high concentration depicting the culture was pure and fresh. In terms of the relationship between impedance magnitude and frequency, the

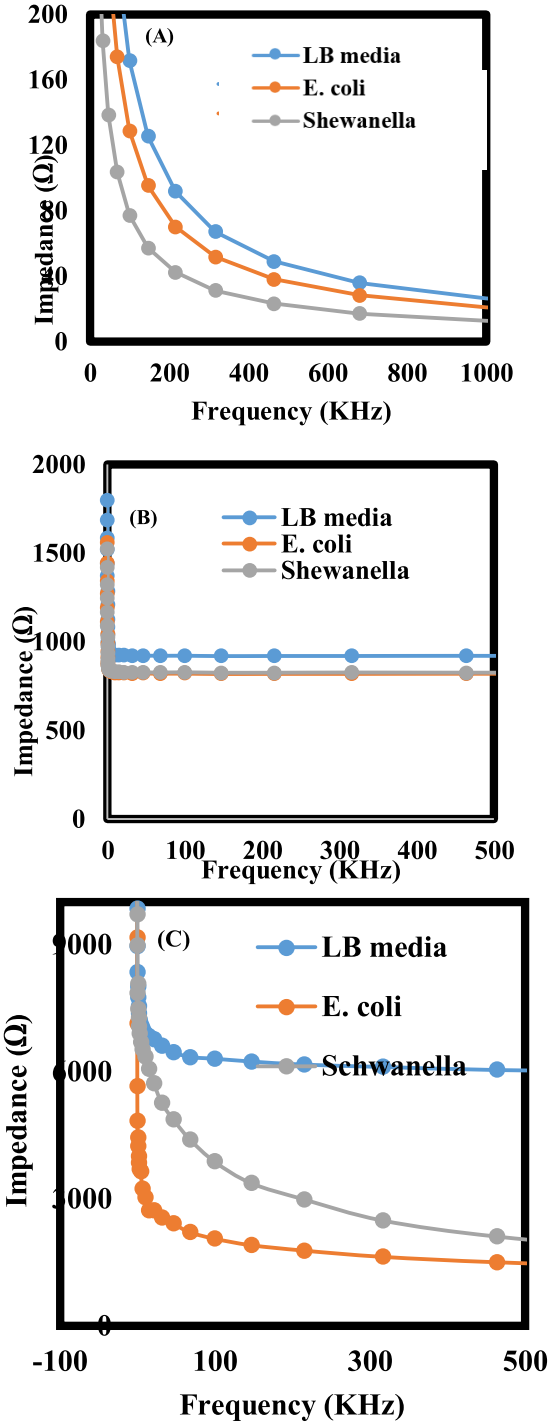


Fig. 5. (A) Interference study of E. coli bacteria using DLW based IDE on Copper Cladded Board. (B) Interference study of E. coli bacteria using LIG-based IDE. (C) Interference study of E. coli bacteria using 3D printed device IDE.

EIS spectra exhibit a similar pattern. When the frequency was less than 4 Hz, the impedance magnitude increases, and when the frequency is greater than 4 MHz, it converges.

C. Hand-Held Multiplexed Impedance Sensor System Testing

Fig. 4(A) shows the response of a LIG-based IDE sensor to varying concentrations of E. coli bacteria. Fig. 4(B) shows

TABLE II
COMPARISON SUMMARY OF ALL THREE SUBSTRATES

	Copper Cladded IDE	Laser induced graphene IDE	3D printed graphene filament IDE
Ease/Time of fabrication	More time	Less time consuming/ single step	More time consuming
Impedance change with addition of E.Coli	Less increment	Significant increment	Moderate increment
Effect of E.Coli concentration	Not much difference in impedance with change in concentration	High linear range and can detect as low as 2.5CFU/mL	Less linear range and cannot detect lower concentration
Interference effect	Significant interference from Schwanella	No interference	Moderate interference from Schwanella

the results of sensing with a DLW-Photolithography-based IDE sensor. Fig. 4(C) shows the results of sensing with a 3D printer-based IDE sensor. The charts show how impedance values fluctuate with frequency. Solution resistance and double-layer capacitance were critical in describing EIS behaviour. As a result of the solution resistance, resistance at higher frequencies increases. However, it was clear that LIG-based IDE sensors can detect lower quantities of E. coli bacteria, such as 2.5CFU/mL. The impedance from a control panel was measured that included broth but no bacteria to see how the sample impacted the stability of the impedance signal. In most cases, a measurable decrease in impedance over time was observed.

D. Interference Effect

The selectivity of the device was tested using the interference effect in the presence of 1.008×10^5 CFU/mL E. coli and the same concentration of *Shewanella Oneidensis* bacteria on all substrates. The LIG-based IDE sensor produced the least amount of interference. The Shewanella bacteria, as shown in Fig. 5, responses at significantly different potentials than E. coli. Furthermore, among the three sensors, the LIG-based IDE sensor exhibits the least interference, which was almost non-existent. Thus, the result obtained shows that the LIG-based IDE sensor shows better selectivity than the other two sensors. Table II summarizes the comparison of various parameters of all three substrate based IDEs.

Overall, it was observed that LIG played a key role in improving the sensitivity of the IDE. Offlately, LIG has been applied to develop several flexible devices and sensors. One such significant application is gas sensing. Stanford et. al., studied the LIG based flexible and embedded gas sensors [45]. Likewise, chemical modification of LIG with metal coatings has also proven to be efficient for gas sensing [46]. The furture porspects has substantial applications of LIG based IDE in varied fields.

IV. CONCLUSION

Herein, a comparative study of different fabricated interdigitated electrodes (IDEs), concerning the impedance change as a function of *E.coli*concentration is reported. Direct sensing of *E.coli* in real-time samples has been attempted. The bio-microdevices prepared were LIG-based IDE sensor, DLW-Photolithography based IDE sensor, and 3D printer-based IDE sensor. The results obtained showed that the LIG-based IDE sensor was an effective approach for the detection of *E.coli* bacteria amongst all. Hence, can be used for biomedical applications. The capability to detect the *E.coli* bacteria using all three fabricated IDE was done and reported. The LIG-based IDE sensor produces better sensitivity than the other two approaches mentioned. Moreover, the LIG-based IDE sensor was fabricated in less time and with areduced cost. The study was carried out at room temperature with all the external conditions intact. The comparative study of these various IDEs fabricated for selective *E. coli* detection opened a gateway for designing a point of care, selective analysis in real time scenario.

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Modified Ultra Micro-Carbon Electrode for Efficient Ammonia Sensing for Water Quality Assessment

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***Abstract*— Ammonia is one of the most prominent and hazardous water pollutants; hence its selective and sensitive detection in water is crucial for monitoring water quality and determining its usability. In the present work, a simple, cost-effective electrochemical sensor for the detection of ammonia is presented. Multi-walled carbon nanotubes modified ultra-micro-carbon thread electrode (UME/MWCNT) has been realized. The electro-catalytic activity of ammonia is studied by voltammetry and amperometry techniques and the results are presented. The microscopic characterization of UME/MWCNT for surface morphology analysis was also carried out. Further, the UME/MWCNT based electrochemical sensor was tested for its practical application by exploring various parameters like the effect of scan rate, pH and interference from co-existing bio-chemicals like nitrate, nitrite, phosphate, hydrazine, H_2 O_2, uric acid, ascorbic acid and dopamine along with real sample analysis. The developed sensor can efficiently detect ammonia in a linear range of 10 μM to 1 mM which is well within the permissible safe drinking water limit. The limit of detection (LoD) and limit of quantification (LoQ) obtained for the developed sensor were 8.69 μM and 26.33 μM respectively. The negligible interference, good reproducibility, and appreciable recovery values indicated the potential of the developed UME for real-time ammonia detection. As a flexible electrode, UME can be further modified and fabricated as a microfluidic or a miniaturized device for a portable electrochemical sensing platform in future.**

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***Index Terms*— Ammonia, carbon thread, multi-walled carbon nanotube (MWCNT), ultra-microelectrode (UME), electrochemical.**

I. Introduction

ACCORDING to the World Health Organization (WHO), 80% of the prevailing diseases are waterborne and ~3% of the overall fatalities across the globe are due to unhygienic water consumption [1]. Ammonia is one of the most common water pollutants and high levels of ammonia in water may lead to yellow fumes and foul odor in the water bodies. The permissible safe and tolerable level of ammonia in water is 0.5 ppm (approximately 29 μM) [1]. Increased level of ammonia concentration can cause serious illnesses like kidney ailments, liver diseases like hepatitis, Reye syndrome, and may even lead to birth disorders in some cases [2]. Additionally, high levels of ammonia cause growth of algae in water bodies and which leads to eutrophication. The major sources contributing to ammonia are agricultural wastes (fertilizers) and industrial wastes. Hence, it becomes necessary for the detection and quantification of ammonia concentration in water bodies. This also helps in assessing quality of the water body as ammonia is one of the high risk water pollutant.

The conventional approaches to detect ammonia in water samples are either by solid-state methods or optical methods [3]. The solid-state method involves metal oxide-based [4]–[6] conducting polymer [7]–[10], electrochemical-based [11]–[14] and field-effect transistor [15]–[17] type sensors, whereas the optical methods involve tunable laser absorption spectroscopy, non-dispersive infrared, cavity ring-down spectroscopy and photoacoustic spectroscopy [18]–[21]. Since in the majority of applications, remote monitoring of water quality is crucial, solid state-based methods are more convenient than optical methods. In solid state-based metal oxide sensors, metal oxides like TiO_2, SnO_2, ZnO are used for sensing various gases with added advantages like low cost, flexibility [22], miniaturization, etc. Despite such benefits, the key disadvantage is the selectivity in detecting a particular substance from the mixture. This makes electrochemical-based analysis a more popular approach for sensing. In the electrochemical

TABLE I
LITERATURE REPORTS ON AMMONIA SENSING

Electrode	Linear Range (μM)	LoD	Technique	Real sample	Ref
Copper composite @ CNT	3 to 100	3.47 μM	Differential pulse voltammetry	Tap water	[35]
Silver wire@ polyaniline (PANI)	0.1 to100	-	Cyclic Voltammetry	Wastewater	[37]
Electrodeposition of Platinum-Polypyrrole on Ni Foam	0.5 to 400	37 nM	Voltammetry	Artificial water sample	[38]
Carbon nanotubes modified by oxygen plasma treatment	-	10 nM	X-ray photoelectron Spectrometry	Artificial water sample	[39]
Enhanced by PANI/TiO_2 Nanocomposites	3 to 500	2.14 μM	-	-	[40]
SnO_2-deposited copper electrode	-	-	Voltammetry	Water sample	[41]
Carbon thread coated with MWCNT	10 to 1000	8.69 μM	Voltammetry	Lake water	This work

methods reported [23], [24], various nanoparticles [25], [26], like carbon nanosheets [27], [28], poly-aniline [29], platinum nanosheets, etc., are deposited on the electrode surface for better electrical conductivity [30], improved sensitivity, and selectivity [31]–[33].

In a recent study, nanosheets ($Ni(OH_2)$) were deposited on carbon cloth by solvothermal method to form ($Ni(OH_2)$) – CC, and then platinum sheets were deposited by amperometric technique to form Pt-($Ni(OH_2)$)–CC [34]. Further, researchers have used carbon nanotube (CNT)/copper composite paste electrodes to demonstrate the detection of ammonia [35]. A thin film of an ionic liquid was used as an electrolyte and a flattened micro-sized electrode was fabricated to form a sensor platform in [25]. This electrochemical sensor effectively detected ammonia of 1 ppm concentration at 25°C, neutral pH, and 1 atm conditions [36]. A summary of the few remarkable approaches reported in the literature for electrochemical detection of ammonia in water samples is presented in Table I.

In this work, a three-electrode system is proposed for the detection of ammonia, in which an ultra-micro carbon thread modified with multiwalled carbon nanotube (MWCNT) designated as UME/MWCNT is used as a working electrode. The presented UME electrode is tested for its stability and repeatability. A real sample analysis is performed using the lake water to validate the efficacy of the proposed sensor.

II. EXPERIMENTAL PROCEDURE

A. Materials and Reagents

Anhydrous monobasic Sodium Phosphate (NaH_2PO_4), Dibasic Sodium Phosphate dihydrate ($Na_2HPO_4.2H_2O$), and Ammonium chloride (NH_4Cl) were acquired from AVRA chemicals. The DI water, used throughout the experiment, was from Milli-Q (18.2) MΩ.cm. Scanning Electron Microscope (SEM) characterization was performed using the Apreo scanning electron microscope from Thermo Fisher Scientific, Massachusetts, USA. MWCNTs, with a purity of approximately 95%, were procured from NanoLab Inc. (Brighton, MA). The carbon thread was acquired commercially from Fuel Cell store, Texas, USA. All the other common chemicals and reagents of analytical grade were used.

B. Ultra-Micro–Electrode (UME) Preparation

The UME was prepared by taking 2 mg of MWCNT in 500 μL of 99.9 % pure ethanol. The proper dispersion of MWCNT was achieved by probe sonicating the mixture for 15 minutes with a start and stop of 30 seconds each. After that, the evenly dispersed MWCNT mixture was drop cast on carbon thread homogeneously, and later oven-dried at a temperature of 60°C for 10 minutes. Fig. 1 (a) demonstrates the procedure for the preparation of UME with MWCNT and Fig. 1 (b) shows the electrochemical sensing setup of ammonia and the possible reaction mechanism of ammonia with modified UME surface.

C. Electrolytic Solution Preparation

0.1 M of phosphate buffer solution (PBS) of pH 7 was prepared. For the preparation of ammonia solution, weighed amount of NH_4Cl salt was homogeneously dissolved in pH 7 PBS. 5 mM $K_3[Fe(CN)_6]$ in 1 mM KCl was prepared.

III. RESULTS AND DISCUSSION

A. Morphological and Electrical Characterization

SEM characterization was performed to understand the distribution of MWCNT on the surface of carbon thread. The images were captured at the same level of magnification of 500 nm for further comparison. Fig. 2 (a) and Fig. 2 (b) show the morphological behavior of UME before and after the surface modification using MWCNT. The even distribution of MWCNT on carbon thread surface is also clearly evident from Fig. 2 (b).

In similar lines, to understand the electron transfer behavior, electrochemical characterization was performed. Herein, 5 mM $K_3[Fe\ (CN)_6]$ with 1 mM KCl as a supporting electrolyte was taken and cyclic voltammetry (CV) was performed at 50 mV/s, for 10 cycles in a potential window of −1 V to +1 V. As it can be seen in Fig. 3 (a), the UME alone

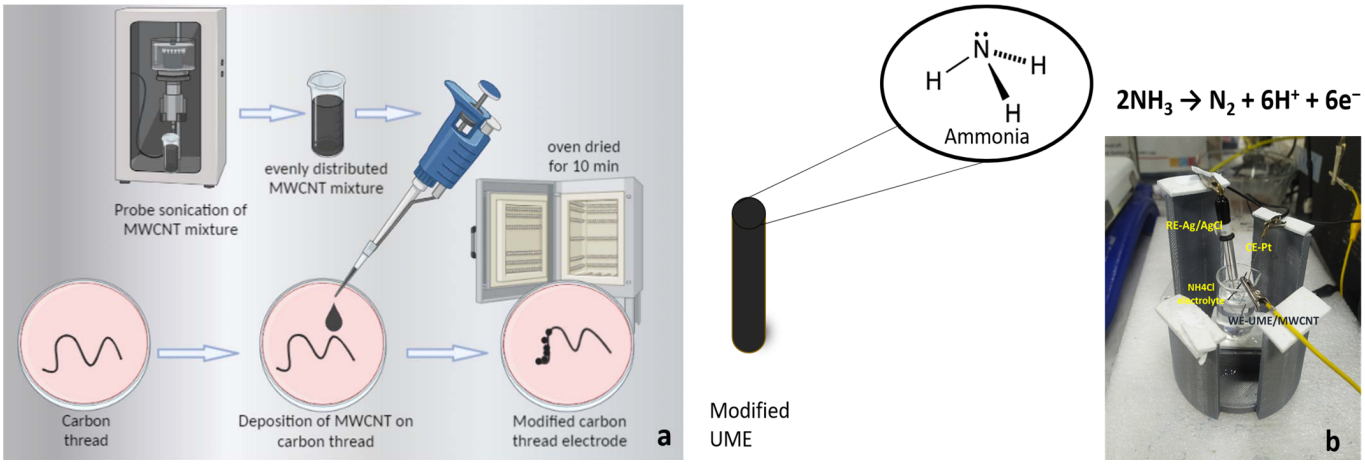

Fig. 1. (a) Schematic representation of UME/MWCNT fabrication. (b) Electrochemical sensing setup of ammonia and the reaction mechanism of ammonia with modified UME surface.

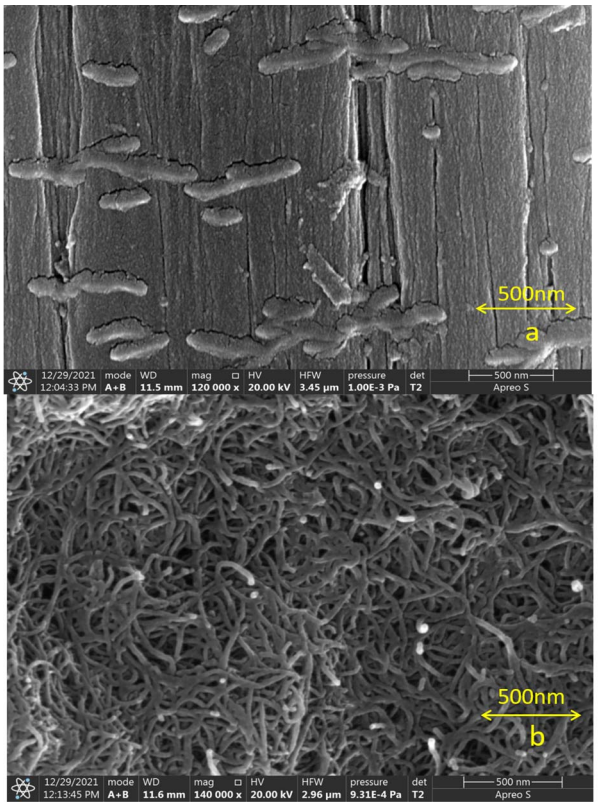

Fig. 2. (a) SEM characterization of carbon thread (b) Carbon thread with MWCNT modification.

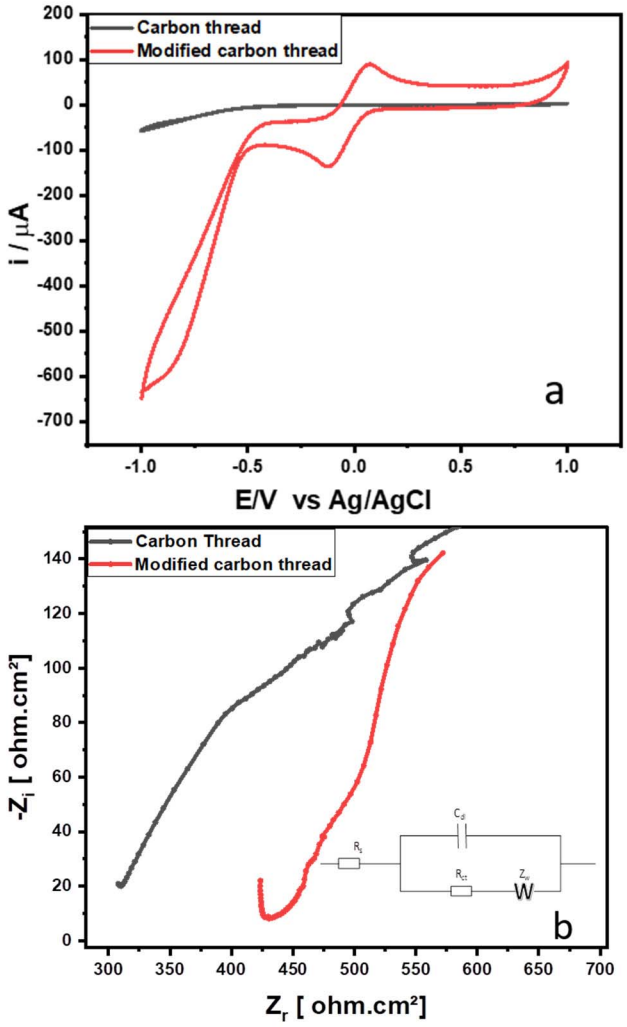

Fig. 3. (a) Comparative CV response of carbon thread and modified UME electrode in $K_3[Fe(CN)_6]$ solution at 25°C and 1atm (b) EIS curve of carbon thread and modified UME electrode along with Randel's circuit.

(without MWCNT) provided lesser current density than the modified UME/MWCNT. A stable redox peak at E'= 0.061 V was observed with ΔEp = 0.187 V. The modification with MWCNT gave enhanced redox peak current and lesser peak-to-peak separation (ΔEp = 0.187 V) thereby improving the electron transfer mechanism.

The Electron Impedance Spectroscopy (EIS) study was also performed with and without MWCNT modified UME in 5 mM $K_3[Fe(CN)_6]$ with 1 mM KCl as a supporting electrolyte. Fig. 3 (b) clearly depicts that the modified and unmodified UME showed semicircle behavior with varied R_{ct} (charge transfer resistance) values. The R_{ct} for UME alone was 3.89 Ω

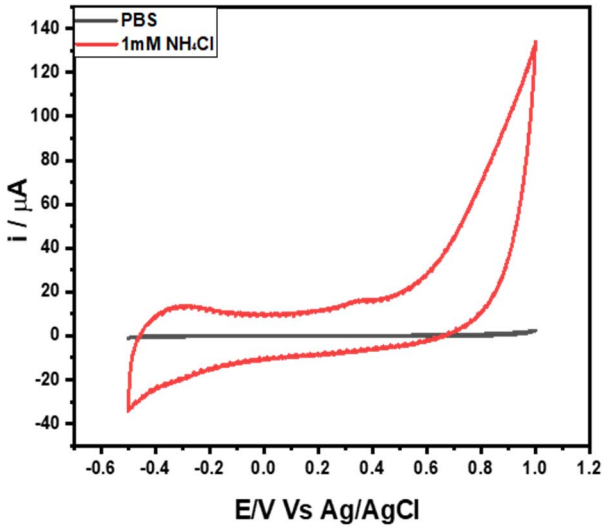

Fig. 4. Comparative CV responses of UME/MWCNT in PBS and 0.5 mM ammonia in pH 7 PBS at 10 mV/s.

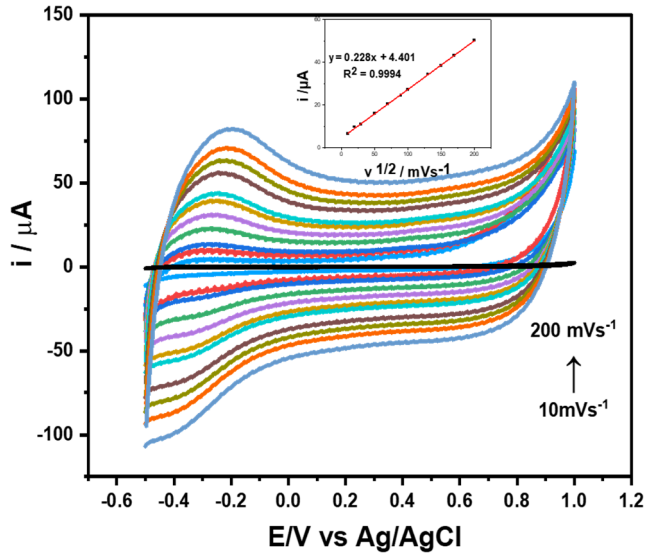

Fig. 5. CV responses of UME/MWCNT at various scan rates from 10 mVs^{-1} to 200 mVs^{-1} in 0.5 mM ammonia in pH 7 PBS. Inset is the linear plot of current v/s $v^{1/2}$.

whereas, for UME/MWCNT was 435 Ω, further proving the enhanced electron transfer behavior [42].

B. Electro-Catalytic Behavior

To examine the electro-catalytic behavior of UME/MWCNT when exposed to ammonia, the prepared electrode was subjected to CV analysis at 10 mV/s scan rate, number of electrons (n = 2), in a potential window of −0.5 V to +1 V in 0.5 mM ammonia solution taken in pH 7 PBS at room temperature. As it can be observed from Fig. 4, UME/MWCNT gave no significant response in PBS alone, whereas a sharp oxidation current of 16.32 μA at the E^0 = 0.37V was observed in the presence of ammonia indicating the electro-catalytic oxidation of ammonia. Similarly, a control experiment was performed wherein bare UME was exposed to ammonia gave negligible response, henceforth, proving the role of MWCNT in detection of ammonia.

C. Scan Rate Effect

The consequence of potential scan rate on UME/MWCNT with ammonia in pH 7 PBS was investigated by varying the scan rate from 10 mV/s to 200 mV/s to understand the optimum electron transfer mechanism. The obtained CV responses are presented in Fig. 5. The subsequent increase of cathodic peak current as the scan rate increases can be clearly noted from Fig. 5. The corresponding linear fit plot of the maximum current v/s the scan rate shows that the modified electrode has a surface-confined electron transfer behaviour as per Randel's Sevcik equation given in (1) [43].

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

where n = 2, A is the surface area of the electrode (410 mm^2), D is the coefficient of diffusion, v is the scan rate (0.01 V/s) and C is the concentration of the NH_4Cl (1 mM). Using (1), the diffusion co-efficient, D is calculated as 8.5×10^{-6} $cm^2 s^{-1}$.

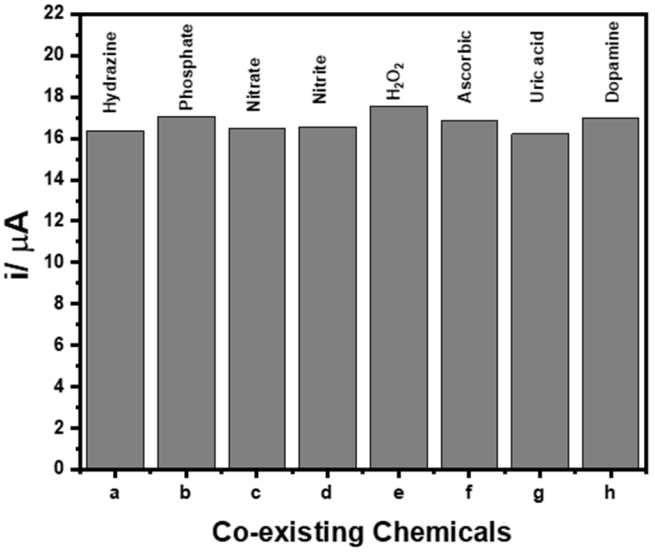

Fig. 6. Interference effect from co-existing chemicals like Hydrazine, Phosphate, Nitrate, Nitrite, H_2O_2, ascorbic acid, uric acid and dopamine with UME/MWCNT electrode.

D. Interference Effect

In general, in real water sample, many other chemicals, like nitrate, nitrite H_2O_2, hydrazine, phosphate, uric acid, dopamine and ascorbic acid are also present and therefore may cause interference during ammonia detection. Hence, the selectivity of the UME/MWCNT towards ammonia has been evaluated. 0.5 mM of each of the interfering agents were tested by exposing it to the fabricated electrode and CV was performed in the potential window of −0.5 V to 1 V, at a scan rate of 10 mV/s. Fig. 6 shows that there is no significant peak is observed with other analytes whereas, a characteristic peak of ammonia was seen at E^0 = 0.37V.

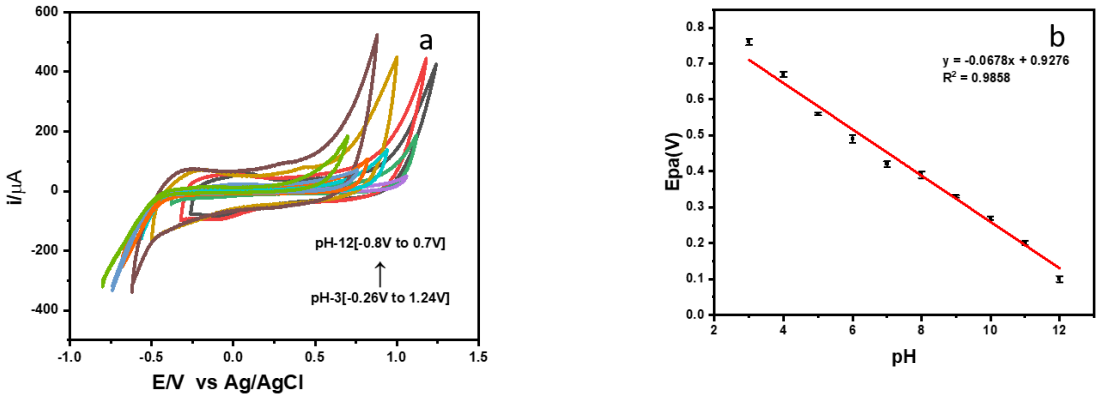

Fig. 7. (a) CV response of UME/MWCNT electrode for pH values ranging from 3 to 12. (b) Calibration plot showing E_{pa} v/s pH with error bars.

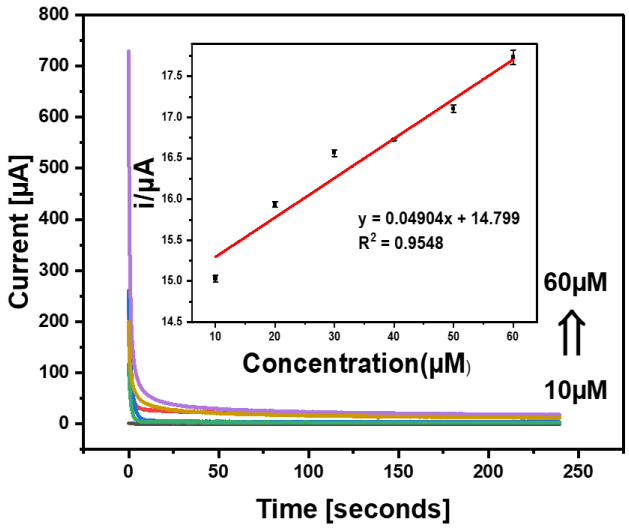

Fig. 8. Chrono-amperometry response for of various concentrations for LoD study. Corresponding calibration plot for oxidation peak currents ipa vs v1/2.

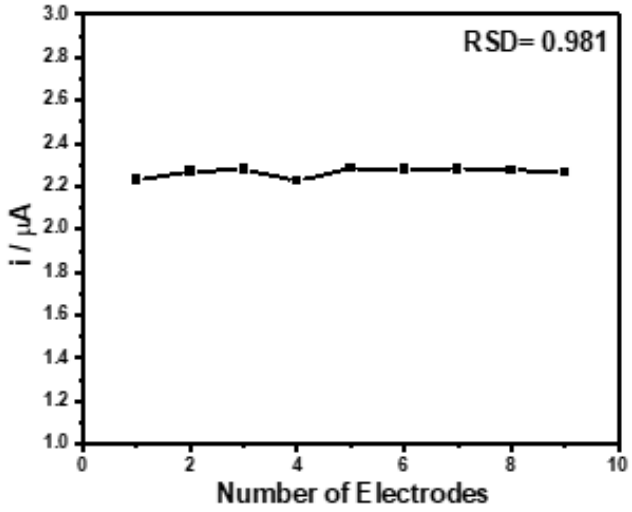

Fig. 9. Repeatability study of the developed sensor using various electrodes with a concentration of 0.5 mM of ammonia.

E. Influence of Solution pH

The influence of solution pH on the UME electrode was studied by preparing various pH solutions ranging from pH values of 3 to 12 of 0.5 mM ammonia in PBS and the results are plotted and shown in Fig. 7 (a). The calibration plot for the same is plotted between pH and peak potential and is shown in Fig. 7 (b). The variation in the pH from acidic to basic did not change the value of the peak current which confirms that the UME electrode is pH independent [44].

F. Concentration Effect

The consequence of increase in concentration was studied by chronoamperometry from 10 μM to 1 mM at 0.37 V. Fig. 8 clearly shows that there is a substantial rise in the amount of current from lower to higher concentration. The respective calibration plots gave LoD and LoQ as 8.69 μM and 26.33 μM respectively.

TABLE II
REAL SAMPLE ANALYSIS

Sample	Ammonia added (μM)	Ammonia Recovered (μM)	Recovery (%)
Pond water	100	102.4	102.4
	200	214.1	107
	300	293	97.6

G. Sensor Reproducibility and Stability

The reproducibility of the proposed UME electrode is tested with nine different fabricated electrodes in 0.5 mM ammonia solution and the relative standard deviation (RSD) is found to be 0.981 as shown in Fig. 9. The stability study was also performed for a period of 7 days and the graph is shown in Fig. 10. From the results it is observed that the proposed sensor has a good reproducibility and stability towards ammonia sensing.

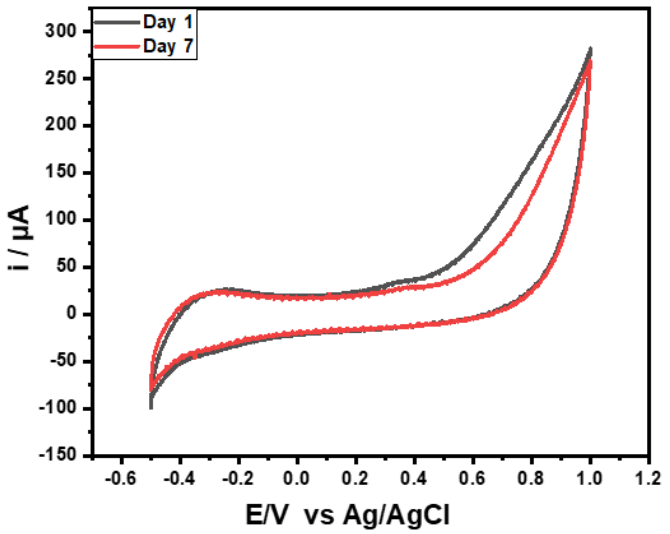

Fig. 10. Stability study of the proposed system for a period of 7 days with a concentration of 0.5 mM ammonia.

H. Real Sample Analysis

In order to understand the practicality of the proposed sensor in ammonia detection, a real sample testing was performed using pond water from BITS Pilani Hyderabad Campus. The standard addition method was used, where 10 ml of pond water was taken and 100 μM of ammonium chloride was added by the method of spiking for three subsequent times. The concentrations were chosen from the linear range of the fabricated UME, wherein 100 μM of NH_4Cl was spiked in real water sample solution. The results are summarized in Table II, manifesting appreciable recovery values.

IV. Conclusion

This work presents the utilization of modified carbon thread as working electrode for sensing ammonia while employing Pt and Ag/AgCl as a counter and reference electrodes respectively. A significant increase in peak current has been observed for various concentrations utilizing CV. The effect of pH, scan rate and concentration were also studied and the results have been presented. Further, reproducibility and stability analysis are also performed. The linear range is found to be from 10 μM to 1 mM and the LoD obtained is 8.69 μM. The electrode was subjected to interference test with co-existing water pollutants and it was found that the device is not interfering with any other electrolyte. Further, real sample analysis was conducted using pond water and the proposed sensor showed good recovery. Hence, the proposed sensor can be effectively utilized for real-time detection of ammonia in water. In future, the proposed sensor can be fabricated as a device for point-of-care applications.

Acknowledgment

The authors would like to thank the Central Analytical Laboratory of BITS-Pilani Hyderabad campus for the characterization and the MMNE Laboratory of BITS-Pilani Hyderabad campus for the fabrication. They also acknowledge Jaligam Murali Mohan for his help during electrochemical studies.

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Portable Electrochemical Platform With Carbon Fibre Microelectrodes Integrated on an OHP Sheet for Snake Venom Analysis

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***Abstract*— Snake bite is a serious medical emergency often leading to untimely fatalities. Serotherapy is the only treatment method adapted for this, whose efficacy depends on identification of the Snake species and venom type. As a specific antivenom has to be implicated for saving the victim, in most of the cases, such identification is challenging, thus, leading to mortality due to delay in treatment or side effects of injecting polymeric non-specific antivenom. Therefore, a point-of-care, venom specific detection device could be an impactful diagnostic tool. Herein, a prototype of miniaturized electrochemical sensing platform is presented for detection of Crotaline, venom from various common pit viper snakes. A three electrode based micro-platform with carbon fibre microelectrode, modified with mesoporous carbon, embedded and laminated in commercial OHP sheet, has been developed. The dimensions of the miniaturized platform was 25 mm × 35 mm, size of electrode was 0.5 mm × 25 mm with an electrochemical testing zone of diameter 10 mm, electrode spacing as 3 mm. The microscopic characterization revealed immobilization of porous carbon on fine fibrous structure. The device gave highly stable and sensitive electro-catalytic oxidation of Crotaline at E'= at 0.81 V, and provided a linear range of 50-300 μM, limit of detection as 18.98 μM and limit of quantification as 63 μM. The device exhibited negligible interference from physiological blood serum biochemicals, high stability and reproducibility. Further, real blood serum samples, analysis via standard addition approach, was performed which showcased appreciable recovery values confirming the practical applicability of the device.**

***Index Terms*— Microelectrode, carbon fibre, electrochemical analysis, snake venom, crotaline.**

I. Introduction

EVEN though snakebite is quite a common public health concern, it is often underrated in most of the tropical regions globally. Owing to this, several fatalities, especially in the remote areas, takes place. As per the stated reports of world health organization (WHO), around 5.4 million cases are reported every year out of which about 1.8 million mortalities are there. India was found to be the epicenter of snake bites and the most affected one, with the highest cases reported (81000) per year off which approximately 35000 casualties takes place [1]. However, the problem is way deeper than reported as precise estimation of the incidents are difficult to record as many of these occurs in countryside areas deprived of appropriate medical, clinical and logistical facility. Frequently, a significant number of cases are left unreported [2]. Most of the snake species are non-venomous, but some of them utilizes venom as their defense mechanism [3]. Venom is a viscous biological fluid comprising of enzymes, peptides, amino acids and glycoproteins [4]. Broadly, it is classified into three types (i) neurotoxic: paralyze the central nervous system, (ii) cytotoxic: destroys the cells, (iii) hemotoxic: effects the cardiovascular system [5]. These toxins induce an immunogenic rigorous reaction in the body of the patient. Injection of antivenom - immunoglobin is the only treatment to save the victim which is termed as serotherapy. Herein, a monovalent or polyvalent antibody containing serum is inoculated [6]. Nevertheless, for a pertinent and an immediate treatment, detection of venom type is crucial. Toru et. al. have reviewed in a detailed about diagnosis and treatment of snakebites and stated that there is no specific detection kit available to identify the category of venom [7].

In real time, clinical diagnosis is based on blood sample testing, analysis based on symptoms like swelling, impaired visions, chest pains, frothing, blistering, and description of the snake type by the patient [8]. Hence, in several cases, this grounds to morbidities either due to delay in identification or side effect of polyvalent antibody. Therefore, a point of care testing (POCT), offering faster and on-field results, could play a crucial role in saving many lives. In this context, quite a few advances have been reported. Enzyme based immunosensing technique is the most popularly adapted. For instance, Selvanayagam et. al. developed an ELISA (enzyme-linked immunosorbent assay) for four snake species found in India namely *E. carinatus, Bungaruscaeruleus, Daboia Russelli* and *Nataraja*. The antivenom specific antibodies were used here and venom detection was carried out in multiple organs like brain, heart, kidneys, spleen of mice and autopsy specimen of human victims. The study was conducted at

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specific time intervals up to 72 hours after the bite and death of the victim. This method could detect venom upto 100 pg ml^{-1} [9]. Likewise, Shaikh et. al. reported a similar dot- ELISA method for selective detection with about 1ng/mL limit of detection (LoD) [10]. Recently, Maduwage et. al. designed a sandwiched enzyme based immunosensing method for analysis of venomous snakes of Sri Lanka. This could detect venom samples up to 24 hours after the bite with upto 1.5 ng/mL LoD [11]. However, enzyme based approaches has limitations in terms of cross reactivity and cost-affectivity.

Optical and fluorescence based techniques have also been explored. For example, Dong et. al. designed an optical immunosensing procedure wherein, an optical layer, that reflects light, is sandwiched between a silicon wafer base and a top layer which is a substrate for binding the capture antibody. The formation of antigen-antibody complex associated with HRP enzyme and the linkers leads to development of a thin film. The thickness of this film causes reflection of incident light and color change from golden yellow to blue or purple which is then recorded [12]. In another work, Gao et. al. developed immunofluorescence based approach wherein, a platform with polystyrene beads and semiconducting quantum dots were employed as a fluorescence tag. The capture antibodies complexes with the venom. The secondary antibody with fluorescent tag binds to this venom-antibody complex which was studied under a UV microscope [13]. An enzyme based immunoassay for venom from *Pseudonajaspp*, (brown snake) was developed for quantifying the venom and antivenom [14]. Similarly, Kaul et. al. established a colorimetric dependent detection kit for field application. Herein, monoclonal antibody against the cytotoxic protein were used for capturing the elapid venom [15]. In another study, Slagboom et. al. utilized nanofractionation method to quantify and identify the proteins in snake venom like phospholipase A2 (anticoagulant), metallo protease and serine protease (procoagulant). The detection of these proteins and peptide chains in the sample were associated with an indirect approach of detection [16].

Although these techniques are highly selective and showed good sensitivity but overall procedure is time consuming, requires skilled laboratory procedures and costly labeling tags. Hence, a simpler way is the potential dependent electrochemical analysis offering rapid and highly efficient strategies for medical applications. To the best of our knowledge, only a few reports are presented in the literature covering this aspect. For example, Zehani et. al. developed an impedance based method wherein, phospholipase A2, an enzyme from the snake venom, was immobilized over a gold electrode and its activity was studied [17]. Further, several biosensors, developed using isolated antibodies via immunization in rabbits, hens, rats etc., has been demonstrated [18]. Similarly, Mars et. al. developed graphene quantum dots based electrochemical immunosensing platform to detect venom from scorpion [19]. A cell based electrochemical bioassay was developed by Ozge Er *et al.* wherein Vipera Anatolica Venom was detected. Here, a radiolabelled detection technique was adapted [20]. Table I presents the comparative representation of these reported works.

The conventional electrochemical approach of using bulk metallic and glassy carbon electrode has limitations in terms of portability and regeneration of the electrode surface. Also, mostly enzyme dependent or antibody dependent approaches has been reported using bulk volume. In the present study, a miniaturized, electrochemical platform is reported for an enzymeless and rapid sensing of *Crotaline*, venom from common pit viper snakes. Herein, a chemically modified carbon fibre microelectrode (CFME) was used as an electrode system. Owing to several crucial properties, like reduced signal-to-noise ratio, low sample volume requirement, lesser response time, enhanced mass transport and more stable diffusion, such microelectrodes are being employed broadly in electroanalytical sensing [21].

TABLE I
LITERATURE REPORTS FOR ELECTROCHEMICAL VENOM DETECTION

Analyte	Electrode	Technique	LoD
Leicthin	Gold [17]	Impedance	10^{-11} M
Scorpio venom	SPCE/GQDS/Antibody [19]	Chronoamperometry	0.55 pg·mL^{-1}
Vipera Anatolica Venom	PGE/ f HEK293/U87MG [20]	Differential pulse voltammetry	--

SPCE: Screen printed electrodes; PGE: Pencil graphite electrode; GQDS: graphene quantum dots; HEK293/U87MG: cancerous and non-cancerous cell lines.

A carbon fibre is thread like structures taken from an electrically conductive, high mechanical strength carbon cloth. However, the smaller electroactive surface area of pure carbon fibre gives poor resolution signals. Henceforth, to improve the conductivity, these fibres can be functionalized via chemical modification. In this case, a fine layer of graphitized mesoporous carbon (GMC) was coated and used as a working electrode to achieve an increased conductivity and electro-catalytic activity. The literature suggests that modification of underlying electrodes with various nanomaterials like carbon, metal organic frame works (MOF), metallic oxides, redox mediators etc. enhances the conductivity of the electrodes [22]–[24]. In the present case, GMC was used owing to its porous nature which could enhance the surface area and conductivity significantly [25].

Furthermore, Ag/AgCl conductive ink coated CFME and bare CFME were casted off as counter and reference electrodes respectively. These electrodes were integrated in an overhead projector (OHP) sheet via double sided adhesive to obtain a compact electrochemical miniaturized sensing device. This fabricated device was realized as a prototype sensor for an enzymeless, rapid testing of snake venom. After fine-tuning and modification with different carbon matrices, this device can be further studied for biosensing application of venom in real time samples.

II. EXPERIMENTAL SECTION

A. Materials

The chemicals utilized in this present work were of analytical grade purity and used as it is. Snake venom: Crotaline,

graphitized mesoporous carbon (GMC) were attained from Sigma Aldrich. A commercially available hydrophillic carbon fibre from Fuel cell store, USA, Part number W0S1009 was used. Sodium phosphate monobasic anhydrous (NaH_2PO_4), and sodium phosphate dibasic dihydrate ($Na_2HPO_4.2H_2O$) was procured from SRL Chemicals (Mumbai, India). Electrochemical experiments were conducted in 0.1 mol/L phosphate buffer solution (PBS) (pH = 7) as a supporting electrolyte. Conductive Ag/AgCl ink was purchased from ALS Co. Ltd., Tokyo Japan. OHP sheets were taken from a local stationary shop.

B. Apparatus

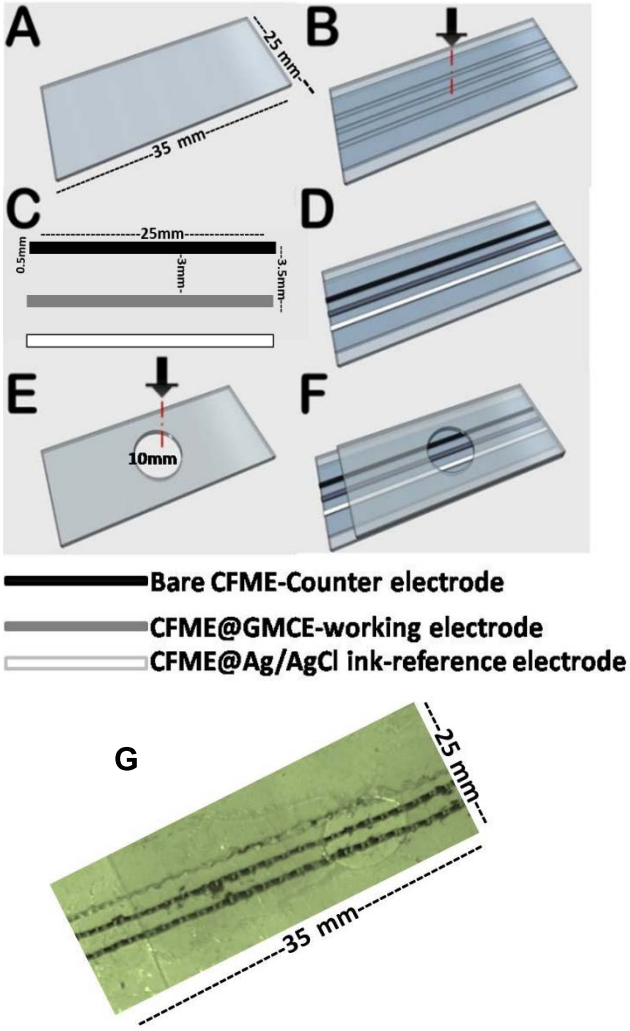
Electrochemical experiments were performed using a potentiostat (OrigaFlex-OGF500 from Origalys, France). Cyclic voltammetry (CV) technique and Differential pulse voltammetry (DPV) technique based experiments were carried out in the potential window - 0.5 V and 1 V with different parameters (pulse height (PH) as 50 mV, pulse width as 15 ms, step height 30 mV and step time as 30 ms). These parameters were scanned and based on the maximum current value the aforementioned parameters were finalized. A miniaturized platform with carbon fibre microelectrode (CFME) - 3 electrode system, embedded in an OHP sheet was fabricated and used for electrochemical studies. A CFME, modified with GMC, was the working electrode, bare CFME as the counter and Ag/AgCl conductive ink coated CFME was used as a reference electrode. The microscopic characterization was done by Scanning Electron Microscope (SEM) (Apreo S, FEI from Thermo Fisher Scientific, USA). Other instruments include pH meter (PC 700 from Oakton Instruments, USA), ultrasonication water bath (from Life-Care, Mumbai, India), hot air oven (CIC-15Afrom Cintex, Mumbai, India) and CO_2 laser machine (VLS 3.60 from Universal Laser System, USA).

C. Electrode Preparation

2 mg of GMC was weighed, dispersed and sonicated in 500 μL of ethanol using an ultrasonication bath for about 15 min. 2 μL of this prepared solution was dropcoated on the carbon fibre and air dried 4±2 mins to give CFME/GMC working electrode (WE). Ag/AgCl conductive ink was coated on CFME and left for drying in hot air oven at 70^0C for 10 mins. This was referred as a reference electrode (RE) and bare CFME was used as a counter electrode (CE).

D. Assembling of the Electrochemical Platform

Scheme I gives the diagrammatic representation of the fabrication of the CFME electrochemical platform. The overall platform size was 25 mm × 35 mm, size of electrode was 0.5 mm × 25 mm with an electrochemical testing zone of diameter 10 mm. Other parameters like electrode spacing was kept as 3 mm was kept small, length of electrode was 25 mm, for good resolution. The CFME electrode was positioned on the custom designed electrode holder using an OHP sheet. Firstly, on the OHP sheet, the transparent double sided tape was attached on which the CFME electrodes were placed. For proper electrode placement, laser mark was made using



SCHEME 1. Fabrication steps: (A) OHP Plastic sheet, (B) CO_2 laser cutting and patterning on double-sided adhesive tapes attached on OHP sheet, (C) Carbon fibre microelectrode equidistant at 3 mm (D) attaching CFME on the laser marked pattern, (E) Laser cut reservoir region on the OHP sheet (F) Laminating the OHP sheet onto the CFME integrated platform. (G) The real image of the fabricated platform.

a CO_2 laser [26], [21] on the double sided tape and then carefully the CFME was placed on the laser marked area. For obtaining an electrochemical testing reservoir, on the other OHP sheet a punch hole was made of 10 mm diameter and it was positioned on the CFME attached OHP sheet followed by the application of heat and pressure using a lamination machine. The as developed CFME/OHP sheet platform can be used for electro-analytical sensing. The fabrication process can be easily carried out at a room temperature. The main benefit of using this fabrication process is in the fact that the working electrode surface can be modified with ease. The working electrode surface can be used for multiple electrochemical analysis. Scheme I gives the diagrammatic representation of the procedure.

III. Results and Discussions

A. Microscopic Characterization

To comprehend the morphological surface characteristics of the CFME, SEM characterization were performed. Fig. 1

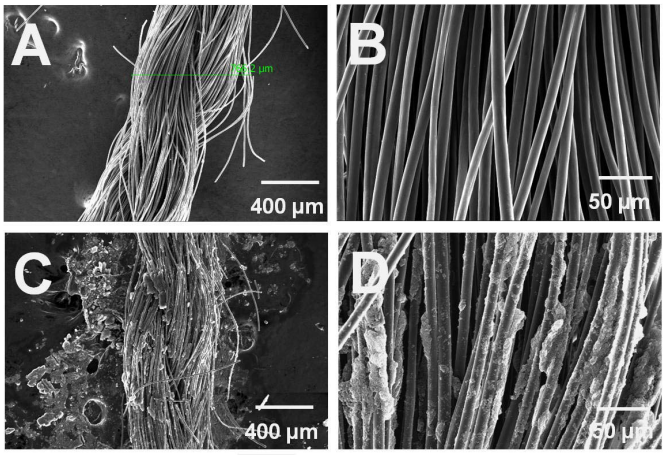


Fig. 1. SEM characterization images of carbon fibres at different magnifications. (A and B) bare CFME, (C and D) CFME/GMC.

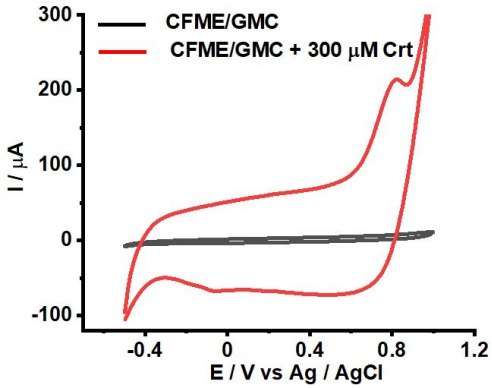


Fig. 2. A typical CV response of CFME/GMC with and without 300 μm Crotaline in pH 7 PBS at 10 mV s-1 for 2 cycles.

depicts the SEM images of CFME and CFME/GMC. A cylindrical fibrous structure with a diameter ~750 μm was seen for bare CFME, whereas, post modification with GMC, immobilization of porous structure over fibres with a consolidated diameter of ~200 nm was observed indicating the improvement in the surface area. GMC is reported to have an average macropores of about 50 nm in size, hence, it is plausible that post modification, the overall size of the fibre was increased [25]. In further, the material of the carbon fibre, hydrophobicity/hydrophilic nature plays the key role in electron transfer behavior.

B. Electrocatalysis of Crotaline

The CFME platform was subjected for electrocatalytic study of Crotaline with a standard concentration of 300 μM taken in pH 7 PBS. CV was carried out in the potential window - 0.5 to 1 V, at 10 mV s^{-1} for number of cycles; n=2. CFME/GMC gave a clear oxidation peak at E'=~0.81- V, which the control CFME/GMC in pH PBS alone failed to give. Hence, it was found that the modified electrode could oxidize the Crotaline (Fig. 2).

C. Effect of Crotaline Concentration

Fig. 3A showcases a DPV response of CFME/GMC with increasing Crotaline concentration in a linear range

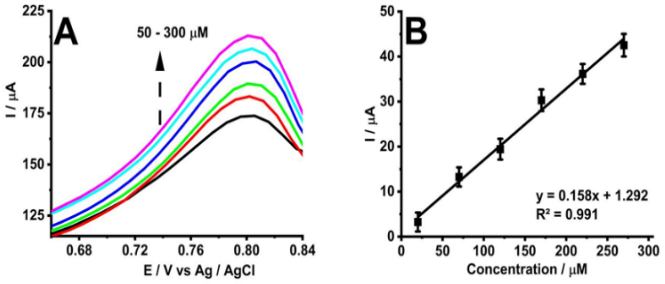


Fig. 3. (A): DPV responses of CFME/GMC within a linear calibration range of 50-300 μM (The colored lines represents the response of various concentrations). (B) Corresponding base line corrected calibration plot with the error percentage bars.

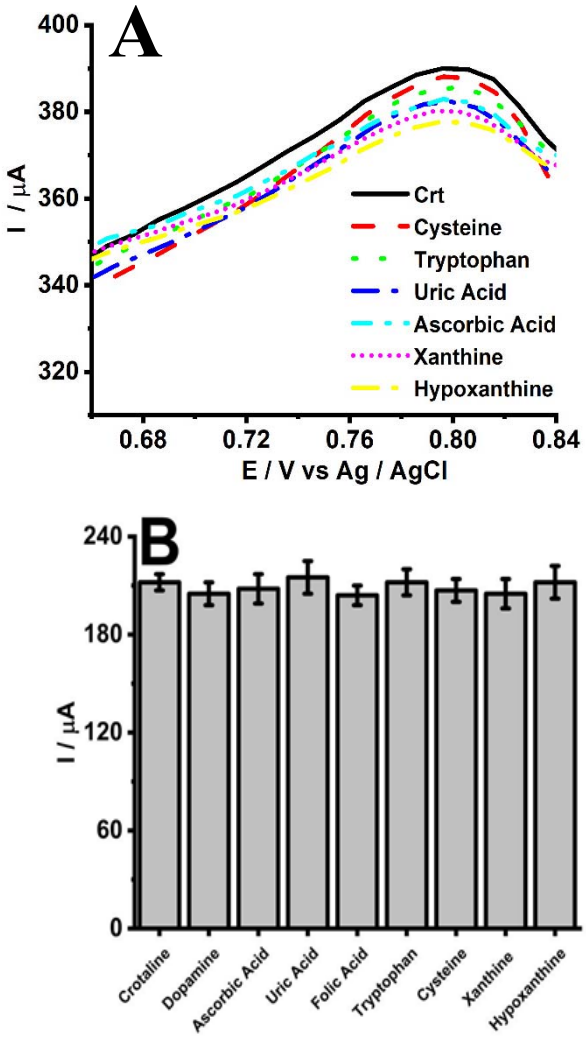


Fig. 4. (A) Comparative DPV responses of 300 μM Crotaline + other biochemicals in pH 7 PBS. (B) Bar graph with error percentage bars for the corresponding current values.

of 50-300μM. As can be seen, a sharp oxidation peak at E'=~0.81 V is obtained whose corresponding current increases linearly with the increase in concentration. A triplicated experiment was performed and the base line corrected calibration plot showed linearity with R2 0.99, sensitivity as 0.158 $\mu A^{-1}\mu M^{-1}$ (Fig. 3B). The calculated limit of

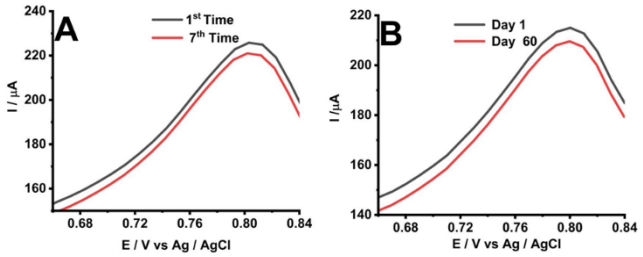


Fig. 5. Comparative DPV response of (A) different devices (B) same device stored for 60 days with 300 μM *Crotaline* taken in pH 7 PBS.

TABLE II
REAL SAMPLE ANALYSIS

Found In sample	CRT Added	Calculated In Sample	RSD	Recovery %
100	20	124.12	5.042016807	103
100	40	146.2	3.151095006	104
100	60	162.23	1.47275405	101

detection (LoD) using the formula [(3.3∗Standard Deviation (SD))/slope] was 18.98 μM, limit of quantification (LoQ) using the formula [(10∗Standard Deviation (SD))x/slope] was 63 μM.

D. Effect of Biochemical Interference

For the real time practical application, the physiological sample has a non–ideal condition wherein, several other biochemicals are present. Therefore, study on effect of these interferences over the *Crotaline* response was crucial. 300 μM of the *Crotaline* sample taken in pH 7 PBS and spiked with other biochemicals like dopamine, ascorbic acid, uric acid, folic acid, tryptophan, cysteine, xanthine and hypoxanthine (1mM each). Fig. 4A is the successive DPV response recorded after addition of each biochemical. Fig. 4B is a bar graph of the corresponding current observed. Negligible interference was seen as the graph shows the current alteration by 0.5 $\pm$ 5%. No significant alteration in the peak current was found justifying zero interference.

E. Reproducibility and Stability

To investigate the reproducibility of the device, 7 CFME devices were prepared and their DPV with 300 μM was checked. It was observed that RSD for n= 7 was 1.4 % indicating a good reproducibility (Fig. 5A). To examine the stability of the created device, the device was stored at a room temperature upto 60 days (Fig. 5B). The DPV response of the device with 300 μM of *Crotaline,* was recorded on every 15th day. Upto 45 days there was no significant reduction observed in the oxidation peak current. Post which, the peak current intensity showed a decrement with a relative standard deviation (RSD) of 3.2 %. These observations indicate that the prepared device was highly stable.

F. Real Sample Analysis

For understanding the selectivity and sensitivity of the realized platform for real time sensing in a non-ideal condition, blood serum real sample was used for the analysis. Standard addition approach with triplicated experiments were performed. The results obtained are showcased in Table II which gave good recovery values.

IV. CONCLUSION

In the present work, a three electrode based miniaturized platform with carbon fibre microelectrodes (CFME) embedded in an overhead projector sheet (OHP) substrate was developed. CFME modified with GMC was used as a working electrode, CFME coated with Ag/AgCl was reference electrode and a bare CFME was a counter electrode. A well-defined electrocatalytic oxidation peak at 0.81 V corresponding to Crotaline was obtained in pH 7 PBS supporting electrolyte. The fabricated device proffered linear of 50-300 μM with limit of detection as 18.98 μM and limit of quantification as 63 μM. 18. The platform was applied to real human blood serum analysis and an interference mitigated, exceedingly stable and reproducible results were observed. The platform is a prototype device for detection of venom in real non-ideal scenarios. Herein, specific one type of venom was tested as a proof of principle. In future, this miniaturized device can be made into a multiplexed testing platform wherein, multiple venom detection electrodes can be integrated as array for simultaneous detection. This could lead to a potable lab on chip development for snake venom analysis.

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Optimality Conditions and Saddle Point Criteria for Fractional Interval-Valued Optimization Problem via Convexificator

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Abstract. In this work, we use the notion of convexificators to discuss optimality conditions for a fractional interval-valued optimization problem. We illustrate the sufficient optimality conditions established in the paper by the example of a nonconvex fractional interval-valued optimization problem with the help of generalized invex functions. Further, we study saddle point criteria of a Lagrange function defined for a fractional interval-valued optimization problem.

Keywords: Convexificator; Fractional problem; LU optimal solution; Lagrange functions; Saddle point.

1. Introduction

In the last two decades, a large number of research has been devoted for solving fractional programming problems. This follows from the fact that optimization problems with the objective function of ratio of two functions have a wide range

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of applications in engineering and economics, game theory, and many more (cf. [12, 18, 22]).

Interval-valued optimization problem is used to tackle interval uncertainity that appears in many real world mathematical problems. For example, it is applied to solve the fixed-charge transportation problem [15], chemical engineering problem [17] and municipal solid waste management [20], etc. Interval-valued programming problem was first studied by Ben-Israel and Robers [2]. Wu [21] formulated four kinds of interval-valued optimization problems and discussed optimality conditions. Further, they also established duality results to relate the primal and dual problems. Singh et al. [16] proposed a theoretical and practical solution method for a multiobjective interval-valued programming problem. In the recent past, many mathematicians have shown their interest to study different types of interval-valued programming problems [1, 3, 11, 14, 19].

The notion of convexificators introduced by Demyanov [6] and extended further by Jeyakumar and Luc [10]. Convexificators can be viewed as weaker versions of the notion of subdifferentials as they are in general closed sets unlike the well-known subdifferentials which are convex and compact sets. In literature, a lot of research has been carried out for convexificators regarding its theoretical properties (see, e.g. [5, 7, 8, 11, 13] and the references therein). Recently, making use of these notions, Karush-Kuhn-Tucker necessary optimality conditions for local weak efficient solutions were established by Hejazi and Nobakhtian [9] for a multiobjective fractional programming problem. Also, Hejazi and Nobakhtian [9] gave some constraint qualifications and subsequently they discussed relationship between these constraint qualifications.

In this paper, by using the idea of convexificators, we study optimality conditions for a fractional interval-valued optimization problem. Further, we establish equivalence between the saddle point and LU optimal solution of the fractional interval-valued optimization problem involving generalized invex functions.

2. Preliminaries

In this section, we give a number of basic definitions and lemmas which will be used in the paper. Let R^n be the n-dimensional Euclidean space and R^n_+ be its non-negative orthant. Throughout this paper, we shall be concerned with Banach spaces. Let X^* be topological dual of a given Banach space X with the canonical dual pairing $\langle ., . \rangle$. Let X and Y be Banach spaces and we denote by $L(X, Y)$ the set of continuous linear mappings between X and Y.

Let $f : X \to R \cup \{+\infty\}$, be an extended real-valued function. Then

$$f^-(x, d) = \lim_{t \to 0+} \inf \frac{f(x + td) - f(x)}{t},$$

$$f^+(x, d) = \lim_{t \to 0+} \sup \frac{f(x + td) - f(x)}{t}$$

denote, respectively, the lower and upper Dini directional derivatives of f at

$x \in X$ in the direction of d.

Now, we begin with the definition of convexificator given by Jeyakumar and Luc [10].

Definition 2.1. *A function $f : X \to R \cup \{+\infty\}$ is said to have a convexificator $\partial^* f(x)$ at x if $\partial^* f(x) \subset X^*$ is weak* closed and*

$$f^+(x,d) \geq \inf_{x^* \in \partial^* f(x)} \langle x^*, d\rangle \text{ and } f^-(x,d) \leq \sup_{x^* \in \partial^* f(x)} \langle x^*, d\rangle\,,\ \forall d \in X.$$

Along the lines of Gadhi [8], we now give the definitions of generalized invex functions by using the concept of convexificators. Assume that $f : X \to R$ admits a convexificator $\partial^* f(\bar{x}) \subset L(X,R)$ at $\bar{x} \in X$.

Definition 2.2. *A function $f : X \to R$ is said to be $(\eta, \partial^* f)$-invex at $\bar{x} \in X$ if there exists $\eta : X \times X \to X$ such that,*

$$f(x) - f(\bar{x}) \geq \langle \xi, \eta(x,\bar{x})\rangle\,,\ \textit{for all } \xi \in \partial^* f(\bar{x}) \textit{ and } x \in X.$$

If strict inequality holds in above definition for $x \neq \bar{x}$, then f is said to be strict $(\eta, \partial^* f)$-invex at $\bar{x}$.

Definition 2.3. *A function $f : X \to R$ is said to be $(\eta, \partial^* f)$-pseudoinvex at $\bar{x} \in X$ if there exists $\eta : X \times X \to X$ such that,*

$$f(x) < f(\bar{x}) \Rightarrow \langle \xi, \eta(x,\bar{x})\rangle < 0,\ \textit{for all } \xi \in \partial^* f(\bar{x}) \textit{ and } x \in X,$$

equivalently

$$\langle \xi, \eta(x,\bar{x})\rangle \geq 0 \Rightarrow f(x) \geq f(\bar{x}),\ \textit{for all } \xi \in \partial^* f(\bar{x}) \textit{ and } x \in X.$$

Definition 2.4. *A function $f : X \to R$ is said to be strict $(\eta, \partial^* f)$-pseudoinvex at $\bar{x} \in X$ if there exists $\eta : X \times X \to X$ such that,*

$$f(x) \leq f(\bar{x}) \Rightarrow \langle \xi, \eta(x,\bar{x})\rangle < 0,\ \textit{for all } \xi \in \partial^* f(\bar{x}) \textit{ and } x \in X,$$

equivalently

$$\langle \xi, \eta(x,\bar{x})\rangle \geq 0 \Rightarrow f(x) > f(\bar{x}),\ \textit{for all } \xi \in \partial^* f(\bar{x}) \textit{ and } x \in X.$$

Definition 2.5. *A function $f : X \to R$ is said to be $(\eta, \partial^* f)$-quasiinvex at $\bar{x} \in X$ if there exists $\eta : X \times X \to X$ such that,*

$$f(x) \leq f(\bar{x}) \Rightarrow \langle \xi, \eta(x,\bar{x}\rangle \leq 0,\ \textit{for all } \xi \in \partial^* f(\bar{x}) \textit{ and } x \in X,$$

equivalently

$$\langle \xi, \eta(x, \bar{x}\rangle > 0 \Rightarrow f(x) > f(\bar{x}),\ \textit{for all } \xi \in \partial^* f(\bar{x})\ \textit{and } x \in X.$$

In order to proceed further, we need the following fundamental concepts of interval mathematics:

Let $\dfrac{\mathbb{A}}{\mathbb{B}} = \left[\dfrac{\alpha_1^L}{\gamma_1^L}, \dfrac{\alpha_1^U}{\gamma_1^U}\right]$ and $\dfrac{\mathbb{C}}{\mathbb{D}} = \left[\dfrac{\alpha_2^L}{\gamma_2^L}, \dfrac{\alpha_2^U}{\gamma_2^U}\right]$ be two fractional closed intervals with $\dfrac{\alpha_1^L}{\gamma_1^L} \leq \dfrac{\alpha_1^U}{\gamma_1^U}$ and $\dfrac{\alpha_2^L}{\gamma_2^L} \leq \dfrac{\alpha_2^U}{\gamma_2^U}$, $\gamma_1^L, \gamma_1^U, \gamma_2^L, \gamma_2^U \neq 0$.

(i) $\dfrac{\mathbb{A}}{\mathbb{B}} + \dfrac{\mathbb{C}}{\mathbb{D}} = \left[\dfrac{\alpha_1^L}{\gamma_1^L} + \dfrac{\alpha_2^L}{\gamma_2^L}, \dfrac{\alpha_1^U}{\gamma_1^U} + \dfrac{\alpha_2^U}{\gamma_2^U}\right]$,

(ii) $\dfrac{-\mathbb{A}}{\mathbb{B}} = \left[\dfrac{-\alpha_1^U}{\gamma_1^U}, \dfrac{-\alpha_1^L}{\gamma_1^L}\right]$,

(iii) $\dfrac{\mathbb{A}}{\mathbb{B}} - \dfrac{\mathbb{C}}{\mathbb{D}} = \dfrac{\mathbb{A}}{\mathbb{B}} + \left(\dfrac{-\mathbb{C}}{\mathbb{D}}\right) = \left[\dfrac{\alpha_1^L}{\gamma_1^L} - \dfrac{\alpha_2^U}{\gamma_2^U}, \dfrac{\alpha_1^U}{\gamma_1^U} - \dfrac{\alpha_2^L}{\gamma_2^L}\right]$,

(iv) $\beta\left(\dfrac{\mathbb{A}}{\mathbb{B}}\right) = \begin{cases} \left[\dfrac{\alpha_1^L}{\gamma_1^L}, \dfrac{\alpha_1^U}{\gamma_1^U}\right], & \text{if } \beta \geq 0, \\ \left[\dfrac{\alpha_1^U}{\gamma_1^U}, \dfrac{\alpha_1^L}{\gamma_1^L}\right], & \text{if } \beta < 0. \end{cases}$

An order relation $\leq_{LU}$ between two intervals $\dfrac{\mathbb{A}}{\mathbb{B}}$ and $\dfrac{\mathbb{C}}{\mathbb{D}}$ are defined as

(i) $\dfrac{\mathbb{A}}{\mathbb{B}} \leq_{LU} \dfrac{\mathbb{C}}{\mathbb{D}}$ iff $\dfrac{\alpha_1^L}{\gamma_1^L} \leq \dfrac{\alpha_2^L}{\gamma_2^L}$ and $\dfrac{\alpha_1^U}{\gamma_1^U} \leq \dfrac{\alpha_2^U}{\gamma_2^U}$.

(ii) $\dfrac{\mathbb{A}}{\mathbb{B}} < \dfrac{\mathbb{C}}{\mathbb{D}}$ iff $\dfrac{\mathbb{A}}{\mathbb{B}} \leq \dfrac{\mathbb{C}}{\mathbb{D}}$ and $\dfrac{\mathbb{A}}{\mathbb{B}} \neq \dfrac{\mathbb{C}}{\mathbb{D}}$, equivalently

$$\begin{cases} \dfrac{\alpha_1^L}{\gamma_1^L} < \dfrac{\alpha_2^L}{\gamma_2^L} \\ \dfrac{\alpha_1^U}{\gamma_1^U} \leq \dfrac{\alpha_2^U}{\gamma_2^U} \end{cases}, \text{ or } \begin{cases} \dfrac{\alpha_1^L}{\gamma_1^L} \leq \dfrac{\alpha_2^L}{\gamma_2^L} \\ \dfrac{\alpha_1^U}{\gamma_1^U} < \dfrac{\alpha_2^U}{\gamma_2^U} \end{cases}, \text{ or } \begin{cases} \dfrac{\alpha_1^L}{\gamma_1^L} < \dfrac{\alpha_2^L}{\gamma_2^L} \\ \dfrac{\alpha_1^U}{\gamma_1^U} < \dfrac{\alpha_2^U}{\gamma_2^U}. \end{cases}$$

Consider the following non-differentiable fractional interval-valued optimization problem:

$$\begin{aligned} &\min \left[\frac{f^L(x), f^U(x)}{g^L(x), g^U(x)}\right] \\ &\text{subject to} \\ &h_i(x) \leq 0, i = 1, 2, ..., m, \\ &x \in X, \end{aligned}$$

which further reduces to the problem

$$\begin{aligned}&\min \left[\frac{f^L(x)}{g^U(x)}, \frac{f^U(x)}{g^L(x)}\right]\\&\text{subject to}\\&h_i(x) \leq 0, i = 1, 2, ..., m,\\&x \in X,\end{aligned}$$

where $f^L(x),\ f^U(x) \geq 0,\ g^L(x),\ g^U(x) > 0$, and $h_i,\ i = 1, 2, \ldots, m$ are continuous functions on X. Set $f^L = p^L,\ g^U = q^L,\ f^U = p^U,\ g^L = q^U$. Then, the above problem reduces to

$$\begin{aligned}\text{(NFIVP)} \qquad &\min \left[\frac{p^L}{q^L}(x), \frac{p^U}{q^U}(x)\right]\\&\text{subject to}\\&h_i(x) \leq 0, i = 1, 2, ..., m,\\&x \in X.\end{aligned}$$

Let $\mathbb{F}$ be the feasible set for the problem (NFIVP).

Definition 2.6. [21] *A feasible point $\bar{x}$ is said to be a LU optimal solution for* (NFIVP) *if and only if there exists no feasible point x such that*

$$\left[\frac{p^L}{q^L}(x), \frac{p^U}{q^U}(x)\right] <_{LU} \left[\frac{p^L}{q^L}(\bar{x}), \frac{p^U}{q^U}(\bar{x})\right].$$

3. Optimality Conditions

For the given feasible solution $\bar{x}$, consider two fractional problems as given below:

$$\begin{aligned}\text{(FP1)} \qquad &\min \phi^L(x) = \frac{p^L}{q^L}(x) \qquad\qquad \text{(FP2)} \qquad \min \phi^U(x) = \frac{p^U}{q^U}(x)\\&\text{subject to} \qquad\qquad\qquad\qquad\quad \text{subject to}\\&h_i(x) \leq 0,\ i = 1, 2, \ldots, m, \qquad\quad h_i(x) \leq 0,\ i = 1, 2, \ldots, m,\\&\frac{p^U}{q^U}(x) \leq \frac{p^U}{q^U}(\bar{x}), \qquad\qquad \frac{p^L}{q^L}(x) \leq \frac{p^L}{q^L}(\bar{x}),\\&x \in X. \qquad\qquad\qquad\qquad\quad x \in X.\end{aligned}$$

The following result gives the relationship between (NFIVP) and (FP1) and (FP2).

Lemma 3.1. [4] *If $\bar{x}$ is a LU optimal solution for the problem (NFIVP) if and only if $\bar{x}$ is an optimal solution for the problems (FP1) and (FP2).*

Lemma 3.2. [4] *$\bar{x}$ is a LU optimum of the problem (NFIVP) if and only if $\bar{x}$ minimizes $\frac{p^L}{q^L}(x)$ on the following constraint set*

$$N = \left\{ x \in X | \frac{p^U}{q^U}(x) \leq \frac{p^U}{q^U}(\bar{x}), h_i(x) \leq 0, i = 1, 2, \ldots, m \right\}.$$

Considered the following single-objective fractional problem:

$$\begin{aligned} \text{(D)} \qquad & \min \phi(x) = \frac{p_1}{q_1}(x) \\ & \text{subject to} \\ & \ell_i(x) \leq 0, i = 1, 2, ..., m, \\ & x \in X, \end{aligned}$$

where p_1, q_1 and ℓ_i, $i = 1, 2, ..., m$ are continuous functions on X such that $p_1(x) \geq 0$ and $q_1(x) > 0$, for all $x \in X$.

On the lines of Theorem 6 of Gadhi [8], we state the following theorem for the problem (D):

Theorem 3.3. *Suppose that $\bar{x}$ is an optimal solution of the problem (D) and a suitable constraint qualification is satisfied at $\bar{x}$. Assume that p_1, q_1 and ℓ_i, $i = 1, 2, ..., m$ are continuous and admit bounded convexificators $\partial^* p_1(\bar{x})$, $\partial^* q_1(\bar{x})$ and $\partial^* \ell_i(\bar{x})$, $i = 1, 2, ..., m$ at $\bar{x}$ respectively and that $\partial^* p_1(\bar{x})$, $\partial^* q_1(\bar{x})$ and $\partial^* \ell_i(\bar{x})$, $i = 1, 2, ..., m$ are upper semicontinuous at $\bar{x}$, then there exist $\lambda > 0$, and $\mu \in R^m_+$ such that*

$$0 \in \lambda \left(\partial^* p_1(\bar{x}) - \phi(\bar{x}) \partial^* q_1(\bar{x}) \right) + \sum_{i=1}^{m} \mu_i \partial^* \ell_i(\bar{x}), \tag{1}$$

$$\mu_i \ell_i(\bar{x}) = 0, j = 1, 2, ..., m, \tag{2}$$

$$\mu_i \geq 0 \text{ and } \ell_i(\bar{x}) \leq 0, \ i = 1, 2, ..., m. \tag{3}$$

Theorem 3.4. (Karush-Kuhn-Tucker Necessary Optimality Conditions) *Suppose that $\bar{x}$ is a LU optimal solution of the problem* (NFIVP) *and a suitable constraint qualification is satisfied at $\bar{x}$. Assume that p^L, q^L, p^U, q^U and h_i, $i = 1, 2, ..., m$ are continuous and admit bounded convexificators $\partial^* p^L(\bar{x})$, $\partial^* q^L(\bar{x})$, $\partial^* p^U(\bar{x})$, $\partial^* q^U(\bar{x})$ and $\partial^* h_i(\bar{x})$, $i = 1, 2, ..., m$ at $\bar{x}$ respectively and that $\partial^* p^L(\bar{x})$, $\partial^* q^L(\bar{x})$, $\partial^* p^L(\bar{x})$, $\partial^* q^L(\bar{x})$, and $\partial^* h_i(\bar{x})$, $i = 1, 2, ..., m$ are upper semicontinuous at $\bar{x}$, then there exist $\lambda^L > 0$, $\lambda^U > 0$ and $\mu \in R^m_+$ such that*

$$\begin{aligned} 0 \in & \lambda^L \left(\partial^* p^L(\bar{x}) - \phi^L(\bar{x}) \partial^* q^L(\bar{x}) \right) \\ & + \lambda^U \left(\partial^* p^U(\bar{x}) - \phi^U(\bar{x}) \partial^* q^U(\bar{x}) \right) + \sum_{i=1}^{m} \mu_i \partial^* h_i(\bar{x}), \end{aligned} \tag{4}$$

$$\mu_i h_i(\bar{x}) = 0, j = 1, 2, ..., m, \tag{5}$$

$$\mu_i \geq 0 \text{ and } h_i(\bar{x}) \leq 0, \ i = 1, 2, ..., m. \tag{6}$$

Proof. By assumption, $\bar{x}$ is a LU optimal solution for the problem (NFIVP), and a suitable constraint qualification is satisfied at $\bar{x}$. Since $\bar{x}$ is an LU optimal solution, by Lemma 3.1, $\bar{x}$ is also a optimal solution for the problems (FP1) and (FP2). Hence, by Lemma 3.2, at $\bar{x}$ the minimum value of $\frac{p^L}{q^L}(x)$ is obtained on the constraint set

$$N_L = \left\{ x \in X | \frac{p^U}{q^U}(x) \leq \frac{p^U}{q^U}(\bar{x}), h_i(x) \leq 0, i = 1, 2, ..., m \right\},$$

and the minimum value of $\frac{p^U}{q^U}(x)$ is obtained at $\bar{x}$ on the constraint set

$$N_U = \left\{ x \in X | \frac{p^L}{q^L}(x) \leq \frac{p^L}{q^L}(\bar{x}), h_i(x) \leq 0, i = 1, 2, ..., m \right\}.$$

By Theorem 3.3, it follows that there exist $\lambda^{LL} > 0, \lambda^{LU} > 0, \mu^L \in R_+^m$ and $\lambda^{UL} > 0, \lambda^{UU} > 0, \mu^U \in R_+^m$ such that

$$\begin{aligned} 0 \in \lambda^{LL} \left(\partial^* p^L(\bar{x}) - \phi^L(\bar{x}) \partial^* q^L(\bar{x}) \right) \\ + \lambda^{LU} \left(\partial^* p^U(\bar{x}) - \phi^U(\bar{x}) \partial^* q^U(\bar{x}) \right) + \sum_{i=1}^{m} \mu_i^L \partial^* h_i(\bar{x}), \end{aligned} \tag{7}$$

$$\mu_i^L h_i(\bar{x}) = 0, j = 1, 2, ..., m, \tag{8}$$

$$\mu_i^L \geq 0 \text{ and } h_i(\bar{x}) \leq 0, \ i = 1, 2, ..., m. \tag{9}$$

and

$$\begin{aligned} 0 \in \lambda^{UL} \left(\partial^* p^L(\bar{x}) - \phi^L(\bar{x}) \partial^* q^L(\bar{x}) \right) \\ + \lambda^{UU} \left(\partial^* p^U(\bar{x}) - \phi^U(\bar{x}) \partial^* q^U(\bar{x}) \right) + \sum_{i=1}^{m} \mu_i^U \partial^* h_i(\bar{x}), \end{aligned} \tag{10}$$

$$\mu_i^U h_i(\bar{x}) = 0, j = 1, 2, ..., m, \tag{11}$$

$$\mu_i^U \geq 0 \text{ and } h_i(\bar{x}) \leq 0, \ i = 1, 2, ..., m. \tag{12}$$

From (7) to (12), we have

$$\begin{aligned} 0 \in [\lambda^{LL} + \lambda^{UL}] \left(\partial^* p^L(\bar{x}) - \phi^L(\bar{x}) \partial^* q^L(\bar{x}) \right) \\ + [\lambda^{LU} + \lambda^{UU}] \left(\partial^* p^U(\bar{x}) - \phi^U(\bar{x}) \partial^* q^U(\bar{x}) \right) + \sum_{i=1}^{m} [\mu_i^L + \mu_i^U] \partial^* h_i(\bar{x}), \end{aligned} \tag{13}$$

$$[\mu_i^L + \mu_i^U] h_i(\bar{x}) = 0, j = 1, 2, ..., m, \tag{14}$$

$$[\mu_i^L + \mu_i^U] \geq 0 \text{ and } h_i(\bar{x}) \leq 0, \ i = 1, 2, ..., m. \tag{15}$$

Let us denote $\lambda^{LL} + \lambda^{UL} = \lambda^L$, $\lambda^{LU} + \lambda^{UU} = \lambda^U$ and $\mu^L + \mu^U = \mu$. Thus, from

(13)-(15), it yields

$$0 \in \lambda^L \left(\partial^* p^L(\bar{x}) - \phi^L(\bar{x})\partial^* q^L(\bar{x})\right)$$
$$+\lambda^U \left(\partial^* p^U(\bar{x}) - \phi^U(\bar{x})\partial^* q^U(\bar{x})\right) + \sum_{i=1}^{m} \mu_i \partial^* h_i(\bar{x}),$$
$$\mu_i h_i(\bar{x}) = 0, j = 1, 2, ..., m,$$
$$\mu_i \geq 0 \text{ and } h_i(\bar{x}) \leq 0,\ i = 1, 2, ..., m.$$

This completes the proof. ∎

Theorem 3.5. (Sufficient Optimality Conditions) *Suppose that $\bar{x}$ is a feasible solution of* (NFIVP) *and there exist $\lambda^L > 0, \lambda^U > 0, \mu \in R_+^m$ such that* (4)–(6) *are satisfied at $\bar{x}$. Also, assume that*

(i) *$p^L(.) - \phi^L(\bar{x})q^L(.)$ and $p^U(.) - \phi^U(\bar{x})q^U(.)$ are respectively $(\eta, \partial^* p^L - \phi^L(\bar{x})\partial^* q^L)$-invex and $(\eta, \partial^* p^U - \phi^U(\bar{x})\partial^* q^U)$-invex at $\bar{x}$,*

(ii) *$\mu_i h_i$, for $i = 1, 2, ..., m$, is $(\eta, \partial^* h_i(.))$-invex at $\bar{x}$.*

Then $\bar{x}$ is a LU optimal solution for (NFIVP).

Proof. By assumption, (4)-(6) are satisfied at $\bar{x}$ with Lagrange multipliers $\lambda^L > 0, \lambda^U > 0, \mu \in R_+^m$. As it follows from (4), there exist $\xi^L \in \partial^* p^L(\bar{x})$, $\nu^L \in \partial^* q^L(\bar{x})$, $\xi^U \in \partial^* p^U(\bar{x})$, $\nu^U \in \partial^* q^U(\bar{x})$, and $\zeta_i \in \partial^* h_i(\bar{x}),\ i = 1, 2, ..., m$, such that

$$\lambda^L \left[\xi^L - \phi^L(\bar{x})\nu^L\right] + \lambda^U \left[\xi^U - \phi^U(\bar{x})\nu^U\right] + \sum_{i=1}^{m} \mu_i \zeta_i = 0. \tag{16}$$

Suppose contrary to the result, that $\bar{x}$ is not a LU optimal solution for (NFIVP). Hence, by Definition 2.6, there exists a feasible solution x such that

$$\left[\frac{p^L}{q^L}(x), \frac{p^U}{q^U}(x)\right] <_{LU} \left[\frac{p^L}{q^L}(\bar{x}), \frac{p^U}{q^U}(\bar{x})\right]$$

that is $\begin{cases} \frac{p^L}{q^L}(x) < \frac{p^L}{q^L}(\bar{x}) \\ \frac{p^U}{q^U}(x) \leq \frac{p^U}{q^U}(\bar{x}) \end{cases}$, or $\begin{cases} \frac{p^L}{q^L}(x) \leq \frac{p^L}{q^L}(\bar{x}) \\ \frac{p^U}{q^U}(x) < \frac{p^U}{q^U}(\bar{x}) \end{cases}$, or $\begin{cases} \frac{p^L}{q^L}(x) < \frac{p^L}{q^L}(\bar{x}) \\ \frac{p^U}{q^U}(x) < \frac{p^U}{q^U}(\bar{x}) \end{cases}$.

This implies

$$p^L(x) - \phi^L(\bar{x})q^L(x) < p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x}),$$
$$p^U(x) - \phi^U(\bar{x})q^U(x) \leq p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x}),$$

or

$$p^L(x) - \phi^L(\bar{x})q^L(x) \leq p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x}),$$
$$p^U(x) - \phi^U(\bar{x})q^U(x) < p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x}),$$

or

$$p^L(x) - \phi^L(\bar{x})q^L(x) < p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x}),$$
$$p^U(x) - \phi^U(\bar{x})q^U(x) < p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x}).$$

From hypothesis (i), $p^L(.) - \phi^L(\bar{x})q^L(.)$ and $p^U(.) - \phi^U(\bar{x})q^U(.)$ are respectively $(\eta, \partial^* p^L - \phi^L(\bar{x})\partial^* q^L)$-invex and $(\eta, \partial^* p^U - \phi^U(\bar{x})\partial^* q^U)$-invex at $\bar{x}$ and therefore, there exists $\eta : X \times X \to X$ such that

$$\left\langle \left[\xi^L - \phi^L(\bar{x})\nu^L\right], \eta(x, \bar{x}) \right\rangle \leq 0, \text{ for all } \xi^L \in \partial^* p^L(\bar{x}), \text{ and } \nu^L \in \partial^* q^L(\bar{x}),$$

$$\left\langle \left[\xi^U - \phi^U(\bar{x})\nu^U\right], \eta(x, \bar{x}) \right\rangle < 0, \text{ for all } \xi^U \in \partial^* p^U(\bar{x}), \text{ and } \nu^U \in \partial^* q^U(\bar{x}),$$

or

$$\left\langle \left[\xi^L - \phi^L(\bar{x})\nu^L\right], \eta(x, \bar{x}) \right\rangle < 0, \text{ for all } \xi^L \in \partial^* p^L(\bar{x}), \text{ and } \nu^L \in \partial^* q^L(\bar{x}),$$

$$\left\langle \left[\xi^U - \phi^U(\bar{x})\nu^U\right], \eta(x, \bar{x}) \right\rangle \leq 0, \text{ for all } \xi^U \in \partial^* p^U(\bar{x}), \text{ and } \nu^U \in \partial^* q^U(\bar{x}),$$

or

$$\left\langle \left[\xi^L - \phi^L(\bar{x})\nu^L\right], \eta(x, \bar{x}) \right\rangle < 0, \text{ for all } \xi^L \in \partial^* p^L(\bar{x}), \text{ and } \nu^L \in \partial^* q^L(\bar{x}),$$

$$\left\langle \left[\xi^U - \phi^U(\bar{x})\nu^U\right], \eta(x, \bar{x}) \right\rangle < 0, \text{ for all } \xi^U \in \partial^* p^U(\bar{x}), \text{ and } \nu^U \in \partial^* q^U(\bar{x}).$$

From the fact $\lambda^L > 0$, $\lambda^U > 0$ and by above inequalities, we have

$$\left\langle \lambda^L \left[\xi^L - \phi^L(\bar{x})\nu^L\right] + \lambda^U \left[\xi^U - \phi^U(\bar{x})\nu^U\right], \eta(x, \bar{x}) \right\rangle < 0. \tag{17}$$

On the other hand, by using the feasibility of x, $\mu_i \geq 0$, $i = 1, 2, ..., m$ and (5), we obtain

$$\mu_i h_i(x) \leq \mu_i h_i(\bar{x}), i = 1, 2, ..., m,$$

which by hypothesis (ii), we get

$$\langle \mu_i \zeta_i, \eta(x, \bar{x}) \rangle \leq 0, \text{ for all } \zeta_i \in \partial^* h_i(\bar{x}), \ i = 1, 2, ..., m. \tag{18}$$

On adding (17) and (18), we have

$$\left\langle \lambda^L \left[\xi^L - \phi^L(\bar{x})\nu^L\right] + \lambda^U \left[\xi^U - \phi^U(\bar{x})\nu^U\right] + \sum_{i=1}^{m} \mu_i \zeta_i, \eta(x, \bar{x}) \right\rangle < 0,$$

which contradicts (16). Hence, $\bar{x}$ is a LU optimal solution for (NFIVP). ■

In order to illustrate the sufficient optimality conditions established in the Theorem 3.5, we consider the following example:

Example 3.6.

$$\begin{aligned}(\text{IVP1}) \qquad & \min\left[\frac{f_1^L(x), f_1^U(x)}{g_1^L(x), g_1^U(x)}\right] \\ & = \min\left[\frac{2x^2, x^2+1}{-x+4, -x^2+6}\right] \\ & \text{subject to} \\ & h_1(x) = -x+2 \leq 0, x \in X = R.\end{aligned}$$

Now, we rewrite the considered optimization problem in the following manner

$$\begin{aligned}& \min\left[\frac{2x^2}{-x^2+6}, \frac{x^2+1}{-x+4}\right] \\ & \text{subject to} \\ & h_1(x) = -x+2 \leq 0, x \in X = R.\end{aligned}$$

where $\frac{p_1^L}{q_1^L}(x) = \frac{2x^2}{-x^2+6}$, $\frac{p_1^U}{q_1^U}(x) = \frac{x^2+1}{-x+4}$. The feasible set is $\mathbb{F}_1 = \{x : -x+2 \leq 0, x \in S\}$. By simple calculations, for the feasible point $\bar{x} = 2$, we see that $\partial^* p^L(\bar{x}) = \{-8, 8\}$, $\partial^* q^L(\bar{x}) = \{-4, 4\}$, $\partial^* p^U(\bar{x}) = \{-4, 4\}$, $\partial^* q^U(\bar{x}) = \{-1, 1\}$ and $\partial^* h_1(\bar{x}) = \{-1, 1\}$. Also, we see that for the feasible point $\bar{x} = 2$, there exist $\lambda^L > 0, \lambda^U > 0, \mu \in R_+^m$ such that (4)-(6) are satisfied at $\bar{x}$ and it is easy to see that

(i) $p_1^L(.) - \phi_1^L(\bar{x})q_1^L(.)$ and $p_1^U(.) - \phi_1^U(\bar{x})q_1^U(.)$ are respectively $(\eta, \partial^* p_1^L - \phi_1^L(\bar{x})\partial^* q_1^L)$-invex and $(\eta, \partial^* p_1^U - \phi_1^U(\bar{x})\partial^* q_1^U)$-invex at $\bar{x}$,

(ii) μh_1 is $(\eta, \partial^* h_1(.))$-invex at $\bar{x}$.

Therefore, by Theorem 3.5, $\bar{x} = 2$ is a LU optimal solution for (IVP1).

Theorem 3.7. (Sufficient Optimality Conditions) *Suppose that $\bar{x}$ is a feasible solution of* (NFIVP) *and there exist $\lambda^L > 0, \lambda^U > 0, \mu \in R_+^m$ such that* (4)–(6) *are satisfied at $\bar{x}$. Also, assume that*

(i) $\lambda^L[p^L(.) - \phi^L(\bar{x})q^L(.)] + \lambda^U[p^U(.) - \phi^U(\bar{x})q^U(.)]$ *is* $(\eta, \lambda^L[\partial^* p^L - \phi^L(\bar{x})\partial^* q^L] + \lambda^U[\partial^* p^U - \phi^U(\bar{x})\partial^* q^U])$*-pseudoinvex at $\bar{x}$,*

(ii) $\mu_i h_i$*, for $i = 1, 2, ..., m$, is $(\eta, \partial^* h_i(.))$-quasiinvex at $\bar{x}$.*

Then $\bar{x}$ is a LU optimal solution for (NFIVP).

Proof. By assumption, (4)-(6) are satisfied at $\bar{x}$ with Lagrange multipliers $\lambda^L > 0, \lambda^U > 0, \mu \in R_+^m$. As it follows from (4), there exist $\xi^L \in \partial^* p^L(\bar{x})$, $\nu^L \in \partial^* q^L(\bar{x})$, $\xi^U \in \partial^* p^U(\bar{x})$, $\nu^U \in \partial^* q^U(\bar{x})$, and $\zeta_i \in \partial^* h_i(\bar{x})$, $i = 1, 2, ..., m$, such that

$$\lambda^L\left[\xi^L - \phi^L(\bar{x})\nu^L\right] + \lambda^U\left[\xi^U - \phi^U(\bar{x})\nu^U\right] + \sum_{i=1}^{m}\mu_i\zeta_i = 0. \tag{19}$$

Suppose contrary to the result, that $\bar{x}$ is not a LU optimal solution for (NFIVP). Hence, by Definition 2.6, there exist a feasible solution x such that

$$\left[\frac{p^L}{q^L}(x), \frac{p^U}{q^U}(x)\right] <_{LU} \left[\frac{p^L}{q^L}(\bar{x}), \frac{p^U}{q^U}(\bar{x})\right]$$

that is

$$\begin{cases} \frac{p^L}{q^L}(x) < \frac{p^L}{q^L}(\bar{x}) \\ \frac{p^U}{q^U}(x) \leq \frac{p^U}{q^U}(\bar{x}) \end{cases}, \text{ or } \begin{cases} \frac{p^L}{q^L}(x) \leq \frac{p^L}{q^L}(\bar{x}) \\ \frac{p^U}{q^U}(x) < \frac{p^U}{q^U}(\bar{x}) \end{cases}, \text{ or } \begin{cases} \frac{p^L}{q^L}(x) < \frac{p^L}{q^L}(\bar{x}) \\ \frac{p^U}{q^U}(x) < \frac{p^U}{q^U}(\bar{x}) \end{cases}.$$

This implies

$$p^L(x) - \phi^L(\bar{x})q^L(x) < p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x}),$$
$$p^U(x) - \phi^U(\bar{x})q^U(x) \leq p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x}),$$

or

$$p^L(x) - \phi^L(\bar{x})q^L(x) \leq p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x}),$$
$$p^U(x) - \phi^U(\bar{x})q^U(x) < p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x}),$$

or

$$p^L(x) - \phi^L(\bar{x})q^L(x) < p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x}),$$
$$p^U(x) - \phi^U(\bar{x})q^U(x) < p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x}).$$

From the fact $\lambda^L > 0$, $\lambda^U > 0$ and by above inequalities, we have

$$\begin{aligned} &\lambda^L[p^L(x) - \phi^L(\bar{x})q^L(x)] + \lambda^U[p^U(x) - \phi^U(\bar{x})q^U(x)] \\ &< \lambda^L[p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x})] + \lambda^U[p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x})]. \end{aligned}$$

From hypothesis (i), $\lambda^L[p^L(.) - \phi^L(\bar{x})q^L(.)] + \lambda^U[p^U(.) - \phi^U(\bar{x})q^U(.)]$ is $(\eta, \lambda^L[\partial^* p^L - \phi^L(\bar{x})\partial^* q^L] + \lambda^U[\partial^* p^U - \phi^U(\bar{x})\partial^* q^U])$-pseudoinvex at $\bar{x}$ and therefore, there exists $\eta : X \times X \to X$ such that

$$\left\langle \lambda^L\left[\xi^L - \phi^L(\bar{x})\nu^L\right] + \lambda^U\left[\xi^U - \phi^U(\bar{x})\nu^U\right], \eta(x, \bar{x})\right\rangle < 0, \tag{20}$$

for all $\xi^L \in \partial^* p^L(\bar{x}), \nu^L \in \partial^* q^L(\bar{x}), \xi^U \in \partial^* p^U(\bar{x})$, and $\nu^U \in \partial^* q^U(\bar{x})$.

On the other hand, by using the feasibility of x, $\mu_i \geq 0$, $i = 1, 2, ..., m$ and (5), we obtain

$$\mu_i h_i(x) \leq \mu_i h_i(\bar{x}), i = 1, 2, ..., m,$$

which by hypothesis (ii), we get

$$\langle \mu_i \zeta_i, \eta(x, \bar{x})\rangle \leq 0, \text{ for all } \zeta_i \in \partial^* h_i(\bar{x}),\ i = 1, 2, ..., m. \tag{21}$$

On adding (20) and (21), we have

$$\left\langle \lambda^L \left[\xi^L - \phi^L(\bar{x})\nu^L\right] + \lambda^U \left[\xi^U - \phi^U(\bar{x})\nu^U\right] + \sum_{i=1}^{m} \mu_i \zeta_i, \eta(x, \bar{x}) \right\rangle < 0,$$

which contradicts (19). Hence, $\bar{x}$ is a LU optimal solution for (NFIVP). ∎

4. Lagrangian Type Function and Saddle-point Analysis

In this section, for the feasible point $\bar{x} \in \mathbb{F}$, we define the Lagrangian type function for the primal problem (NFIVP) as follows:

$$L(x, \lambda^L, \lambda^U, \mu) = \lambda^L \left(p^L(x) - \phi^L(\bar{x})q^L(x)\right) + \lambda^U \left(p^U(x) - \phi^U(\bar{x})q^U(x)\right) + \sum_{i=1}^{m} \mu_i h_i(x)$$

where $x \in X$, $\lambda^L \geq 0$, $\lambda^U \geq 0$ and $\mu \in R_+^m$. Now, we define a saddle-point of $L(x, \lambda^L, \lambda^U, \mu)$ and subsequently we discuss its relation to the problem (NFIVP).

Definition 4.1. *A point $(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu}) \in X \times R_+ \times R_+ \times R_+^m$ is said to be a saddle point for $L(x, \lambda^L, \lambda^U, \mu)$, if*

(i) $L(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \mu) \leq L(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu})$, *for all* $\mu \in R_+^m$,
(ii) $L(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu}) \leq L(x, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu})$, *for all* $x \in X$.

Theorem 4.2. *Let $\bar{\lambda}^L > 0$, $\bar{\lambda}^U > 0$ and $(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu})$ be a saddle point for $L(x, \lambda^L, \lambda^U, \mu)$. Then $\bar{x}$ is a LU optimal solution to* (NFIVP).

Proof. Suppose contrary to the result, that $\bar{x}$ is not a LU optimal solution for (NFIVP). Hence, by Definition 2.6, there exists a feasible solution x such that

$$\left[\frac{p^L}{q^L}(x), \frac{p^U}{q^U}(x)\right] <_{LU} \left[\frac{p^L}{q^L}(\bar{x}), \frac{p^U}{q^U}(\bar{x})\right]$$

that is

$$\begin{cases} \dfrac{p^L}{q^L}(x) < \dfrac{p^L}{q^L}(\bar{x}) \\ \dfrac{p^U}{q^U}(x) \leq \dfrac{p^U}{q^U}(\bar{x}) \end{cases}, \text{ or } \begin{cases} \dfrac{p^L}{q^L}(x) \leq \dfrac{p^L}{q^L}(\bar{x}) \\ \dfrac{p^U}{q^U}(x) < \dfrac{p^U}{q^U}(\bar{x}) \end{cases}, \text{ or } \begin{cases} \dfrac{p^L}{q^L}(x) < \dfrac{p^L}{q^L}(\bar{x}) \\ \dfrac{p^U}{q^U}(x) < \dfrac{p^U}{q^U}(\bar{x}) \end{cases}$$

This implies

$$p^L(x) - \phi^L(\bar{x})q^L(x) < p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x}),$$

$$p^U(x) - \phi^U(\bar{x})q^U(x) \leq p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x}),$$

or

$$p^L(x) - \phi^L(\bar{x})q^L(x) \leq p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x}),$$
$$p^U(x) - \phi^U(\bar{x})q^U(x) < p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x}),$$

or

$$p^L(x) - \phi^L(\bar{x})q^L(x) < p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x}),$$
$$p^U(x) - \phi^U(\bar{x})q^U(x) < p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x}).$$

By above inequalities and from $\lambda^L > 0$, $\lambda^U > 0$, we have

$$\begin{aligned} &\bar{\lambda}^L[p^L(x) - \phi^L(\bar{x})q^L(x)] + \bar{\lambda}^U[p^U(x) - \phi^U(\bar{x})q^U(x)] \\ &< \bar{\lambda}^L[p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x})] + \bar{\lambda}^U[p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x})]. \end{aligned} \tag{22}$$

Since $(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu})$ is a saddle point for $L(x, \lambda^L, \lambda^U, \mu)$, by Definition 4.1 (i), we get

$$L(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \mu) \leq L(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu}),$$

that is,

$$\sum_{i=1}^{m} \mu_i h_i(\bar{x}) \leq \sum_{i=1}^{m} \bar{\mu}_i h_i(\bar{x}). \tag{23}$$

Taking $(\mu_1, \mu_2, ...\mu_{i-1}, \mu_i, \mu_{i+1}, ..., \mu_m) = (\bar{\mu}_1, \bar{\mu}_2, ...\bar{\mu}_{i-1}, \bar{\mu}_i + 1, \bar{\mu}_{i+1}, ..., \bar{\mu}_m)$ in the above inequality (23), we obtain

$$h_i(\bar{x}) \leq 0, i = 1, 2, ..., m,$$

which shows that $\bar{x}$ is a feasible solution to (NFIVP).

Using $\bar{\mu} \in R_+^m$, above inequality implies

$$\bar{\mu}_i h_i(\bar{x}) \leq 0, i = 1, 2, ..., m. \tag{24}$$

Again taking $\mu_i = 0$, $i = 1, 2, ..., m$, in the inequality (23), we get

$$\bar{\mu}_i h_i(\bar{x}) \geq 0, i = 1, 2, ..., m. \tag{25}$$

From the inequalities (24) and (25), we conclude that

$$\bar{\mu}_i h_i(\bar{x}) = 0, i = 1, 2, ..., m. \tag{26}$$

On other hand, since $(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu})$ is a saddle point for $L(x, \lambda^L, \lambda^U, \mu)$, by Definition 4.1 (ii), we get

$$L(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu}) \leq L(x, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu}),$$

that is

$$\begin{aligned} &\bar{\lambda}^L \left(p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x})\right) + \bar{\lambda}^U \left(p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x})\right) + \sum_{i=1}^{m} \bar{\mu}_i h_i(\bar{x}) \\ &\leq \bar{\lambda}^L \left(p^L(x) - \phi^L(\bar{x})q^L(x)\right) + \bar{\lambda}^U \left(p^U(x) - \phi^U(\bar{x})q^U(x)\right) + \sum_{i=1}^{m} \bar{\mu}_i h_i(x) \end{aligned}$$

Using the feasibilty of x of the problem (NFIVP) together with $\bar{\mu} \in R_+^m$ and (26), above inequality gives

$$\begin{aligned} &\bar{\lambda}^L \left(p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x})\right) + \bar{\lambda}^U \left(p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x})\right) \\ \leq\ &\bar{\lambda}^L \left(p^L(x) - \phi^L(\bar{x})q^L(x)\right) + \bar{\lambda}^U \left(p^U(x) - \phi^U(\bar{x})q^U(x)\right). \end{aligned}$$

This contradicts (22). Hence the proof. ∎

Theorem 4.3. *Let $\bar{x}$ be a LU optimal solution to* (NFIVP) *and assume that there exist $\bar{\lambda}^L > 0, \bar{\lambda}^U > 0, \bar{\mu} \in R_+^m$ such that* (4)–(6) *are satisfied at $\bar{x}$. Also, assume that*

(i) *$p^L(.) - \phi^L(\bar{x})q^L(.)$ and $p^U(.) - \phi^U(\bar{x})q^U(.)$ are respectively $(\eta, \partial^* p^L - \phi^L(\bar{x})\partial^* q^L)$-invex and $(\eta, \partial^* p^U - \phi^U(\bar{x})\partial^* q^U)$-invex at $\bar{x}$.*

(ii) *$\mu_i h_i$, for $i = 1, 2, ..., m$, is $(\eta, \partial^* h_i(.))$-invex at $\bar{x}$.*

Then $(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu})$ is a saddle point for $L(x, \lambda^L, \lambda^U, \mu)$.

Proof. By assumption, (4)–(6) are satisfied at $\bar{x}$ with Lagrange multipliers $\bar{\lambda}^L > 0, \bar{\lambda}^U > 0, \bar{\mu} \in R_+^m$. As it follows from (4), there exist $\xi^L \in \partial^* p^L(\bar{x})$, $\nu^L \in \partial^* q^L(\bar{x})$, $\xi^U \in \partial^* p^U(\bar{x})$, $\nu^U \in \partial^* q^U(\bar{x})$, and $\zeta_i \in \partial^* h_i(\bar{x})$, $i = 1, 2, ..., m$, such that

$$\bar{\lambda}^L \left[\xi^L - \phi^L(\bar{x})\nu^L\right] + \bar{\lambda}^U \left[\xi^U - \phi^U(\bar{x})\nu^U\right] + \sum_{i=1}^{m} \bar{\mu}_i \zeta_i = 0. \tag{27}$$

From the hypothesis (i), $p^L(.) - \phi^L(\bar{x})q^L(.)$ and $p^U(.) - \phi^U(\bar{x})q^U(.)$ are respectively $(\eta, \partial^* p^L - \phi^L(\bar{x})\partial^* q^L)$-invex and $(\eta, \partial^* p^U - \phi^U(\bar{x})\partial^* q^U)$-invex at $\bar{x}$, therefore, there exists $\eta : X \times X \to X$ such that

$$\begin{aligned} &[p^L(x) - \phi^L(\bar{x})q^L(x)] - [p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x})] \\ \geq\ &\left\langle \left[\xi^L - \phi^L(\bar{x})\nu^L\right], \eta(x, \bar{x}) \right\rangle, \text{ for all } \xi^L \in \partial^* p^L(\bar{x}), \text{ and } \nu^L \in \partial^* q^L(\bar{x}), \end{aligned}$$

and

$$\begin{aligned} &[p^U(x) - \phi^U(\bar{x})q^U(x)] - [p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x})] \\ \geq\ &\left\langle \left[\xi^U - \phi^U(\bar{x})\nu^U\right], \eta(x, \bar{x}) \right\rangle, \text{ for all } \xi^U \in \partial^* p^U(\bar{x}), \text{ and } \nu^U \in \partial^* q^U(\bar{x}). \end{aligned}$$

From the fact $\bar{\lambda}^L > 0$, $\bar{\lambda}^U > 0$ and by above inequalities, we have

$$\begin{aligned} &\bar{\lambda}^L[p^L(x) - \phi^L(\bar{x})q^L(x)] - \bar{\lambda}^L[p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x})] \\ \geq\ &\left\langle \bar{\lambda}^L \left[\xi^L - \phi^L(\bar{x})\nu^L\right], \eta(x, \bar{x} \right\rangle, \text{ for all } \xi^L \in \partial^* p^L(\bar{x}), \text{ and } \nu^L \in \partial^* q^L(\bar{x}), \end{aligned} \tag{28}$$

and

$$\begin{aligned} &\bar{\lambda}^U[p^U(x) - \phi^U(\bar{x})q^U(x)] - \bar{\lambda}^U[p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x})] \\ \geq\ &\left\langle \bar{\lambda}^U \left[\xi^U - \phi^U(\bar{x})\nu^U\right], \eta(x, \bar{x}) \right\rangle, \text{ for all } \xi^U \in \partial^* p^U(\bar{x}), \text{ and } \nu^U \in \partial^* q^U(\bar{x}). \end{aligned} \tag{29}$$

From hypothesis (ii), we get

$$\sum_{i=1}^{m} \bar{\mu}_i h_i(x) - \sum_{i=1}^{m} \bar{\mu}_i h_i(\bar{x}) \geq \left\langle \sum_{i=1}^{m} \bar{\mu}_i \zeta_i, \eta(x, \bar{x}) \right\rangle, \tag{30}$$

for all $\zeta_i \in \partial^* h_i(\bar{x}),\ i = 1, 2, ..., m.$

On adding (28)-(30), we have

$$\begin{aligned}
&\bar{\lambda}^L[p^L(x) - \phi^L(\bar{x})q^L(x)] + \bar{\lambda}^U[p^U(x) - \phi^U(\bar{x})q^U(x)] + \sum_{i=1}^{m} \bar{\mu}_i h_i(x) \\
&- \left[\bar{\lambda}^L[p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x})] + \bar{\lambda}^U[p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x})] + \sum_{i=1}^{m} \bar{\mu}_i h_i(\bar{x})\right] \\
&\geq \left\langle \bar{\lambda}^L\left[\xi^L - \phi^L(\bar{x})\nu^L\right] + \bar{\lambda}^U\left[\xi^U - \phi^U(\bar{x})\nu^U\right] + \sum_{i=1}^{m} \bar{\mu}_i \zeta_i,\ \eta(x, \bar{x}) \right\rangle,
\end{aligned}$$

which by (27), yields

$$\begin{aligned}
&\bar{\lambda}^L[p^L(x) - \phi^L(\bar{x})q^L(x)] + \bar{\lambda}^U[p^U(x) - \phi^U(\bar{x})q^U(x)] + \sum_{i=1}^{m} \bar{\mu}_i h_i(x) \\
&\geq \left[\bar{\lambda}^L[p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x})] + \bar{\lambda}^U[p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x})] + \sum_{i=1}^{m} \bar{\mu}_i h_i(\bar{x})\right],
\end{aligned}$$

that is

$$L(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu}) \leq L(x, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu}). \tag{31}$$

On the other hand, using the feasibility of $\bar{x}$ of the problem (NFIVP) and the fact $\mu \in R^m_+$, we have

$$\mu_i h_i(\bar{x}) \leq 0, i = 1, 2, ..., m, \tag{32}$$

By using (32) and the optimality conditions (5), we get

$$L(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \mu) \leq L(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu}). \tag{33}$$

By inequalities (31) and (33) we conclude that $(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu})$ is a saddle point for $L(x, \lambda^L, \lambda^U, \mu)$. Hence the proof. ∎

5. Conclusion

In this paper, with the idea of convexificators, we have discussed optimality conditions and saddle point criteria for a nonconvex fractional interval-valued optimization problem. Also, we provided an example to validate the results of sufficient optimality conditions established in this paper. In our opinion,

the techniques employed in this paper can be extended for proving the similar results for other classes of fractional programming problems with the functions involving are convexificators. This may be the topic of some of our forthcoming papers.

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Morphological and physiological changes in the *Sorghum bicolor* plants under zinc treatment

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Abstract

Zinc, a micro nutrient is essential for plant growth. It plays a vital role in metabolic activities of plant. A laboratory study was conducted to estimate the effect of excess zinc on growth of sorghum plant. The plants were monitored for 7 days. The plants were grown in different concentrations of zinc taken as $ZnSO_4$. Plants grown in water were taken as control. Morphological parameters like germination, root and shoot length, percent phytotoxicity, fresh, turgid, dry weights, relative water content were measured and biochemical parameters like pigments, proteins and IAA were estimated in seven day old sorghum plants. A gradual decline in growth and physiological parameters was observed with increase in zinc concentration.

Keywords

Zinc , sorghum plant, morphological parameters, biochemical parameters.

Introduction

Zinc , one of the mineral elements, a micronutrient is necessary for plants of all types. It is an integral part of enzymes, which are involved in photosynthesis and other energy processes. Zinc is essential for the development of seeds, generative organs and in maintaining the integrity of bio membranes(1). Proteins and macromolecules also contain zinc as their constituent element. Being a constituent of protein , zinc acts as a structural and functional unit as well as cofactor of many enzymes(2). It's deficiency reduces the crop yield. Soil with high phosphorous and silicon, also peat, sandy, calcareous soils are said to be deficient of zinc(2). Howerver , excess zinc is toxic to plants.

The present study is to understand the effect of excess zinc on morphological and physiological changes in seven day old *Sorghum bicolor* seedlings.

Materials and methods

Sorghum, a member of the grass family Poaceae, is not a single uniform crop, but rather a genus comprising approximately 30 species. Traditionally the plants are grown for grass, grain, and syrup extraction. These plants are grown in tropical and subtropical conditions and does well in arid and semi - arid soils. It is a highly productive crop plant which can be used for alternative energy resource, human food; livestock feed or industrial purposes (3).

Seeds of sorghum were procured from a local seed market, Hyderabad. Seeds of uniform size were chosen for experimental purpose. Glass Petri plates were used for conducting the experiment. Each Petri plate was lined with two layers of filter papers and 10ml of different concentrations of $ZnSO_4$ solution was poured in the plates. The plates were labeled accordingly. Petri treated with distilled water served as control.

Seeds were surface sterilized with 90% alcohol for five minutes and thoroughly washed with distilled water several time to remove any alcoholic traces. Later, 30 seeds were evenly placed in each Petri plate. These plates were left in a place with sufficient sunlight. The experiment was conducted in triplicates.

The Petri plates were monitored at regular intervals to record the various morphological parameters and germination. Sampling was done on the 7^{th} day to analyze the different morphological and physiological parameters like root length, shoot length, seedling vigor index, percent phytotoxicity fresh weight, turgid weight, dry weight, relative water content, chlorophylls, carotenoids, IAA and Protein content.

Germination Percentage - Emergence of radicle from the seed was considered as germination. Germination count was recorded in each concentration after 72 hours of placing the seeds on the moistened filter paper. The %Germination was calculated as = (Number of germinated seeds/Total number of planted seeds) × 100.

Seedling Vigor Index – The vigor index was calculated using the formulas proposed by Abdul-Baki, (1973)(4)

SVI = Germination percentage × Seedling length

Root length and shoot length - Shoot and root lengths were measured using a graph sheet (in cms). Shoot height was measured from the point where seed gave rise to shoot till the tip of shoot. Similarly, root length was measured from the point where seed gave rise to root till the tip of root.

Percent phytotoxicity: was calculated using the formula of Chou and Lin, (1976)(5).

$$\text{Percent phytotoxicity} = \frac{\text{Root/shoot length of control - Root/shoot length of test x 100}}{\text{Radical length of control}}$$

Fresh weight - seedlings from different concentrations and control were taken and weighed using a weighing balance and the weights were recorded in grams (g).

Turgid weight - seedlings from different concentrations and control were placed in different beakers containing distilled water for 4 hours at 4ºC. The water was blotted using a blotting paper and then weighed using a weighing balance.

Dry weight - After taking the turgid weights the seedlings were wrapped in filter papers and labeled. These paper packets were place in hot air oven, then dried for 48 hours at 80ºC and were weighed again to get dry weights.

Relative water content (RWC): According to the formula of Liu and Ding, (2008)(6).
RWC (%) = [(FM - DM)/ (TM - DM)] X100, Where FM = Fresh mass, DM = dry mass and TM = turgid mass.

Estimation of pigments
Extraction - 200 mg of fresh shoots of all treated and control plants were macerated with 10ml of 80% acetone the mixture obtained was centrifuged at 5000 rpm for 10 minutes. The supernatant was collected. The extraction was repeated three times till a colorless pellet was obtained. The supernatant was collected and the volume was made up to 25 ml by adding 80% acetone. The absorbance was measured at 450 nm, 645 nm and 663 nm using a UV- Visible spectrophotometer. The amount of chlorophyll ' a' , chlorophyll ' b' , total chlorophyll and carotenoids were calculated as per Arnons, (1949)(7) method.

Estimation of proteins
Extraction
100 mg of fresh shoots of all concentrations and control were macerated with buffer. The liquid obtained was centrifuged at 5000 rpm for 10 minutes. The supernatant was collected.
Assay - To the crude extract, distilled water and biurette reagent were added and incubated for 30 minutes. The absorbance was recorded at 540 nm using a UV - Visible spectrophotometer.

Estimation of IAA
Extraction - 100 mg of fresh roots and shoots of all concentrations and control were macerated with ethanol thoroughly. This is the crude extract used for assay preparation.
Assay
To 1ml of extract 2ml distilled water and 2ml salkowski reagent was added and was incubated for 30 minutes. The absorbance was recorded at 530 nm using a spectrophotometer and compared with the standard IAA.

Results and Discussions

Fig. 1. Effect of Zinc on seven day old seedlings of Sorghum

Fig. 2. Effect of zinc on germination and germination inhibition percentage in 7 day old Sorghum seedlings.

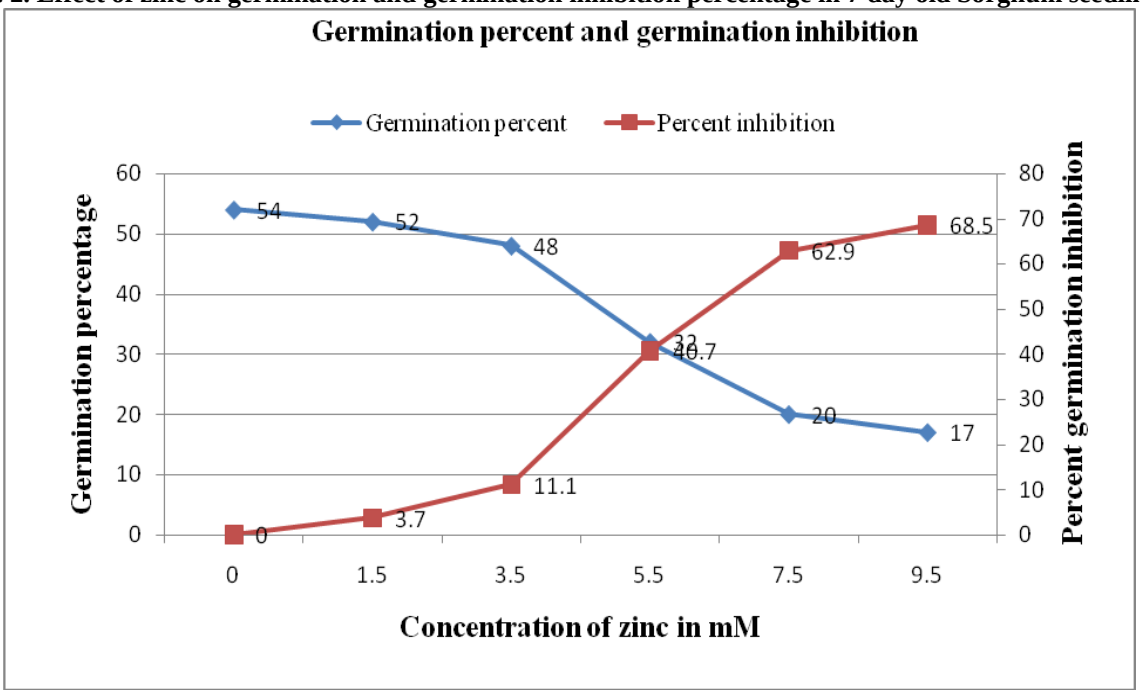


Fig. 3. Effect of zinc on seedling length and seedling vigor index in 7 day old Sorghum seedlings

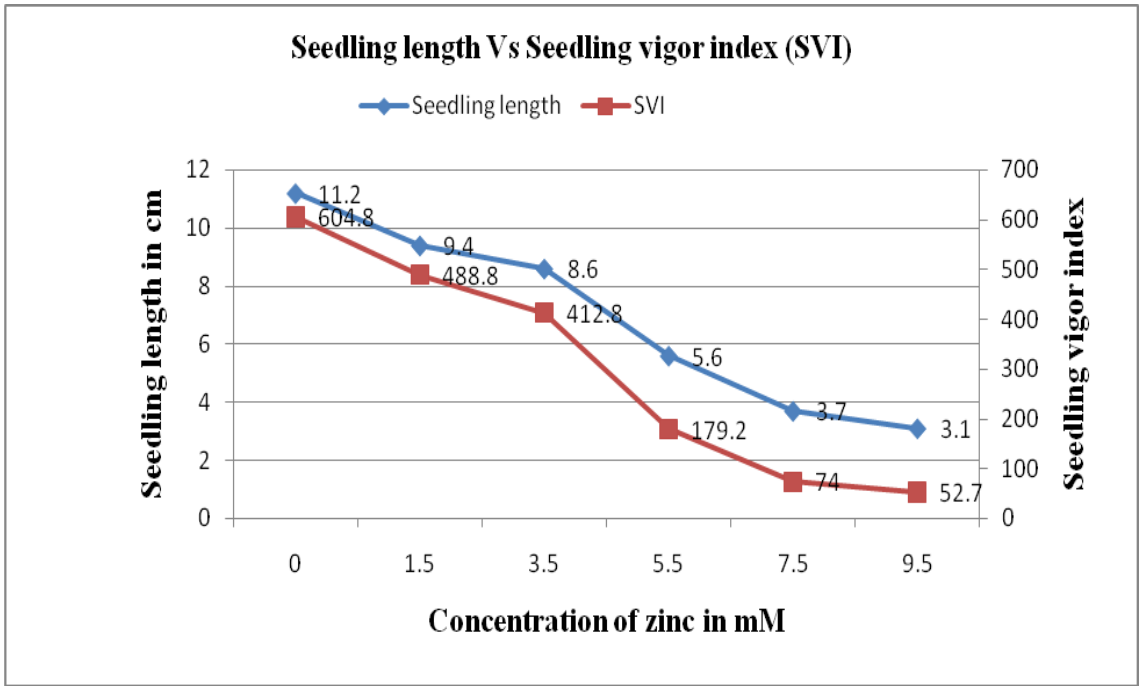


Fig. 4. Effect of zinc on root length and percent phytotoxicity in 7 day old Sorghum seedlings

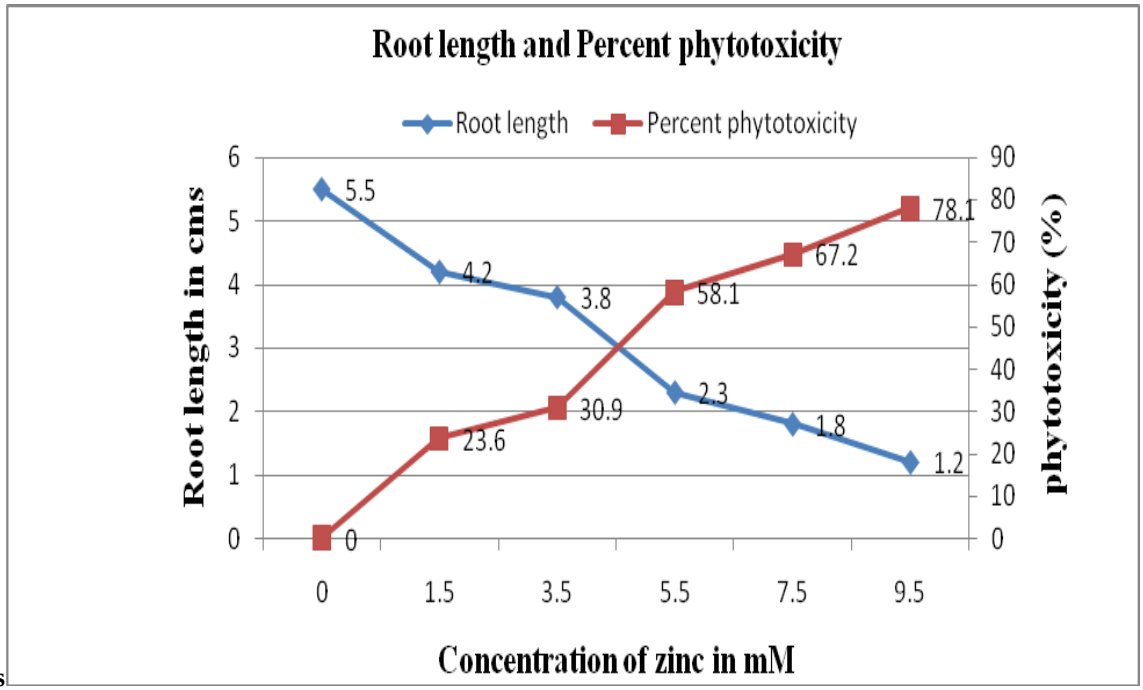


Fig. 5. Effect of zinc on shoot length and percent phytotoxicity in 7 day old Sorghum seedlings

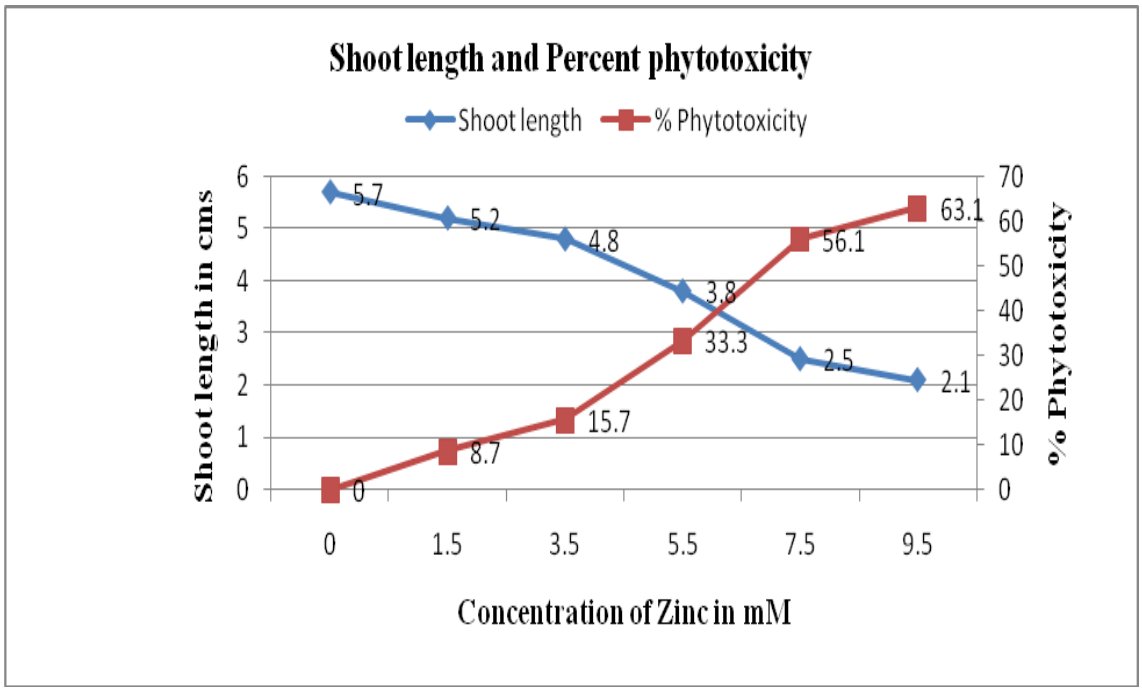


Fig. 6. Effect of zinc on fresh and dry weights of 7 day old Sorghum seedlings

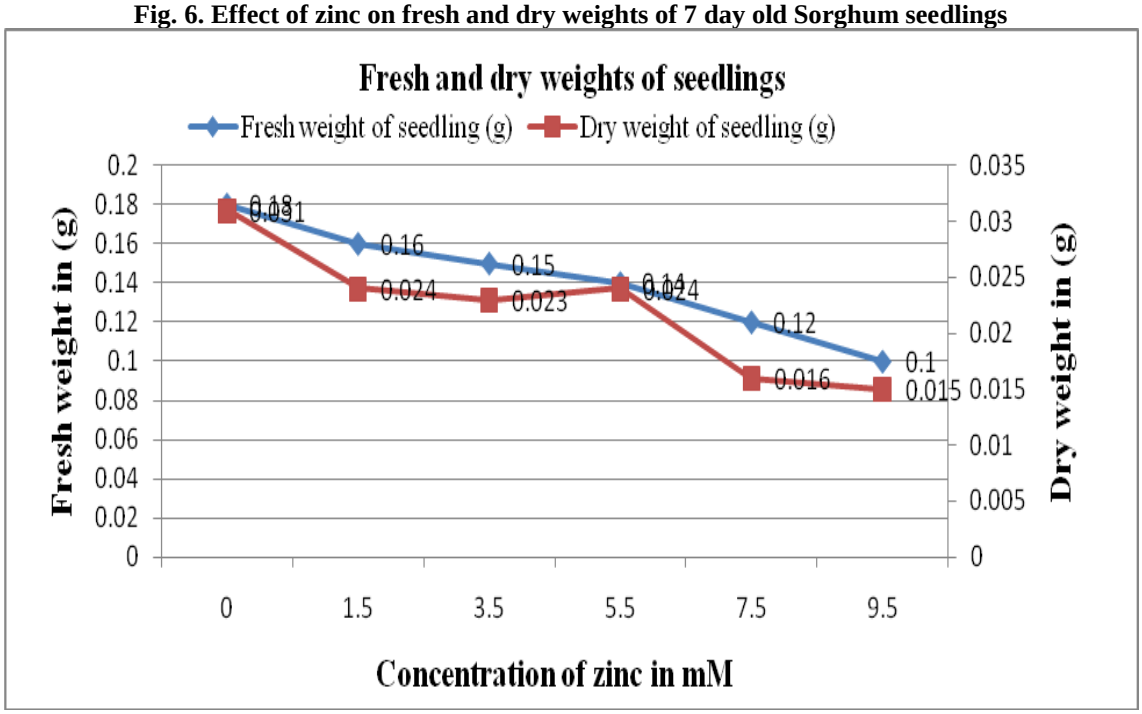


Fig. 7. Effect of zinc on relative water content in 7 day old Sorghum seedlings

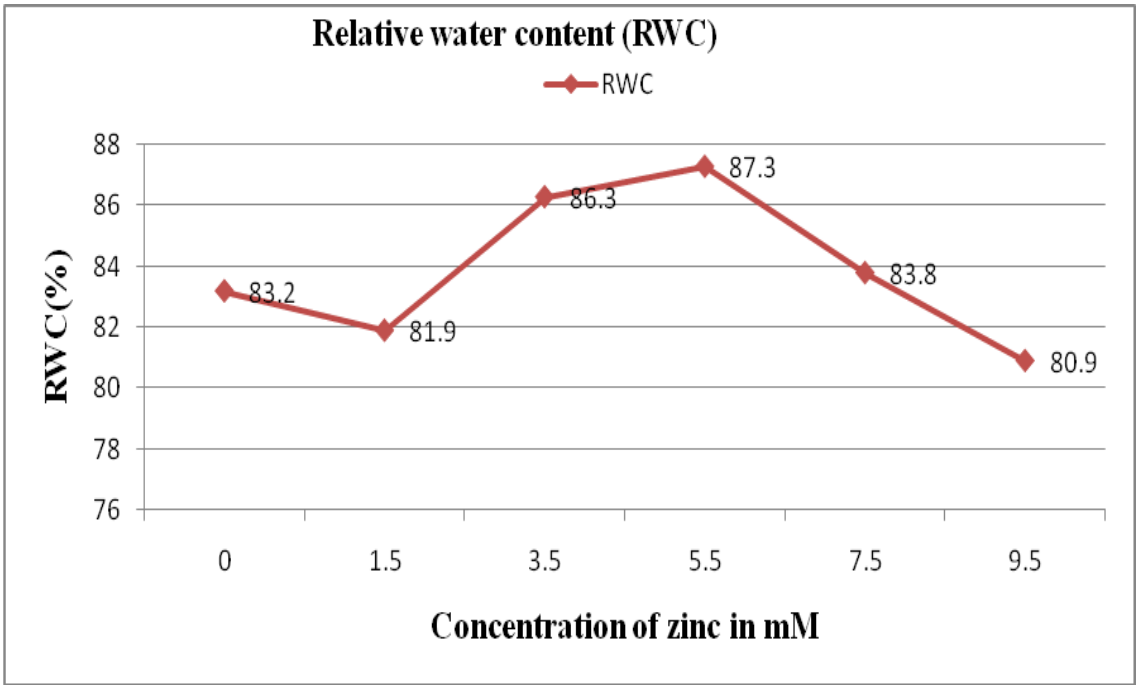


Fig. 8. Effect of zinc on chlorophyll content in the leaves of 7 day old Sorghum seedlings

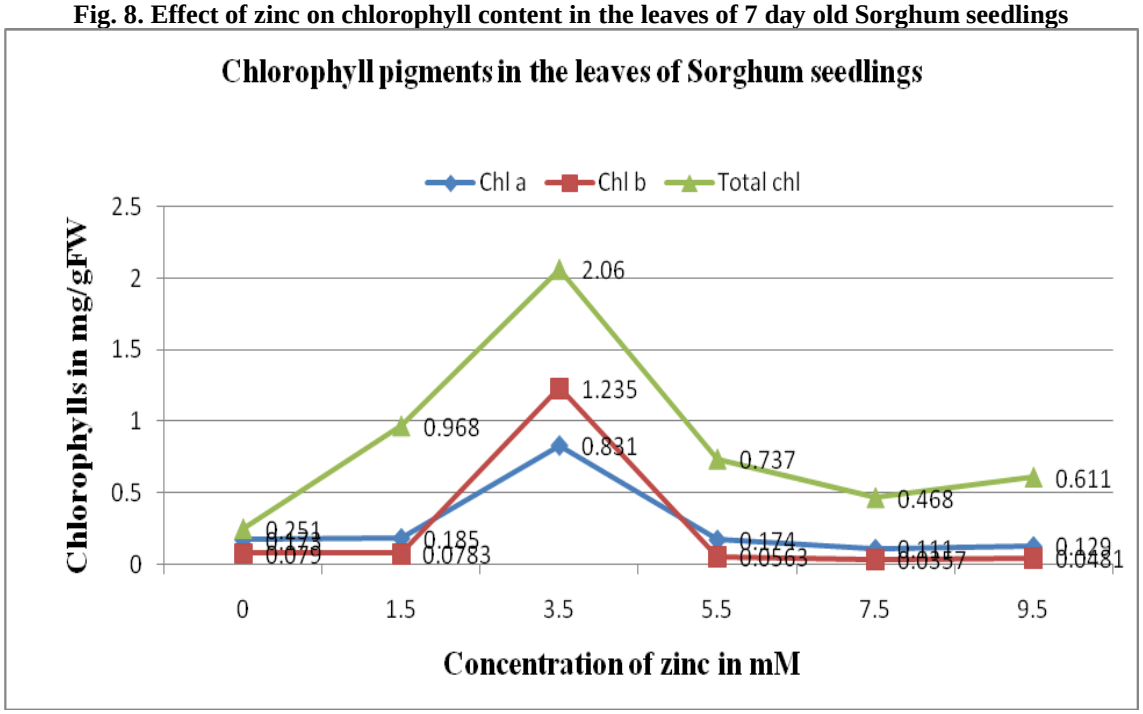


Fig.9. Effect of zinc on a/b ratio and carotenoids in the leaves of 7 day old Sorghum seedlings

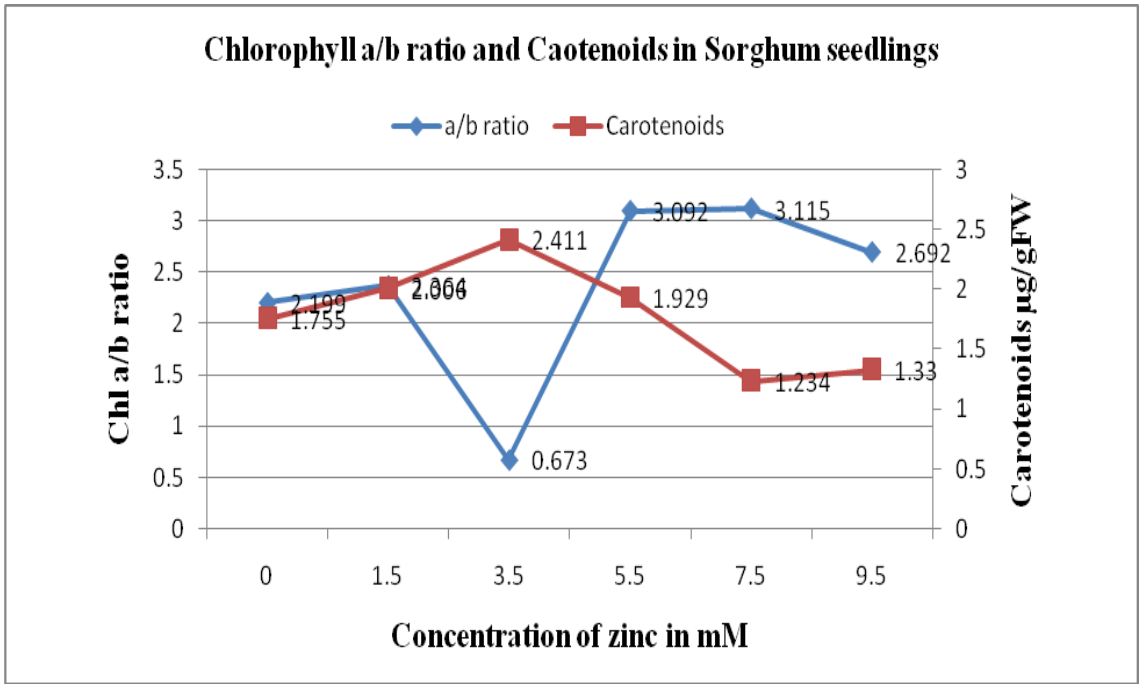


Fig.10. Effect of zinc on IAA content in the shoot and roots of 7 day old Sorghum seedlings

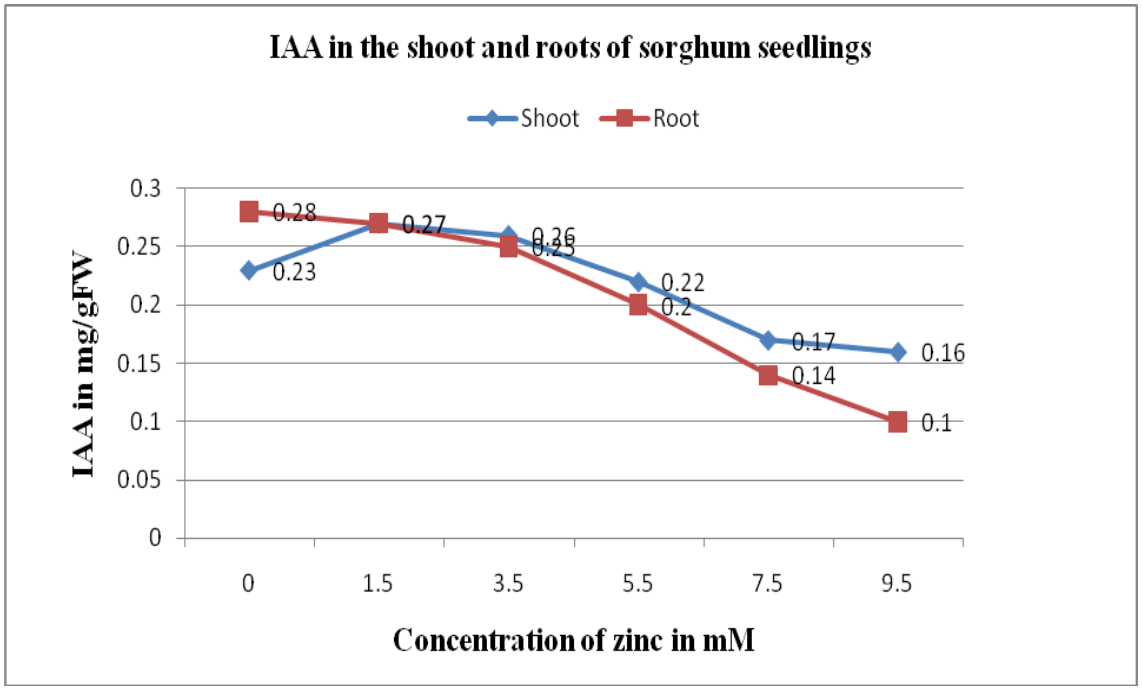
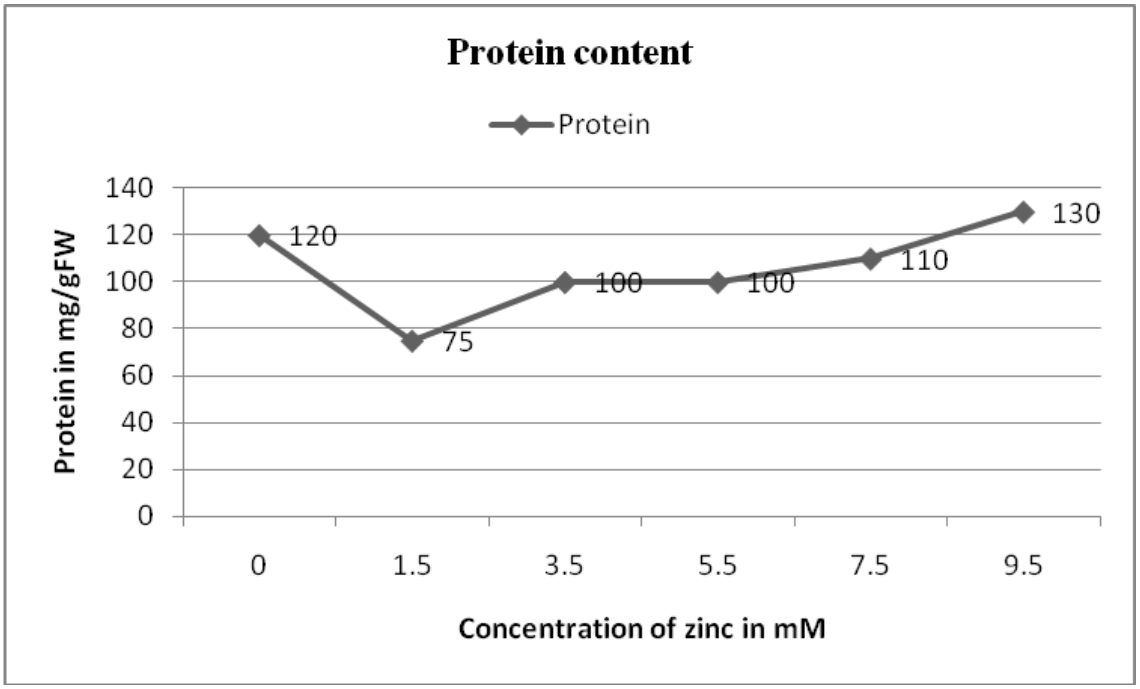


Fig.11. Effect of zinc on protein in the leaves of 7 day old Sorghum seedlings



Zinc is an essential nutrient required in trace quantities with a vital role as a cofactor of a number of enzymes, synthesis of chlorophyll and carotenoids, regulation of nutrients; however, in high doses it causes stress and negatively affects plant growth.

Phytotoxicity symptoms - The visible symptoms showed general retardation in the shoots and roots was observed with increase in zinc treatment (Fig.1.). The leaf size and area reduced in the zinc treated plants. The leaf lamina remained rolled and highly reduced in plants treated with 7.5 and 9.5 mM of zinc.

Germination

Germination and early seedling growths have been regarded as critical phases, which are greatly influenced by stressful conditions (8). In the present study reduction in germination percentage was observed with increase in zinc concentration in the sorghum seeds. More than 50% inhibition was observed in the seeds placed in 7.5 and 9.5mM concentration of zinc (Fig2.). Though seed germination was observed at higher concentration of zinc concentration subsequent growth in early seedling was highly reduced.

Sunil Kumar and Ghosh, 2013(9), reported reduced germination under heavy metal exposure. retarding seed germination and growth of many crops has been reported to affect the break down and mobilization of food reserves like proteins and carbohydrates in germinating seeds reducing plant height, root length, fresh and dry weight, chlorophyll content (10). Mechanisms by which germinating seeds combat heavy metal stress is mainly unknown.

Seedling length and SVI

The results of seedling length and SVI are presented in Fig.3. a gradual decrease in the seedling length and SVI was observed with increase in zinc concentration. Around 50% inhibition in the seedling length was recorded at 5.5mM of zinc treatment whereas around 70% inhibition in SVI was noted at the same treatment.

Increase in the heavy metal concentration in soils, the SVI values decreased. The SVI in the aqueous phase has been widely used to evaluate the phytotoxicity of heavy metals on seedling growth (11). A progressive decrease in SVI was observed with an increase in concentrations was observed in *Trigonella foenum-graceum* L (12). Increasing metal concentrations decreased the tolerance and seedling vigor indexes in wheat and rapeseed (13 & 14)

Root and shoot length

The root and shoot length decreased in all treated plants with increase in zinc concentration. An increase in percent phytotoxicity was observed with increase in zinc treatment. It was noted that the root suffered more toxicity compared to shoots (Fig.4 & 5.). The root length showed a decrease and percent phytotoxicity increased with increase in zinc concentration. 58% reduction in the root length was observed at 5.5mM of zinc however, it was only 33.3% in the shoots at the same concentration. Asfa Rizvi, 2020(15), reported increased percentage phytotoxicity and reduced root growth with the gradual increase in the Cr(VI) level for all wheat cultivars. Tariq mahmood *et al.*, 2007(3), reported a decrease in root length in the cereal crops under different heavy metal treatment The adverse effects of heavy metals reportedly caused structural and morphological changes of roots as well as inhibition of root hair growth of seedlings (16).

Fresh and dry weights

The fresh and dry weights in the sorghum seedling decreased with increase in zinc concentration (Fig.6.). Reduction in dry matter of plants could be due to depletion of moisture content in plant organs and also due to degradation of roots . reduction in the root, shoot and seedling length and dry weight was noticed in *Albizia lebbeck* and *Leucaena leucocephala* on application with different concentration of lead and cadmium (17).

Relative water content (RWC)

Relative water content is the capacity of a plant to withhold maximum amount of water in the leaves under stressful conditions of moisture, also it is an index to determine drought . In the present study the RWC in sorghum seedlings showed an increase at 3.5, 5.5 and 7.5mM of zinc and then declined at 9.5mM as compared to control(Fig.7). Decline of RWC at 9.5mM could be due to decrease in water uptake that could be attributed to highly reduced root growth. Increasing water stress led to decrease in relative water content (18). Similar findings were reported by Haroun *et al.*, (2003) in Sorghum plants under cadmium stress, in Indian mustard by David *et al.*, (1995). Similar observations were also reported by Vassilev and Yordanov, (1997), and Valeria et al, (2006) in different plant species as a result of the effect of heavy metal.

Chlorophyll content

In the present study an increase in chlorophyll a and b was noted in the plants growing in 1.5 and 3.5mM of zinc and from 5.5mM to 9.5mM of zinc treatment there was a decline (Fig.8.). Highest level of chla, b and total chlorophyll was observed at 3.5 mM of zinc treatment. Khurana and Chatterjee, (2001) reported increased content of chlorophyll at lower concentration of zinc and decreased chlorophyll content at elevated zinc concentration in sunflower. Reduced of cholorophyll in Cd, Cu, Mn and Ni treated plant have been reported by Siedlecka *et al*. 2001.(19)

Chlorophyll a/b and carotenoid content is depicted in Fig.9. Carotenoid content showed a gradual increase upto 5,5mM of zinc treatment and decreased in 7.5 and 9.5mM of zinc treatment as compared to control. the a/b ratio decreased in 1.5 and 3.5mM treatment of zinc but sharply increased in plants growing in 5.5, 7.5 and 9.5 mM of zinc treatment. Carotenoids are an indication of stress response as carotenoids increased with decrease in a/b ratio and as carotenoids decreased the a/b ratio increased indicating the the plants were not able to withstand or tolerate stress

Indole acetic acid (IAA)

An increase of IAA was noted at 1.5 and 3.5mM of zinc treatment in the shoot and from 5.5mM a gradual decline was noted as compared to control (Fig.10). However a steep decrease was observed in all treatments in the roots. IAA reduces the negative effects of metal like, retarded shoot and root dry weight, root length, root volume and root surface area . An increase in chlorophyll content in the shoots at 1.5 and 3.5mM can be ascribed to increasing IAA levels of shoots. Plants get adapted to abiotic stresses in the presence of a hormone , Auxin. Singh and Prasad, (2015)(20) reported that photosynthetic efficiency of Cd-treated seedlings was improved by IAA ,by restoring functional and structural attributes of photochemistry system.

Proteins

The protein content showed a decrease in all the treated plants except in the plants growing at 9.5mM where an increase was observed compared to control (Fig.11.). drop in protein content may be due to the breakdown of soluble proteins or due to the accelerated activity of proteins or other catabolic enzymes which were activated and destroyed the proteins (21). Saxena and Harinder , (2006) (22) reported a remarkable increase in the protein content of the moss plants after 6 days of metal (Pb, Ni and Cd) treatment.

Increase was reported in the protein compared to control by several workers after metal exposure in a variety of plants (23 ,24 & 25). This relative increase in the protein content could be due to recycling of nitrogen from porphyrins and apoproteins of pigment-protein complexes in the synthesis of new protein molecules (26). A common feature of many environmental stresses is the cellular dehydration, which serves as the signal for the accumulation of stress induced proteins such as dehydrins in a wide variety of plants, in response to a wide variety of environmental stresses (27).

Conclusion

In the present study it was noted that in spite of high concentration of zinc the plants seemed to be better adapted at 1.5 and 3.5 mM. The morphological parameters like the root and shoot length, root and shoot fresh weight showed a decline, the SVI decreased, RWC declined with an increase in zinc concentration. Changes were observed in the physiological parameters like chlorophylls, carotenoids, IAA and proteins at higher levels of zinc. However, further research is necessary, to analyze and determine the detrimental effect of different metals in the environment on plants

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LANGUAGE STRUCTURE IN MODERN LINGUISTICS IN ENGLISH: A COMPONENTIAL ANALYSIS

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Abstract
In modern linguistics, the study of meaning or associate mantis has remained less developed compared with the advances made in the studies of phonology and syntax. For instance right from Leonard Bloomfield to Noam Chomsky, modern Linguists have set aside the study of semantics in preference to their investigations into the phonology and syntax of language.

Linguists like Lakoff, Ross, Fillmore, McCauley, Halliday, Pike, S. Lamb etc. have attemptedto include the study of meaning in the models of language they forwarded. Thus the study of meaning in language structure has remained a desideratum.

An attempt has been made in this paper to analyse the different shades of meaning across, the most frequently used verbs of motion in English which constitute a single semantic field. The concept of semantic field is explained and illustrated, followed by an explanation of the process of componential analysis employed to decompose the meaning of different member verbs in the semantic field of verbs of motion in English. Finally, the implications for English language teaching are also explained and illustrated.

VERBS OF MOTION IN ENGLISH: A COMPONENTIAL ANALYSIS

Introduction

Modern linguists have conceived the primary function of grammar is to relate sound and meaning and to show how meaning or conceptual world is transformed into utterances or sentences in language. Different theories of grammar and language models have been forwarded to fulfill this goal of grammar. However, the demarcation between semantics and syntax has not been well established because the internal structures of meanings imply certain syntactic properties which should be given proper representation in the grammatical model. For example syntactically indexed arguments of a verb denote the required or acceptable noun phrases and their syntactic function. Furthermore, the selection restrictions impose additional conditions on these co-constituents. Therefore, an essential part of the syntactic behaviour of the lexical element has to be derived directly from its semantic representation.

Even, the syntactic deep structure representation is directly dependent on its semantic structures. It is in this sense that the approach of Generative Grammar seems to be acceptable (as opposed to Chomskyian Generative Transformational Grammar) because this model of language recognizes no other deep structure than the semantic structure on which transformations operate to deride the surface structure, and not on the deep syntactic structure. Lakoff, Ross, Fillmore and McCawley have shown that there is no need to setup an artificial intermediate level, viz. the syntactic deep structure. "It is an artificial intermediate level between the empirically discoverable 'Semantic deep structure', a level the properties of which have more to do with the methodological commitments of grammarians

than with the nature of human languages." (Fillmore 1968: 88) "Ifthe level of structure to which transformations can be most simply applied… has no systematic interconnections with any other facts of language, there does not seem to be any valid motivation for using the level 'deep structure' for it." (Lakoff and Ross, 1967:63) Similarly, "The semantic and syntactic systems of a language comprise the principles that relate conceptual structures and surface structures. For every sentence of a language, these principles specify the relationship between its form as a string of morpheme and its conceptual import" (Langacker, 1968:89) In the same vein Chafe (1970) argues that the 'ideas' exists before and outside of and independently of a language.

Chafe maintains that ideas or concepts are real entities in people's minds, having some physical, electrochemical reality in the human nervous system. The fact that semantics or the study of meaning is an indispensable part of any linguistic investigation has become fully established. However, in modern linguistics the study of meaning or semantics has remained less developed in contrast with the studies of phonology and syntax. Yet linguists have made significant studies in semantics. Two major contributions may be noted in this context. They are a) Semantic fields b) Componential analysis of meaning.

VERBS OF MOTION

- Process

Verbs of Motion indicate any kind of movement as opposed to standstill or resting position. Motion is basically physical and its other implied or figurative meanings are instances of extension of meaning.

The process expressed by a Motion verb requires at least one participant. The participant may be Human or Non-human, Animate or Inanimate. The term Agent stands for the performer of a particular action denoted by a verb and the term patient for the affected. The term participant implied both and its reference is to the process concerned.

- Componential Features

The process involved in motion may be described as movement from one point to another in space; within or around one and the same point of space or with reference to a single point of space. That is, motion implies the presence or absence of a certain Direction which may be of different kinds. To define Direction, a particular spatial point is taken as Reference. This Reference may be Goal - oriented (+Goal) or participant-oriented (-Goal). +Goal includes the speaker's or hearer's standpoint while –Goal refers to that of the participant (Agent/Patient). Another Component is Mode of motion. It may be, for instance, Swift or Slow or neither swift nor slow. The verb 'run' is marked for swiftness whereas the verb 'crawl' is marked for 'slowness' while the verb 'move' is unmarked for either. Thus the componential features of the verbs of motion are as follows:

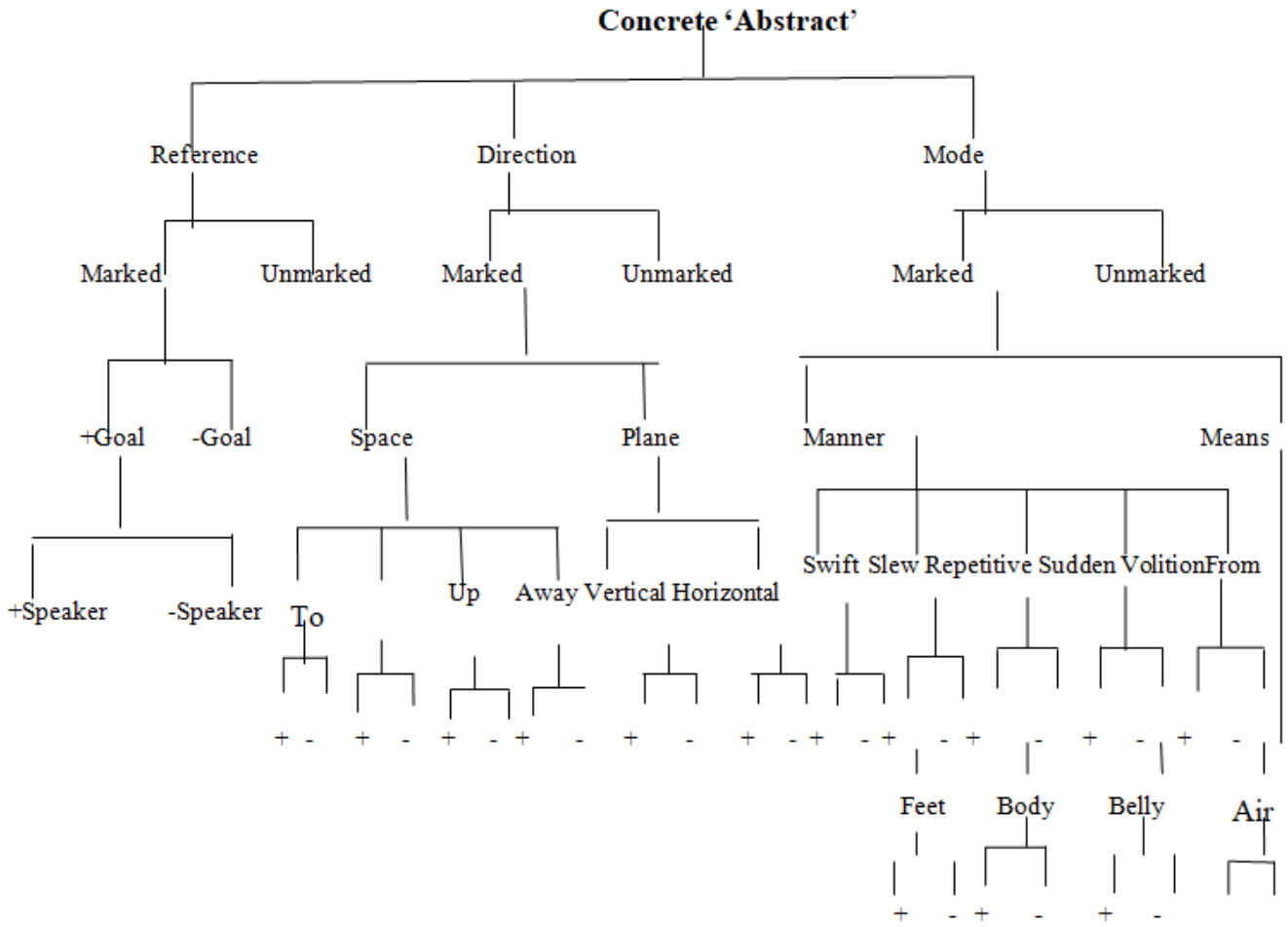
Reference may be marked or unmarked. If it is marked, it may refer to the Goal (+Goal) or may not (-Goal). +Goal comprises the features speaker and hearer; either of them may be specified + or -. If Goal is marked +speaker, it means the Reference is in terms of the speaker's point of view. If it is marked +Hearer, the Reference is to the Hearer's point of view. If a verb is marked – Goal, the Reference is neither to the speaker nor to the Hearer, but to the participant.

Direction is mostly dependent upon Reference. It may be marked or unmarked. It is marked either for space or for plane. Space comprises the features To, From, Up and Away each of which, in turn, may be + or -. Plane includes the features vertical (+ or -) and Horizontal (+ or -).

Mode refers to the kind of movement. It is either marked or unmarked. It is either marked or unmarked. It is marked for Manner and Means. Manner comprises the features swift, slow, repetitive, sudden and volition, each of which may be either + or -. Means covers the features Foot, Body, Belly, and Air. Each of these features may be + or -.

Each of the verbs of motion carries all this information. That is, information about the agent, about the agent-patient relationship if there is a patient, about the spatial point of reference, about the spatial direction and about the spatial direction and about the mode of motion. The semantic description of a motion verb has to present this information as

opposed to that of the other motion verbs.
In the componential feature specification of a verb only those features which distinguish it from the others ('Minimal features') are included. Fig 1 presents a diagrammatic relationship of these componential features.



The Verbs:

Go	Climb	Fly	Move	Come	Jump	Crawl
	Leave	Hop	Creep	Walk	Trot	Rise
	Run	Gallop	Fall			

Contrasts

Go v Come v Leave

Go and Come are logically antonyms while leave and Go are synonyms. A comparison and contrast will throw light on their semantic composition. Consider the following sentences.

1. He has gone to China.
1a. He has come to China.
*1b. He has left to China.
1c. He has left for China.

'He is now in China, or on his way to China' will be a paraphrase of (1) but (1a) cannot be its paraphrase. (1c) can be a paraphrase of (1) with a slight change of stress in meaning. (1c) is different from (1) in the sense that it lays emphasis not on the Goal (China), but on the place thatthe participant has left. In fact (1c) is a contraction of (1d).

1d. He has left (England/India/America) for China.

This kind of expansion is not possible with 1. 1b is not unacceptable syntactically for this reason. 1a gives a different meaning altogether in that it specifies the reference. There, it is implied that the speaker identifies himself with China and the participant also has come to China. In 1 and 1c there is no such reference as to the speaker's situation.

2. Shall we go by train or by steamer?
2a. Shall we leave by train or by steamer?2b.
Shall we come by train or by steamer?

2. Does not make any spatial reference while in 2a. 'Leave' implies 'leave the place where the speaker and the hearer are present.

3. I must be going now
3a. I must be leaving now
*3b. I must be coming now
3c. I must be coming now

3b is semantically anomalous because 'come' implies 'here' or –Away and also it is Goal- oriented in reference. That is, somebody or something other than the speaker 'comes to the place where the speaker is', but not the speaker himself. Therefore 3b is semantically unacceptable. The difference between 3 and 3a is similar to that in 1 and 1d.

4. I wish this pain would go.
4a. I wish this pain would leave me.
*4b. I wish this pain would come.

4 and 4a are synonymous. Here, their difference is rather syntactic than semantic. Leave, being both transitive and intransitive, syntactically requires an object in this context. Also Go in 4 does not imply any physical motion, but implies disappearance of pain or healing. The syntactic difference between Go and Leave is, too partly semantic. Go does not allow a 'place object' whereas Leave allows one.

4b is semantically anomalous because a person is likely to wish for the good things in his favour but not the unpleasant states such as pain. Since Come implies 'near' or –Away a person is unlikely to wish for any pain to come near and in fact itself upon him. Secondly, Come has a restricted semantic range; Go, on the other hand, is flexible and covers a wide semantic range. One can say
But not
5. It goes without saying.

*5a. It comes without saying.

Nevertheless, Go cannot occur at all places where Come does.Compare 6 and 6a.

6. Be coming. ('I request you to come here often)
6a/ Be going.
7. Go away!

*7a. Leave away!
7b. Come away!

7a is semantically irregular and therefore syntactically impossible because Leave includes the features +Away, + From in its semantic composition and does not allow 'away' again syntactically.
7b is semantically different from 7 because come is marked for the referential component whereasGo is unmarked for reference.

8. Winter has gone and spring is here.
*8a. Winter has left and spring is here.
*8b. Winter has come and spring is here.

In 8 'has gone' mean 'ended'. 8a is not acceptable because Leave is marked for reference, +participant. 8b is semantically anomalous being self-contradictory.

9. Don't go doing that!
10. He went red with anger.
11. Fish soon goes bad in hot weather.
12. Tiverton went Liberal at the by-election.
13. When did America go dry?

In the sentences above Go conveys meanings which are idiomatic or figurative or extensionalin nature. Come and Leave cannot express these meanings.

These three verbs take an Agent marked Animate (i.e. +Animate or –Animate).
Thus the following feature configurations will account for the semantic difference between Go,Come and Leave.

Go	Come	Leave
U Ref	M Ref (+Goal)	M Ref (-Goal)
M Dir (+Away)	M Dir (-Away)	M Dir (+Away, +From)
U Mode	U Mode	U Mode
(Animate Agent)	(Animate Agent)	(Animate Agent)

Walk V Run
Walk is marked –Swift and –Slow in mode whereas Run is marked for swiftness (+Swift). Walk is restricted in meaning and cannot be substituted for Run whereas the converse is possible with difference of meaning. Compare the following sentences.

14. We walked three miles.
15. We ran three miles.
16. How old are the babies when they learn to walk?
17. He was walking up and down the station platform.
18. How old are the babies when they learn to run?
18a. He was running up and down the station platform.
The sentences with Run, 15, 16a, 17a may be rare but they are not unacceptable either semantically or syntactically.

However, Walk can replace Run in a few instances syntactically, but with a change of meaning.

19. I can't run fast.
19a. I can't walk fast.
20. The children walked out to see what was happening.
*20a. The children walked out to see what was happening.

19a is semantically anomalous, because walk expresses an idiomatic meaning, 'walk out' being a phrasal verb. Thus 20 is acceptable.

21. The men in the factory walked out yesterday.
21a. She came running towards me.
21b. She came walking towards me. (rare)
22. She ran to meet us.
*22a. She walked to meet us.

Walk is syntactically similar to 'Run' but semantically different.

23. Horses should be walked for a while after a race.
24. He walked his horse up the ill.
25. He put his arm round me and walked me off.
26. You will walk me off my feet.

As a causative verb Walk means 'cause or make somebody or some animal like horse walk'.It also implies that the patient is not physically normal or sound as illustrated in 22 to 25. Run, on the other hand, as a causative implied 'cause to move a vehicle such as a car'. This can be observed in the sentences given below.

27. I will rum you up to town.
28. I will run you back home.

In other words, Walk, when used as a transitive requires a patient marked +Animate whereas Run as transitive takes a patient marked –Animate. Walk is more restricted in meaning than Run.

For mode, Walk is marked –Swift, -Slow and +Foot, while run is only marked for swiftness. Walk requires an Animate Agent or patient whereas it may be Animate for run. These semantic distinctions and selectional restrictions are responsible for the acceptable or non-acceptability of the sentences in which they occur. Thus, in terms of selectional restrictions 28 and 29 are unacceptable.

*28. He walked the car into the garage.
*29. He put his around me and ran me off.

The semantic composition of Walk and Run may be represented as follows:

Walk	Run
U Ref	U Ref
U Dir	U Dir
M Mode (- Swift, +Foot)	M Mode ((+swift)
(Animate Agent/Patient)	(Animate Agent/Patient)

Jump V Hop V Trot V Gallop

Jump
30. He jumped to his feet.
31. The dog jumped over the fence.
32. He was jumping up and down in excitement.
33. The manager jumped out of his chair.
34. She jumped into the taxi.
35. The speaker jumped from religion to politics.
36. Jane jumped down her husband's throat.
37. I jumped the horse over the fence.
38. He jumped for joy.
39. Her heart jumped when she heard the news.
40. Gold shares jumped on the Stock Exchange yesterday.
41. He jumped at the offer.
42. Don't jump to conclusion until you read the letter.
43. The teacher jumped (up) on the inattentive pupil.

None of the other three verbs than Jump, viz., Hop, Trot and Gallop can substituted for Jump in the sentence above.

Jump physically involves a movement that is sudden and non-repetitive. It generally requires an Animate Agent with a few exceptions such as 40 where the meaning is obviously figurative. It ismarked +up, +vertical, +Horizontal for direction and for mode +Sudden, Repetitive.

Hop
44. Sparrows were hopping about on the lawn.
45. He hurt his left foot and had to hop along.

Trot
46. Well, I must be trotting off home.
47. You trot away!
48. Smith was trotting out his knowledge.
49. He trotted Jane off her legs.

Gallop
50. He galloped across the field.
51. I must gallop through my work.

Here, in the sentences above, the semantic distinctions are more crucial than the syntactic differences because none of them can be a substitute semantically to the other two.

Hop is marked +Vertical, +Horizontal for direction and +Repetitive for mode. Trot is unmarked for direction and for mode it is marked –Swift, -Slow. Gallop is also unmarked for direction and marked +Swift and +Repetitive for mode. All these are unmarked for reference and require an Agent/Patient marked +Animate. Their semantic features may be shown as follows:

Jump	Hop	Trot	Gallop
M Dir (+horizontal)	M Dir (+vertical, +horizontal)	U Dir	U Dir (+up, +vertical,
M Mode (+repetitive)	M Mode (+repetitive)	M Mode (-swift, -slow)	M Mode (+sudden, -repetitive) (+swift, +repetitive)

Crawl V Creep

Crawl

52. The snake crawled into the room
53. The wounded soldier crawled into a shell-hole.
54. Don't crawl to your boss.
55. Our train crawled over the damaged bridge.
56. The ground was crawling with ants.
57. She says that the sight of snakes makes her flesh crawl.

Creep

58. The cat crept silently towards the bird.
59. Old age creeps upon one unawares.
60. A feeling of drowsiness crept over him.
61. Ivy had crept over the ruined castle walls.
62. The sight of the cold damp prison cell with rats running about, made her flesh creep.

These two verbs are partially synonymous and hence in 52 and 57 creep can replace crawland vice versa. This is not possible in sentences 53 to 57.

Both crawl and creep are marked +Horizontal and +Vertical for direction. In mode both are marked +Slow and Crawl is marked +Body while Creep is marked +Belly. Crawl always requiresan animate agent whereas it may be animate in the case of Creep. Both are unmarked for reference.

Their minimal destination may be shown as follows:

Crawl	**Creep**
M Mode +Body (Animate Agent)	M Mode +Belly (Animate Agent)

Rise V Fall

Rise and Fall are antonyms in a way look at the following sentences.

63. The wounded man fall and was too weak to rise.
64. His voices rose in angry.
65. His voice fell to a whisper.
66. Mercury is rising.
67. The barometer is falling.
68. The wind is rising.
69. The wind fell during the night.
70. Where does the Nile rise?
71. The river falls into the lake.

But they are not antonyms always as in the following pairs of sentences.

72. The sun rises in the East.
*73. The sun falls in the west.
74. Darkness fell upon the screen.
*75. Light rose upon the scene.

Rise itself has a wide range of meaning, but fall has yet a wider range. The main distinction between Rise and Fall is in that the former is marked +up for Direction and for mode it is Volition while the latter is marked +Down for direction and is –Volition for mode. Both can take Animate Agent.
This distinction is borne out in their syntactic co-occurrence possibilities with reard to prepositions or adverbs. Compare the following.

76. He will rise to greatness.
77. He fell into disgrace.

Both of these verbs are unmarked for reference. The semantic components of Rise and Fallmay be represented as follows:

Rise	**Fall**
M Dir (+Up)	M Dir (+Down)
U mode	M Mode (-Volition)

Conclusion

The implication underlying the foregoing componential analysis of the semantic field, the verbs of motion of English is that the learners of English can use English verbs of motion appropriately and meaningfully in their English. For instance such unacceptable, ungrammatical, deviant and nonsensical sentences such as:

*Sherkhan tried to assassinate Mouglis.
*the police hunted the thief.
*the garden is creeping with ants etc.

Such an awareness of the subtle semantic differences across the member verbs of a given semantic field will enable language learners to appreciate the effect of syntax variation and to employ appropriate styles in their own language.

Similar accounts of other semantic verbs of English and also those of nouns can be undertaken by future researcher to make teaching of English as a second or foreign language more effective and purposeful.

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Bioremediation of Chromium by *Pseudomonas aeruginosa* from polluted water samples

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Abstract

Chromium (Cr) is one of the most common heavy metals affecting the water quality which is introduced into the environment from Industries. Wastewater have resulted in significant quantities of Cr(VI) in the environment, which may constitute toxicological risk to humans, animals, and plants. To reduce the toxicity of Cr (VI) the concept of bioremediation is being employed. Bioremediation techniques can be in situ or ex situ depending on whether the intervention is carried out with suitable bacteria directly on the polluted sites or on portion on environmental samples such as sewage, sludge and soil after being removed and transported appropriately for treatment. In this study, we investigated the efficiency of reduction and biosorption of chromium by chromate-resistant bacteria isolated from tannery effluent. The chromium-resistant strains isolated from Heavy metal polluted samples are further examined for their resistivity to different concentrations based on their growth in Luria Bertini medium containing heavy metals. A laboratory study was carried out to evaluate the bioremediation potential of hexavalent chromium Cr (VI)), taking into account the chromate pollution of various industrial sites namely Chadarghat, and Attapur located in Hyderabad. Results showed the presence of Cr (VI) in polluted samples and Luria Bertini broth, analysis by mass spectrophotometer indicated the degradation of Chromium through chromium resistant organisms. organisms. One of the isolate was selected based on its tolerance and on 16S rRNA sequencing it was identified as Pseudomonas aeruginosa which showed bioremediation of Cr(VI) concentration ranging from 50 to 150 ppm.

Keywords: Bioremediation, Hexavalent chromium, Heavy metal, Bacteria, 16S RNA Sequencing, Biosorption, Toxicological risk, Spectrophotometer.

1. INTRODUCTION

Water is one of the most essential natural resources, necessary for all forms of life. This natural resource is being polluted every day by various human activities such as rapid growth of occupants, modernization, and industrialization which ultimately makes the environment polluted. In recent years, sewage water has been used for irrigation purposes. There are greater threats about heavy metal contamination in the water system and land.

The main reason for this may be traced back to the increase in different industrial activities which takes a great part in producing plenty of waste and untreated water throughout the world. It has been emphasized that the water pollution induced by the large amounts of industrial effluents without proper treatment before disposal to the water bodies is causing a serious threat to entire human health.

The amount of toxic heavy metals in the soil is of geogenic or anthropogenic origin. Heavy metals from the point of origin and various other sources can be transported to distant environments. Heavy metal exposure has been one of the big global concerns due to its high toxicity, high bioaccumulation in the human body and food chain, the essence of nonbiodegradability, and most likely human carcinogenicity[1]. High levels of heavy metals can destroy soil fertility and may affect productivity and quality. Heavy metals in the atmosphere may also change plant diversity and affect aquatic life.

Among toxic heavy metals, chromium is ranked among the top sixteen toxic contaminants that have adverse effects on human health as an essential metal [2]. Wastewater from tanneries is primarily characterized by high levels of chemical demand for oxygen (COD), biochemical oxygen demand (BOD), turbidity, suspended solids (SS), sulfides, and chromium[3]. Cr(III) on reaction with water has the composition of chromium hydroxide ($Cr(OH)_3$) and iron chromium hydroxide ($CrFe(OH)_6$), which is less soluble and tend to precipitate or accumulate under neutral to basic aqueous solutions. In contrast, the other form of chromium that is stable in a nature is the hexavalent chromium, or Cr(VI), is soluble at any pH and is 100-times more lethal than Cr(III). The toxic action of Cr(VI) is due to its ability to easily break through cellular membranes of prokaryotes and eukaryotes.

Cell membrane injury caused by oxidative stress induced by Cr(VI) have also been largely reported [4]. It is being used widely in various industries including leather and tanning industries in metallurgical and electroplating industries in production of pigments and paints. The major chromium pollution is contributed by leather industries through water discharges and poisonous gases released by them, which is responsible for 40% of total industrial waste[5]. Westernization and industrialization has resulted in environmental pollution due to release of heavy metal discharges into water bodies without proper treatment. This has resulted in transfer of toxicity through food chain.

On exposure of chromium (VI) to human results in corrosion and irritation of skin and has carcinogenic effects. The significant route of entry is through inhalation ingestion and skin penetration. The target organ for acute systemic toxicity is the kidney. Usually poisoning by chromium (VI) results in acute tubular necrosis of the kidney, and death[6].

Chromium (VI) is hazardous to health when its limit in potable water exceeds 0.05Mg/L [7]. The oral human lethal dose for Cr(VI) is 71mg/Kg , while according to the occupational safety and health association (OSHA): permissible exposure limit (PET) , time-weighted average (TWA) value is $1mg/m^3$ [8]

It is known that Cr(VI) is many times more toxic than the Cr (III). Hence, reduction of Cr(VI) to Cr (III) by bacteria is a very significant occurrence in terms of environmental pollution control. Microbial reduction

of Cr(VI) to Cr (III) was premeditated widely [9]. Various methods have been followed by bacteria for the reduction of Cr(VI) such as Biosorption, Bioaccumulation Bioremediation, biotransformation, Biodegradation through different estimations [10].

Many microbes have reported to reduce Cr(VI) in aerobic or anaerobic with their exceptional ability to adapt the metal polluted environment and colonize them. These microbes have developed these qualities to be resistant to heavy metal toxicity by various strategies as adsorption, methylation, reduction and etc. Although the availability of efficient Cr(VI conditions) reducing organisms is an essential requirement for bioremediation – biodegradation of contaminated water/sewage.

Bioremediation is the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state. The main principle of this technique is to remove pollutants from the natural environment or convert the pollutants to a less harmful product using the indigenous microbiological community of the contaminated environment. The bioremediation and biotransformation approaches use bacterial catabolic range to damage, transform and accumulate a huge range of compounds including hydrocarbons, polychlorinated biphenyls (PCBs), polyaromatic hydrocarbon (PAHs), pharmaceutical substances and heavy metals through which we can control damage to the environment. Some microbial species can use Cr(VI) as a terminal electron acceptor in their respiratory process and transform Cr(VI) to less toxic Cr(III) compounds [11] [12].

Microorganism-driven methods offer an attractive in terms of low operational cost and efficient treatment which is more beneficial than physical and chemical treatment method for decontamination of soils by heavy metals.

The simple, inexpensive, and eco-friendly solution that can be performed over a wide range of experimental conditions for the detoxification and elimination of Cr(VI)-pollutants is bioremediation, which uses indigenous microorganisms.

2. MATERIALS AND METHODS

2.1 SAMPLING SITES AND SAMPLE COLLECTION

The samples (soil, sludge, water) collected from different locations of the chromium deposited and contaminated site located at Musi river , Karwan road(sewage disposal), Chadarghat were examined to estimate the amount of pollutants in the sample. Environmental samples were collected from various sites exhibiting different characteristics in order to isolate chromate tolerant strains of diverse origin. Isolation of cultures from sample collected was carried out as per standard procedures.

2.2 ISOLATION OF CHROMIUM-RESISTANT BACTERIA

The enriched cultures were first grown on MSM (Mineral Salt Medium) broth and LB (Luria Bertini) broth in presence of 0.02gm (VI), inoculated with 10ml of water sample incubated for 15 days at 37 C. Selective isolation was carried out by spread plating 0.1 ml of the culture onto Luria Bertini agar plates in the presence of the same initial concentration of Cr(VI) and incubated at 35.°C for 24 hours. Individual distinct colonies were further undergone repeated sub-culturing. After this incubation period, the presence of microbial growth was observed and colonies were selected based on morphological characteristics.

2.3 DETERMINATION OF TOLERANCE TO DIFFERENT CHROMIUM CONCENTRATION

For the isolation of chromium resistant bacteria, 0.1 mL of 5 sewage sample were separately spread on Luria–Bertani (LB) agar plates supplemented with Cr(VI) concentration of 10ppm to 50ppm respectively with each sample and was incubated at 37 ◦C for 48h. Microbial growth was observed for three sewage samples. Selected colonies were inoculated in LB broth media supplemented with increasing concentration of Cr(VI) from 100 mg/L to 200 mg/L and incubated for 24 h, 48 h, 72 h respectively at 37C.

Colorimetric assay was thereby conducted, the strains showing highest optical density was selected to expose it further to increasing concentration of chromium. Culture from broth having the highest turbidity and survived a concentration of 150ppm of Cr(VI) was streaked on Luria Bertini plates and observed under microscope and these Cr resistant bacterial colonies was preserved for further study on a LB slant.

2.4 CHARACTERISATION OF BACTERIA BY MICROSCOPIC OBSERVATION AND BIOCHEMICAL TESTS

After incubation at 37C for 24h the colonies on Luria Bertini plates were observed as Small, Round, Entire, Umbonate elevation, Dry, Opaque and Creamy white.
Gram staining is a common practice used to discriminate two large groups of bacteria based on their dissimilar cell wall constituents. Thus, gram staining procedure was performed and the slide was ready to observe under compound microscope at magnifying power of 100x. Short pink rods were observed, Fig 3.

Biochemical Test
Biochemical tests are among the most among the important methods for identification of any microorganisms. Routine biochemical tests include tests for Indole test, Methyl Red test, Voges Proskauer test, Simon citrate test, Catalase test and Oxidase test were carried out

2.5 PHYLOGENETIC DETERMINATION OF SELECTED ISOLATE

Report of Microbial Identification Experimental Method:
DNA was isolated from the culture provided by the scientist. Its quality was evaluated on 1.0 % agarose gel, a single band of high-molecular-weight DNA has been observed. Fragment of 16S rRNA gene was amplified by 16SrRNA-F and 16SrRNA-R primers. A single discrete PCR amplicon band of 1500 bp was observed when resolved on agarose gel and then the PCR amplicon was purified to remove contaminants. Further, forward and reverse DNA sequencing reaction of PCR amplicon was carried out with 16SrRNA-F and 16SrRNA-R primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Consensus sequence of 16S rRNA gene was generated using aligner software. The 16S rRNA gene sequence was used to carry out BLAST with the 'nr' database of NCBI GenBank database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix and phylogenetic tree was constructed was constructed using MEGA 10.
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Microcosm Study

3. ANALYSIS FOR DETERMINATION OF THE CHROMIUM (CR) SPECIES IN DIFFERENT SAMPLES BY PHOTO SPECTROPHOTOMETRY WITH DIPHENYL CARBAZIDE

Chromium speciation analysis methods developed rapidly not only because of the impact of its toxicity but because its concentration is very low in the water system. Chromium(III) is an essential micro-nutrient for the human body, and play an important role in the metabolism of glucose and some fat. While the Cr(VI) is

highly toxic and carcinogenic[13] .

The aim of this study is to optimize a DPC method for low-cost and simple incorporation into a microfluidic detection system. Spectrophotometric methods can be used for selective determination of the different chromium species using reagents to form absorbing species that present selectivity in the response. The most common method for determining Cr(VI) in aqueous solutions is based on the reaction of diphenylcarbazide (DPC) with Cr(VI) at a pH of 1.0 [14] [15] [16] [17]. Spectrophotometric analysis of the magenta chromagen (λ max~540 nm) which is formed by the reaction of Cr(VI) with 1,5-diphenylcarbazide (DPC) in strongly acidic solution [18].

The DPC method was chosen because of the intense color development at 543 nm region [19]. Onocke and Sasu developed a method for Cr VI detection in groundwater and industrial waste samples. In this method, Cr VI was reacted with 1,5-diphenylcarbazide (DPC) dye, which, in acidic conditions, forms a purple-colored species. As a result of a redox reaction, Cr VI is reduced to Cr III, and DPC is oxidized to 1,5-diphenylcarbazone (DPCA). Cr III and DPCA form a purple-colored species with lambda max of 540 nm.

Spectrophotometric methods can be used for selective differentiation & determination of the different chromium species using reagents to form absorbing species that present discernment in the response [20]. The most conventional method for determining Cr(VI) in aqueous solutions is based on the reaction of diphenyl carbazide (DPC) with Cr(VI) at a pH of 1.0 [21]. Spectrophotometric

analysis of the magenta chromagen (λ max~540 nm) which is formed by the reaction of Cr(VI) with 1,5- diphenyl carbazide (DPC) in strongly acidic solution.

Sample collection
Water and sludge sample was collected from Karwan area, Hyderabad.

Apparatus used
PC-Based double beam UV-VIS spectrophotometer 2202 was used with 1mm quartz cuvettes. For the absorbance measurement.

Reagents Used
DPC reagent, H2SO4

Method
A stock solution was prepared with 0.01gm of Cr VI and 10ml of distilled water. Two controls were prepared to compare with the natural samples. 1ml of chromium-resistant isolate was added into 100ml of LB broth having Cr concentrations if 100ppm and 150ppm respectively. Further 1ml of Cr-resistant isolates were added into each natural samples and left for incubation for 24hrs, 48hrs, and 72hrs respectively at 37 C. The sample matrices were analyzed using the DPC method in order to determine whether or not Cr VI was present in concentrations detectable by the method and to check degradation levels of Cr by chromium-resistant isolates.
Sample Preparation: 2ml of sample was collected in an Eppendorf tube and centrifuged for 5mins at 10,000RPM. After centrifugation residue was discarded and supernatant(2ml) was collected was transferred to a glass vial. Sulphuric acid (0.2 M,1 ML) and 1,5-diphenylcarbazide (0.5% w/v, 1mL) were added, and the mixture was gently shaken and left for five minutes. The absorbance was measured at 543nm against a reagent blank.

Result
The absorbance of each solution was extrapolated to the standard curve of Cr(VI) and concentration was noted. The absorbance values decreased over the course of time indicating reduction in chromium concentration by the isolate **Pseudomonas aeruginosa**

4. Results and discussion

4.1 Screening, Isolation, and MTC of Bacteria

The major objective of this work was to isolate and characterize gram negative bacteria that were chromium resistant from several polluted Musi river areas. 50 isolates from LB plates were chosen for this project based on their colour, size, and morphology. A total of 12 bacterial isolates that were tolerant to high concentrations of chromium were further screened based on the highest metal tolerance for chromium.
On the basis of the MTC and co-metal tolerance of the metal, one isolate (1AJU19) was chosen for further study. One bacterial isolate was identified as a gram-negative rod by the results of the morphological and biochemical tests. The sample responded negatively to the indole synthesis test, the methyl red test, and the Voges Proskauer test, but positively to the citrate utilization, catalase, and oxidase test. **Fig 1(A)) & Fig 1 (B)**

4.2 Determination of maximum tolerance concentration (MTC) of heavy metals
The MTC of Chromium was established following preliminary selection of chromium-tolerant isolates. The isolated strains were inoculated in LB broth supplemented with increasing concentrations of chromium for the quantitative assessment of heavy metal tolerance (50 to 150ppm). The broth tubes were kept in an incubator at 37°C for various lengths of time. Maximum tolerance concentration (MTC) of the relevant heavy metal for the isolated strain is the highest

concentration of metal ions at which bacterial growth was observed (as indicated by optical density, OD, at 600 nm) (Schmidt and Schlegel, 1994). **Fig 2(A) (B)**

4.3 Identification of the isolate by 16S rRNA sequencing and phylogenetic analysis.

(A)The 16S rRNA gene sequence and blast analysis: Analysis has led to the identification of the bacterial isolate as Pseudomonas aeruginosa strain IHB B 6863 with 100% genetic sequence similarity (fig ...). The submitted sequences can be found in the NCBI database (accession number KF668476.1). **Figure 3 (A)**

(B)Chromatogram Data

CGGATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCGATAA
GTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGC
CCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCTG
GCCTTGACATGCTGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCAGACAC
AGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAAC
GAGCGCAACCCTTGTCCTTAGTTACCA

GCACCTCGGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGG
ATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCG
GTACAAAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTC
CGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGTGAATCA
GAATGTCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGA
GTGGGTTGCTCCAGAAGTAGCTAGTCTAACCGCAAGGGGGACGGTTACCACGGAGTG
ATTCATGACTGGGGTGAAGTCGTAACAAGGTAACCGTAGA

Data and Result**:**

Sample which was labeled as 1AJU19 was found to be Pseudomonas aeruginosa, showed high similarity based on nucleotide homology and phylogenetic analysis. **Fig 3 (B)**

(C) Gdna and 16S Amplicon QC data: The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model. The tree with the highest log likelihood (-2064.21) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. This analysis involved 11 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 1498 positions in the final dataset. Evolutionary analyses were conducted in MEGA X. **Fig 3 (C)**

(D) Reduction of Cr (VI) by the isolate in sewage effluents after 24, 48, and 72h of incubation at 37°c indicated through absorbance via spectrophotometric analysis. **Fig 3 (D)**

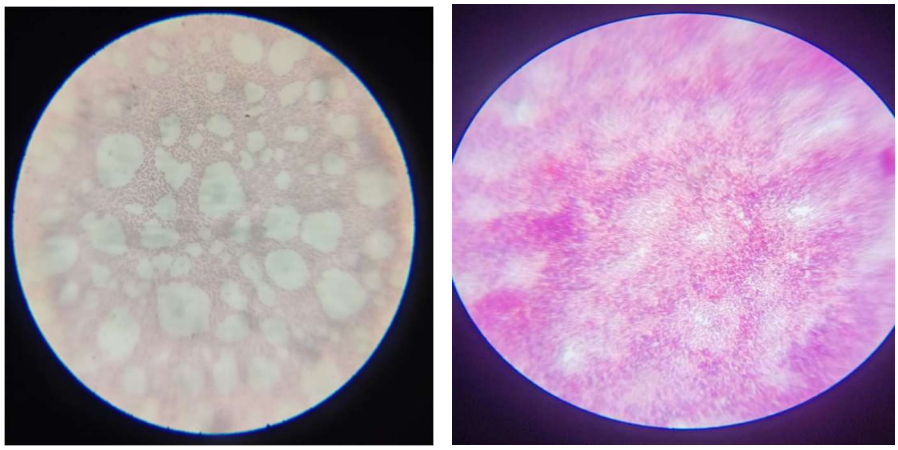
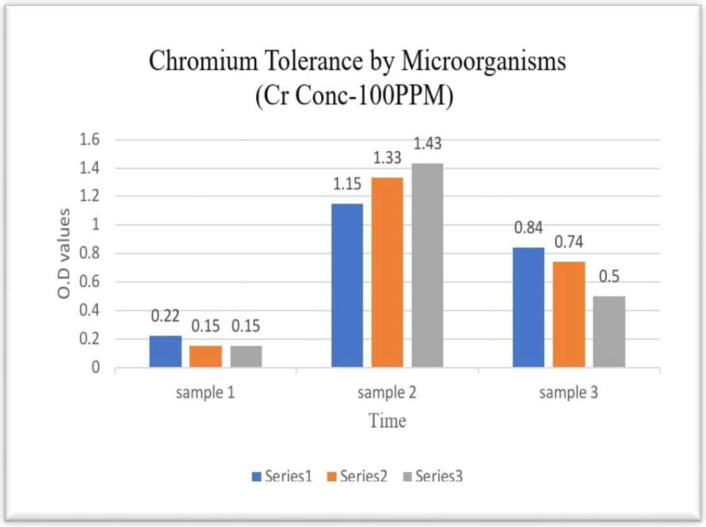


Figure. 1(A) Compound Microscopic analysis of Pseudomonas sp. when examined under oil immersion lens (100x) appeared as pink colour rods

S.No.	Identification	Sample (1AJU19)
1	Colony Characteristics	Pink colored, smooth, glistening colonies
2	Gram staining	Gram negative Rods
3	Indole production	-
4	Methyl Red	-
5	Voges Proskauer Test	-
6	Citrate Utilization	+
7	Catalase test	+
8	Oxidase test	+

Fig 1(B) Morphological and biochemical characterization of selected bacterial isolate.



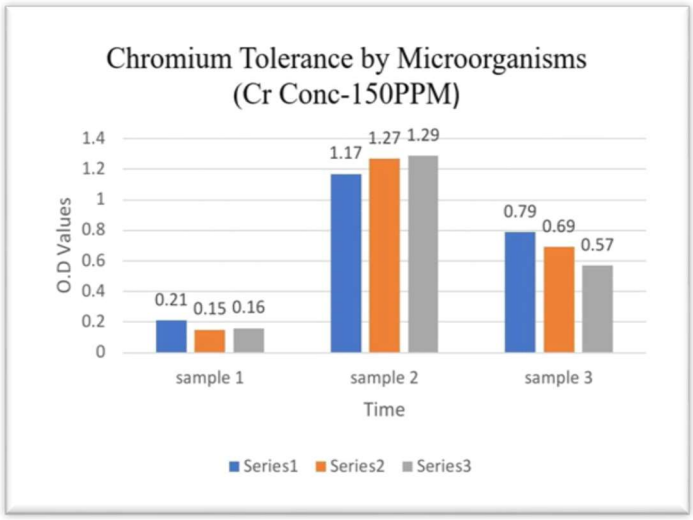


Figure 2(A) (B)

(A). Tolerance of Cr (VI) by the isolate in LB broth with an initial concentration of 100ppm after 24, 48, and 72h of incubation at 37 °c.

(B). Tolerance of Cr (VI) by the isolate in LB broth with an initial concentration of 150ppm after 24, 48, and 72h of incubation at 37 °c.

Sample 1 and 2 were the effluents taken from Chadarghat, Sample 3 effluent was taken from karwan road.

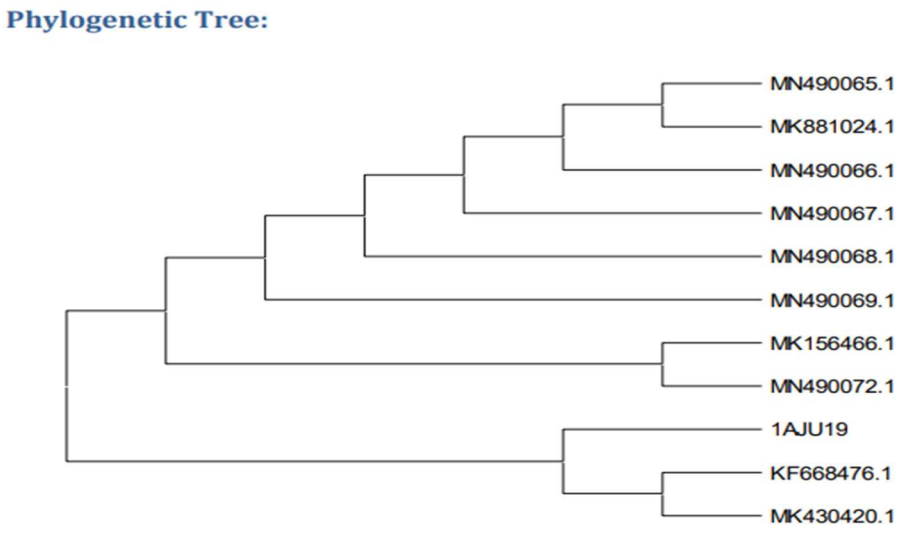
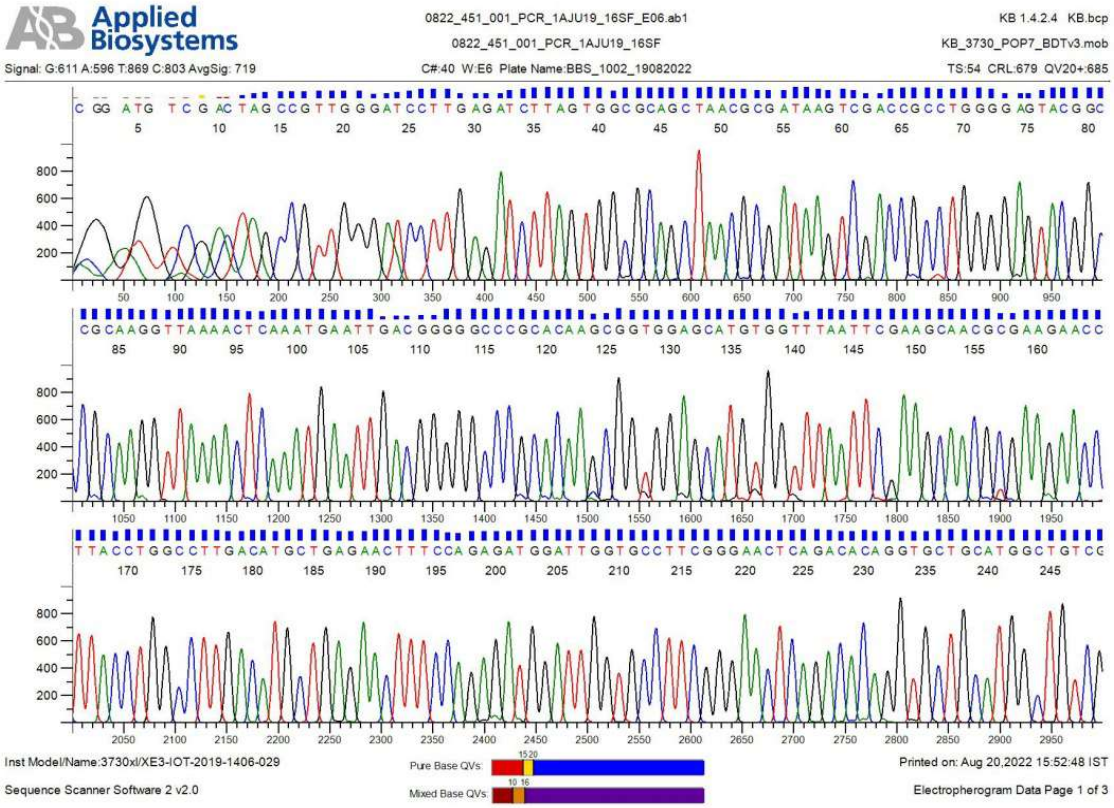


Fig 3(A) Phylogenetic tree showing the relationship of isolate 1AJU19 with closely related neighboring sp.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KB_3730_POP7_BDTv3.mob
TS:54 CRL:679 QV20+:685
Signal: G:611 A:596 T:869 C:803 AvgSig: 719
Inst Model/Name:3730xl/XE3-IOT-2019-1406-029
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Pure Base QVs:
Mixed Base QVs:
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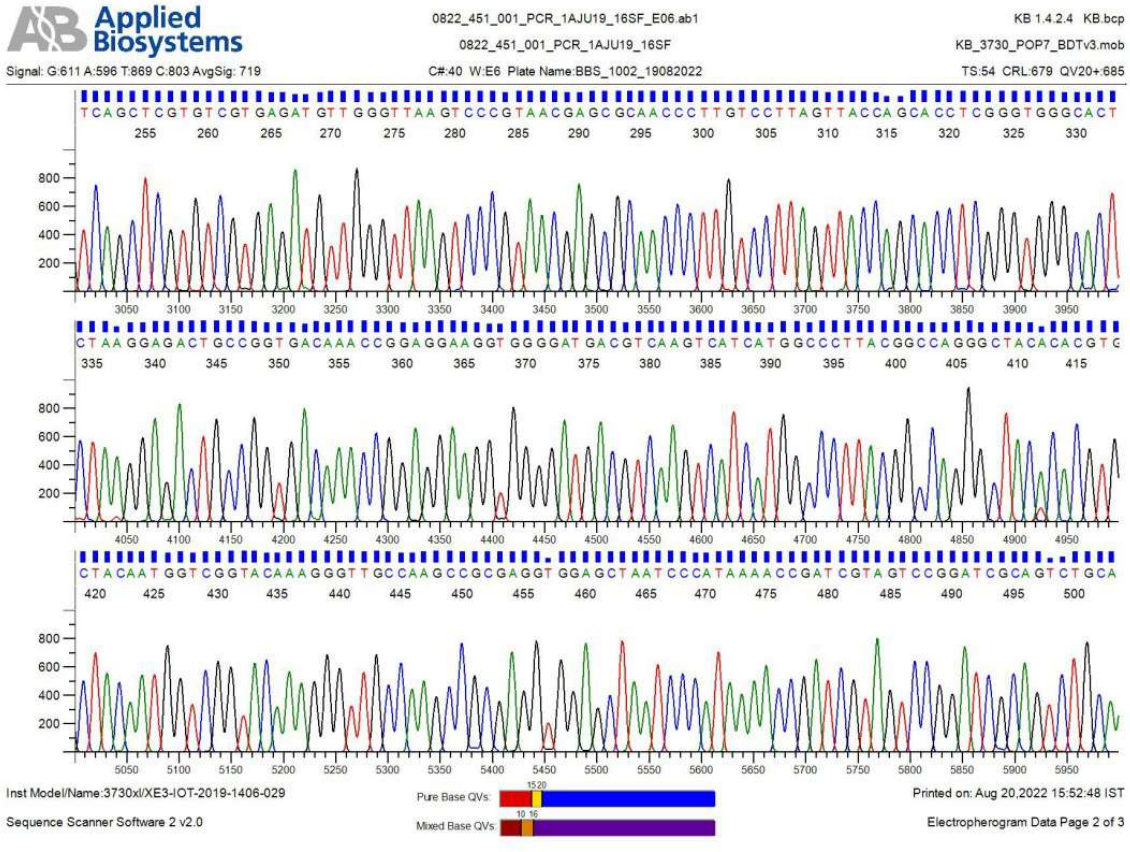
Electropherogram Data Page 1 of 3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Signal: G:611 A:596 T:869 C:803 AvgSig: 719
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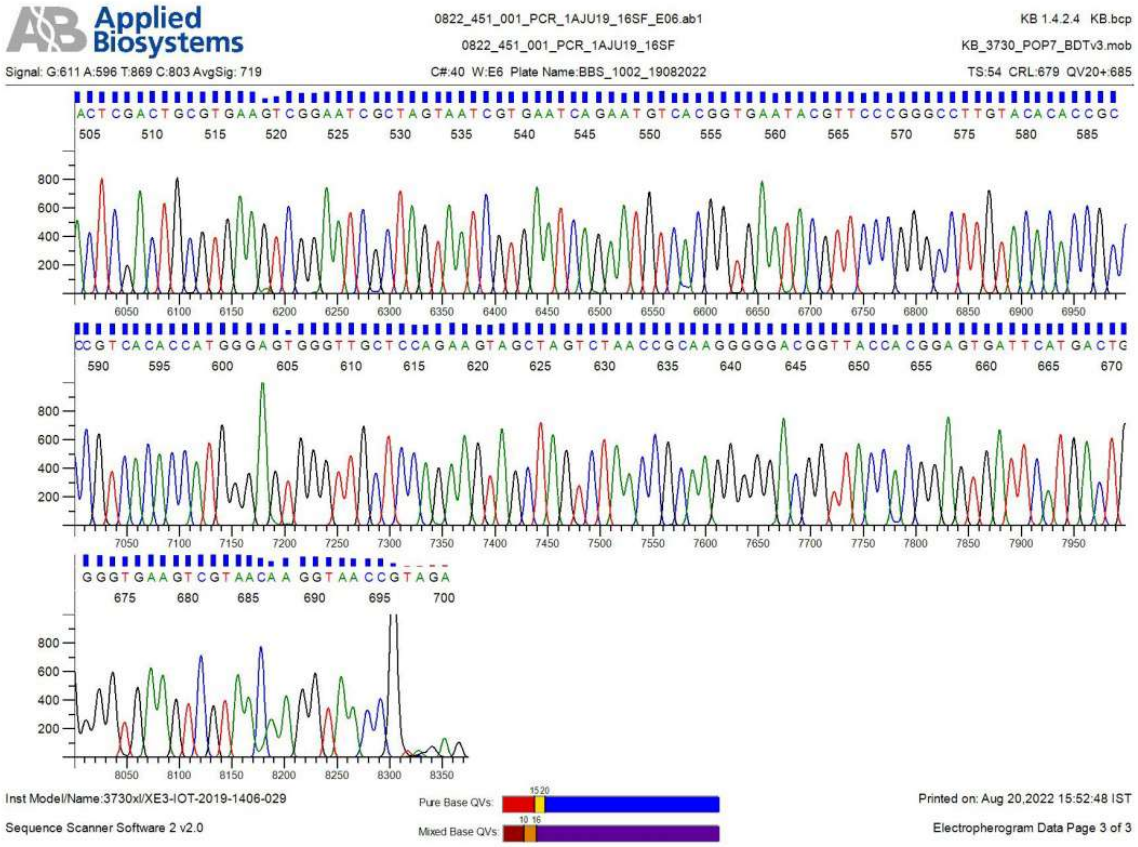

Fig 3 (B) Chromatogram data sequence

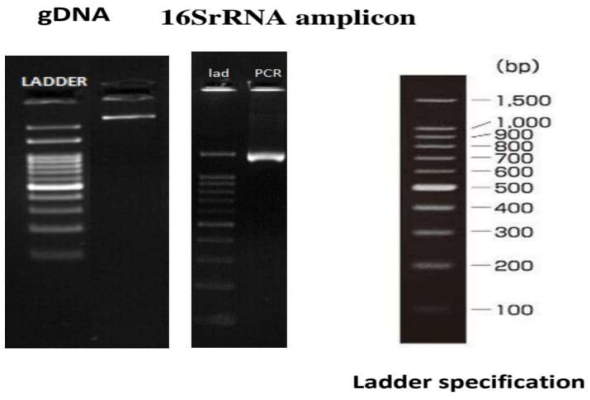

Fig 3(C) Gdna and 16S Amplicon QC data

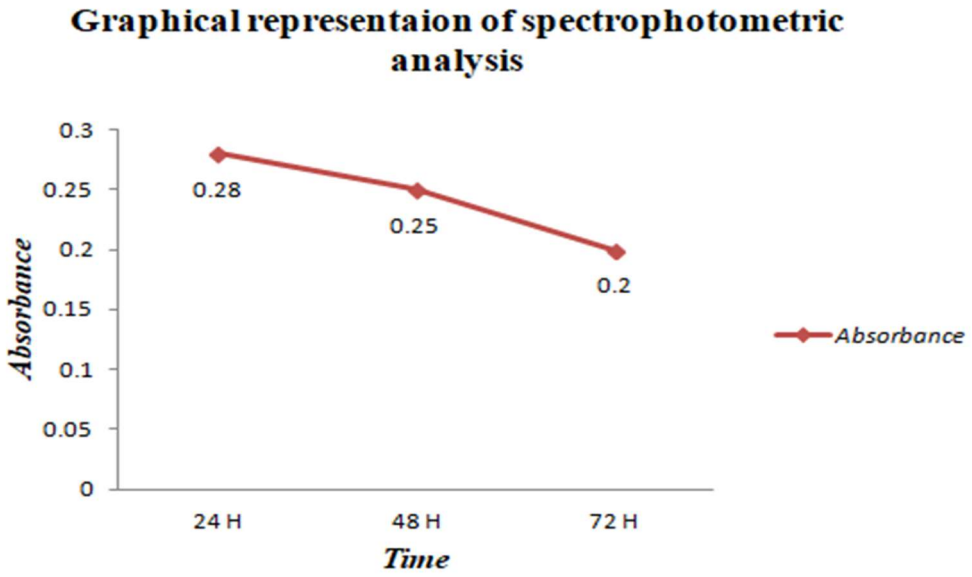

Fig 3 (D). Reduction of Cr (VI) by the isolate in sewage effluents after 24, 48, and 72h of incubation at 37°C.

5. CONCLUSION

The potentiality of chromium-resistant microorganisms in bioremediation of polluted sewage sites was evidenced in this experiment. A case study was reported with the description of bacterial strain isolated from samples contaminated by the metal and tests of Cr(VI) reduction was included. The isolated bacterial strain showed resistance up to 150ppm Cr conc and phylogenetic analysis of 16S RNA gene assigned them to the Domain: Bacteria, Phylum: Proteobacteria, Class: Gammaproteobacteria, Order: Pseudomonadales, Family: Pseudomonadaceae, Genus: Pseudomonas, Species: aeruginosa. These results suggest the use Cr(VI) resistant isolated bacterial strain for possible biodegradation process on contaminated sites.

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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 stake holder 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 affectd group(12%).[13]

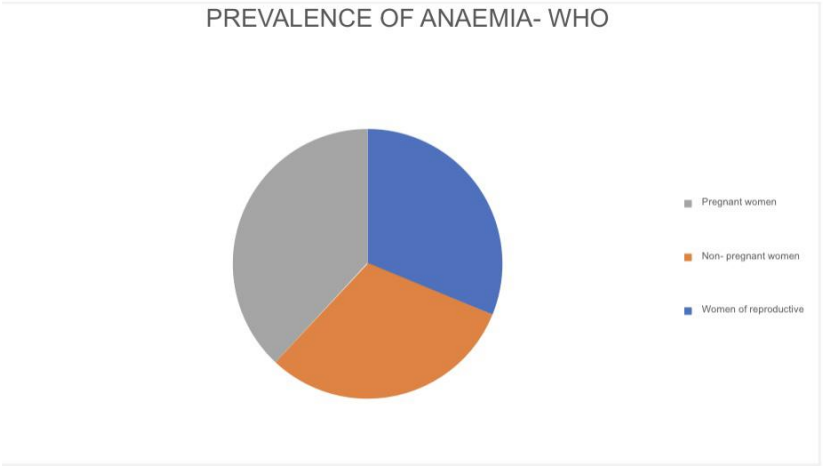


Figure-1

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]

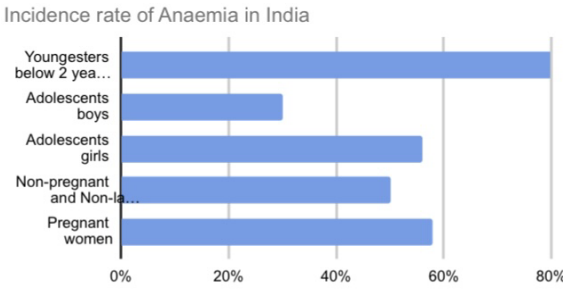


Figure-2

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-ratehazard to maternal and baby survival, contributes to low delivery weight, diminished resistance if infection, poor cognitive improvement and reducedwork productivity. The magnitude of anaemia collectively with the relateddetrimentalfitness, 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 healthproblemover the last few years. According to N Milman,"The maximumcommonnutrition deficiency in eachgrowingin addition to in advancedcountries is iron deficiency"[19]

Adolescence is a transitional duration from childhood to adulthood, for the duration of which positivehealthissues and change behaviour establishedamongstchildrenmay alsohave an impact on their futurefitness. According to WHO, Adolescent (early life) is describedas theperiodamong 10 years and 19 years. Adolescent of each sexes are in particularprone togrowing anaemia due tofast growth, in girls and womenmoreoverdue 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 longeronly in India howeverall over the globe. It has been predicted that, among 2010 and 2050 approximately 14.2 million toddlersmight 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, hemotocrit ,or number ofred blood cells per to litre beneathneath the reference interval forhealthfulpeopleof comparable age ,sex, and race, belowcomparable environmental situations According to the WHO , for belowfive years kids, the edge Hb stage for being anaemic is much less than 11.0g/dl .Anaemia is a common inmedicalcircumstancecharacterisedvia way of means ofreduced Hb rangesthat areinadequate for the body's demand .Anaemia proven to be a public health problem that influences low,middle,and high incomecountries at different ranges and incidence varies with socioeconomic status. The maximumdependable indicator of anaemia on thepopulation stage is blood haemoglobin concentration. Anaemia as a consequence of iron deficiency has been pronounced to well increase morbidity and mortality in preschool-elderlykids and pregnant women. numerouselementsmake contributions to the prevalence of anaemia and almost1/2 of of 43% the anaemia instances in formative years are because of iron deficiency. The deficiency may alsoend result from insufficientnutritionalconsumption of iron,malabsorption of iron multiplied iron demandfor the duration ofrapid growth in kids and persistent blood loss. differentreasons of anaemia consist of folate,vitaminB12 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]

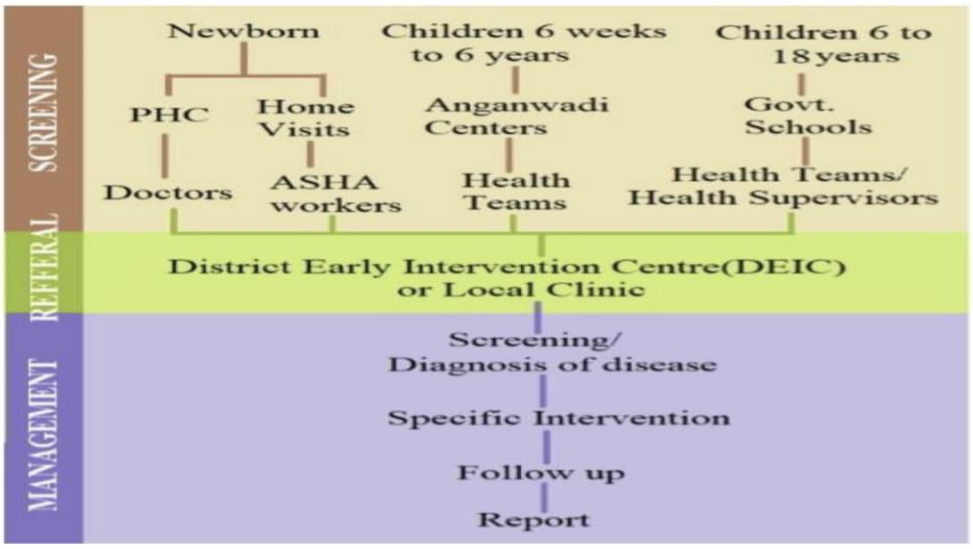
Certain key programmes are initiated by the Government of India to eradicate the prevalence of anaemia in pregnant and lactating women under the ICDS Scheme of MWCD (Ministry of women and child development) by providing supplementary nutrition and for childrens the provision of Iron and Folic acid (IFA) 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 ladoo, 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 Mukt 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 Mukt 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 Mukt 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 lookafter 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.

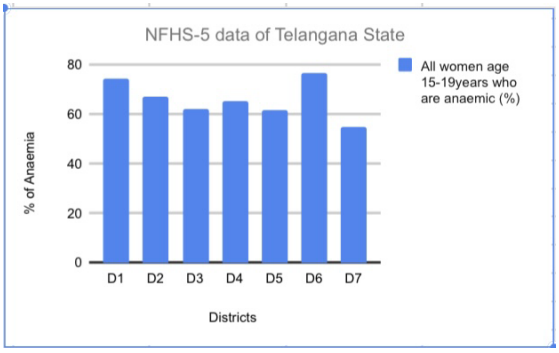


Figure-3

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 prespective 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 ladoo, 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 anaemicProvision of healthy and nutritious diet to the students

- Interventing 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
- lookafter 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 offsprings 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 inconsent by parents

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Elucidation of Antibacterial and Antioxidant Activities of Sesuvium portulacastrum leaf extracts

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Abstract:

Mangroves have evolved several physiological and morphological changes over the course of time to withstand the harsh environmental conditions of the coastal regions. They provide a safe niche for a uniquely diverse flora and fauna. The evolutionary adaptation to harsh climate has provided mangroves with novel secondary metabolites and bioactive compounds which have found to be beneficial for human consumption. These bioactive compounds found in the Mangroves can also curb the growth of bacteria. *Sesuvium portulacastrum*, is a Mangrove, commonly called as the "sea purslane" which has been found to contain Antioxidant and Antibacterial properties. In the present study, crude extracts of *S. portulacastrum* have been made from Acetone, Diethyl ether, Petroleum ether and water. Acetone crude extract was found to exhibit phytochemicals such as alkaloids, saponins, flavonoids, proteins and Anthraquinones. Diethyl ether crude extract has shown the presence of Tannins, Saponins, Phenols and Proteins. Aqueous extract of *S. portulacastrum* has shown the presence of most of the phytochemicals such as alkaloids, tannins, saponins, glycosides, steroids, phenols, anthraquinones and reducing sugars, on the other hand, petroleum ether crude extract had shown the presence of only two phytochemicals *viz.*, glycosides and steroids. Based on the phytochemical screening, the Antibacterial test was assayed through agar well diffusion method using the aqueous extract of *S. portulacastrum* against *Bacillus thuringiensis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. It can be observed from the experiment that the maximum Zone of Inhibition was noted against *Klebsiella pneumoniae* (1.2±0.4 mm) and the minimum Zone of Inhibition was observed against *Bacillus thuringiensis* (0.5±0.02 mm). Similarly assays like DPPH and FRAP of the aqueous leaf extract have shown that *S. portulacastrum* has significant Antioxidant properties as well. The IC_{50} values of DPPH were noted to be 289.92 µl/ml and the FRAP assay has shown an increase in the reduction power with a range between 9.33 ± 0.11 µg/ml at 15.62 µg/ml concentration and 44.16 ±0.21 µg/ml at 250 µg/ml concentration.

Key Words: Mangroves, Antibacterial activity, Phytochemicals, *Sesuvium portulacastrum*

Introduction:

The world is inundated with a vast biodiversity, of which one unique ecosystem is the Mangrove ecosystem. The mangroves have evolved evolutionarily in such a way that several physiological and morphological adaptations help them survive in the harsh climes of the coastal regions. Globally, the mangroves are present in 123 countries and territories, with about 40% of the mangrove cover which is found along the coasts of South and Southeast Asia. India contributes to 3% of the total mangrove cover in South Asia. The area of interest for the present study is in Andhra Pradesh state, which currently has 404 sq. km of mangrove cover, of which Krishna District has 137 sq. km. [8, 28].

There is a need to conserve the mangrove forests as they has the evolutionary advantage of having adaptive features which are endowed with novel bioactive compounds and secondary metabolites which help them sustain in harsh conditions. Metabolites like alkaloids, terpenoids, saponins, phenolics, steroids and flavonoids are found in plants and they provide them with the ability to prevent the growth of unrequired bacteria, fungi and other parasites, they also show sensitivity towards heavy metals which are found in the soil etc. The bioactive compounds are a rich source of therapeutic precursors and also serve as industrial raw materials [22].

World is constantly plagued with an array of infectious diseases which is the leading cause behind premature deaths. Most of the notorious infectious diseases are caused by the bacteria. It has become a challenge for the scientists and pharmacists all over the world to design new therapeutic drugs against superbugs like *Candida auris*, *Clostridiodes difficile*, *Neisseia gonorrheae* etc., which have become resistant to most of the synthetic drugs produced in the world. The need of the hour is to explore the antibacterial potential of mangrove medicinal plants and to exploit them for large-scale drug production [23].

Several independent studies conducted on the mangrove plants have proven their efficacy in treating bacterial, fungal and other microbial infections. Amongst them, *Sesuvium portulacastrum* L., has proven to be an excellent antibacterial and antioxidant agent. Traditionally, *S. portulacastrum*, commonly known as the "sea purslane" is consumed by the native people as a salty, leafy vegetable. It is also used to treat various diseases and ailments such as epilepsy, scurvy, conjunctivitis, leprosy, haematuria, liver or kidney disorders etc. The GC-MS analysis of the methanolic extracts of *S. portulacastrum* has shown the presence of chemical constituents such as pyrrole derivatives, butanoic acid, ascorbic acid, octadecanoic acid and hentriacontane which act as antioxidant, antimicrobial, antiulcerogenic and anticancer agents [3]. Alshrari *et al.,* [2] stated that the methanolic and chloroform extracts of the leaves and stem of *S. portulacastrum* were efficacious in inhibiting the growth of *E. coli, P. vulgaris, S. aureus* and *K. pneumoniae*. The hexane concentrate of *S. portulcastrum* was effective against bacterial strains such as *P. aeruginosa,* and *E. coli* in comparison to ethyl acetate and methanolic extracts [14]. The aim of the present study is to explore the antibacterial as well as antioxidant properties of the leaf extracts of *Sesuvium portulcastrum*.

Materials and methods

Collection of Plants and Extraction:

Leaves of *S. portulacastrum* were collected from Gilakaladindi mangroves (16°0' N latitude and 81°10'E longitude) located in Andhra Pradesh, India. The source was identified at Botanical Survey of India (BSI), Hyderabad. Leaves of the plant were cleaned, shade dried and pulverized into fine powder using pestle and mortar. The powdered plant material was extracted by using acetone, diethyl ether, petroleumether and water as solvents by using Soxhlet apparatus. The extracts obtained were filtered using Whatman No. 1 filter paper and they were stored in a refrigerator for use in subsequent experiments.

Phytochemical screening

Screening for the phytochemicals of the aqueous extract of *S. portulacastrum* was carried out to detect the presence of alkaloids, saponins, glycosides, fats, phenols, tannins, flavonoids, terpenoids, steroids, phytosterols, anthraquinones, cardiac glycosides, reducing sugars and proteins using standard methods [12].

***In-vitro* antibacterial activity**

To determine the antibacterial activity of aqueous extract of *S. portulacastrum* against pathogens like *Staphylococcus aureus* (MTCC737), *Pseudomonas aeruginosa*, (MTCC 1688), *Bacillus thuringiensis* (MTCC 1953) and *Klebsiella pneumoniae* (MTCC 3384), agar well diffusion method was used. Mueller-Hinton Agar medium (MHA) was poured carefully into the petriplates and by using an inoculum with a size of 106 colony forming units (c.f.u)/ml of bacteria [24]. These wells were made in MHA plates using a cork- borer of 8mm. Then, 40 µl of extract was loaded into the wells and amikacin was used as a positive control. The prepared petriplates were incubated at 37ºC for 12 h to initiate the growth of the bacteria. After incubation, zone of inhibition diameter was observed and the wells made in the petriplate were measured and tabulated.

***In-vitro* antioxidant activity**

The antioxidant activity of aqueous extract of *S. portulacastrum* was analysed through DPPH radical scavenging assay and FRAP assay.

DPPH radical scavenging activity

DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging capacity of the aqueous extracts of *S. portulacastrum* was determined and compared with a standard ascorbic acid as positive control and methanol are used as standard and blank [19]. DPPH solution at different concentrations was mixed with leaf extract in 1:1 ratio and incubated for 30 mins and the observations were recorded using spectrophotometer at 517 nm.

The DPPH radical scavenging activity was determined by the following equation:

$$\%\ \text{DPPH radical scavenging activity} = \left[\frac{(A0 - A1)}{A0}\right] \times 100$$

Ferric reducing antioxidant power (FRAP) assay:

FRAP assay was conducted in accordance to the method of Benzie and Strain [5]. The reagent for the experiment was prepared in acetate buffer (300mm) by mixing 10mm 2,4,6-tri (2-pyridyl-s-triazine) (TPTZ) solution in 40mM HCl and 20mM $FeCl_3$ solution in the proportion of 10:1:1 (v/v), which isfollowed by incubation for 15 min at 37 °C for furtheruse. The sample extract and standard ascorbic acid at different concentrations 15.62, 31.25, 62.5, 125, 250 and 500µl/ml are added to FRAP reagent. The absorbance was measured at 593 nm and the results were recorded as µg of ascorbic acid equivalents (AAE) per ml.

Results and Discussion

Phyto-chemical screening

Qualitative estimation of *S. portulacastrum* leaf extracts were analysed for the presence of phytochemicals and the results were **tabulated (1).** The analysis revealed that majority of phytochemicals like alkaloids, tannins, saponins, glycosides, steroids, phenols, anthraquinones and reducing sugars are found to be present in aqueous extract and other solvent extracts like acetone, diethyl ether and petroleum ether showed positive results only for Saponins and Proteins and negative for all other phytoconstituents. In comparison, acetone extract has shown the presence of

alkaloids, saponins, flavonoids, proteins and anthraquinones; Diethyl ether exhibited the presence of tannins, saponins, phenols and proteins. Petroleum ether tested negative for almost all the phytochemicals except glycosides and steroids.

S. portulacastrum has a rich source of phytochemicals like the 22,23-Dihydrostigmasterol, 3,4,5-trihydroxy-(Gallic acid), Benzoic acid, (2R, 3R) -(-)-Epicatechin and also Capsaicin in the ethanolic leaf and stem extracts which were responsible for their antimicrobial nature which was reported by Amad Al-Azzawi *et al.*[1]. Chintalapani *et al*. [9] reported the presence of alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, proteins, saponins, sterols, tannins, terpenoids, quinones, diterpenes in ethanol, methanol, acetone and diethyl ether extracts of *S. portulacastrum.* The phytochemicals such as the saponins, tannins, alkaloids, terpenoids and steroids were observed in the methanolic leaf extracts of *Suaeda maritima* [15]. Three mangrove species viz., *Avicennia schaueriana, Rhizophora mangle* and *Laguncularia racemosa* leaf extracts have shown the presence of phytochemicals like alkaloids, saponins, tannins, steroids, coumarins, and triterpenoids. These phytochemicals were analysed to be incredibly helpful in controlling the parasitosis of the intermediate host; *Biomphalaria glabrata* in the lifecycle of the parasite *Schistosoma mansoni*, which is the causative organism of Schistosomiasis disease. The hydroalcoholic extracts of these mangroves were proven to be effective molluscicides as they acted negatively on the biological activities of the host snails [20]. The phytochemical screening of medicinal plants like *Solanum virgianum* and *Physalis angulate* were revealed the presence of phytochemical compounds like phenols, flavonoids, saponins and glycosides [26]. Usually the phytochemicals are of high medicinal importance as they possess anti-bacterial, anti-fungal, astringent, analgesic, anti-viral, molluscicidal, immune boosting, anti-tumor, anti-malraial, ant-parasitic, anti-inflammatory, anti-oxidant properties [13, 10]. The present study revealed the presence of highest number of phytochemicals in the aqueous extract of *S. portulacastrum* indicating the efficacy of aqueous solvent over other solvents for phytochemical extraction. Extracting compounds in the present study as compared to all other extracts, with acetone, diethyl ether and petroleum ether shown very poor extractability of phytochemicals.

***In vitro* antibacterial activity**

Based on the results of phytochemical screening, the aqueous leaf extract of *S. portulacastrum* was selected for the assessment of *in vitro* antibacterial activity as it extracted a greater number of phytochemicals. The aqueous leaf extract of *S. portulacastrum* was selected for antibacterial activity as it possesses a high number of phytochemicals as their positive results exhibited in phytochemical screening. The assay was carried out *in vitro* by following the protocol for agar well diffusion method against *Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*. As shown in **Table (2),** the highest zones of inhibition were observed against the *Klebsiella pneumoniae* followed by *Staphylococcus aureus*, then in *Pseudomonas aeruginosa* and the lowest zone of inhibition is seen in the

Bacillus thuringiensis. All the tested strains exhibited the zones of inhibition ranging from 0.5 to 1.2 mm. Mangrove species are most significant in exhibiting antibacterial activity, earlier studies on methanolic leaf extract of a marine mangrove plant *Avicennia marina* have shown the highest antimicrobial activity against *Pseudomonas aeruginosa* [29]. Kolli *et al*. [16] reported the antibacterial activity of methanol, ethanol and hexane extracts of *S. portulacastrum* against *staphylococcus aureus, Streptococcus pyogenes, Escherechia coli* and *Pseudomonas aeruginosa*. *Suaeda nudiflora* crude extracts made with different solvents like hexane, methanol, chloroform and ethyl acetate have shown a higher degree of inhibition zones against a selected group of bacterial strains such as four Gram +ve bacteria *viz., Bacillus subtilis, S. aureus, S. haemolycticus* and *Enterococcus faecalis* and five Gram -ve bacteria such as *Citrobacter sp., Pseudomonas sp., E. coli, Klebsiella pneumoniae* and *Stenotrophomonas maltophila*. Similarly, Divya *et al.* [11] reported that the acetone and diethyl ether extracts of *A. marina* has highest inhibitory activity against *K. pneumoniae*, *S. aureus* and *P. aeruginosa.*

***In vitro* antioxidant activity**

The DPPH radical scavenging activity and FRAP assays were used to test the antioxidant activity of the aqueous crude leaf extracts of *S. portulacastrum*. As shown in **Table (3),** percentage of DPPH radical scavenging activity at various concentrations ranging from 31.25 to 500 µg/ml was determined along with 50% inhibitory concentration of extract. DPPH is a synthetic free radical mostly used in the experiments to find the radical scavenging activity of antioxidant compounds in the tested extract. The results in the present study revealed significant free radical scavenging activity on DPPH with IC50 value of 289.92 µg/ml. In previous studies, it was reported that the ethanol leaf extract of *Rhizophora mucronata* has shown antioxidant activity and the free radical scavenging activity of 127.5 µg/mL [25]. A study conducted by Sofia and Teresa [27] determined the DPPH activity in leaf, stem and root samples of *E. agallocha*. The leaf extracts have shown maximum IC_{50} at 141.56µg/ml in the methanolic extract of the leaves and the extracts from the stem showed minimum IC_{50} at 931.3µg/ml. The methanol leaf extract of *Avicennia marina* having phenolic compound namely gallic acid was reported for high antioxidant activity using DPPH assay. Similar studies reported on antioxidant activity in *Acanthus ilicifolius* petroleum ether extract exhibited highest activity of 116.75µg/mL [6].

Antioxidants plays an important role in maintaining the oxidative resistance .According to the results, as shown in **Table (4)**, the reducing potential of aqueous leaf extract of *S. portulacastrun* was expressed in ascorbic acid equivalent. The reducing power gradually increased with increase in concentration just as exhibited in DPPH radical scavenging assay with an AAE value of 44.16µg/ml. From the results, the tested leaf extract exhibited moderate reducing activity. The reducing power of leaf extracts could be estimated from their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe(II) [7]. Similar studies of Kuthi and Basar [18] reported in the methanol extracts of *Pellacalyx axillaris* provides significant reducing power with equivalent values at

varying IC50 concentrations (1.0mm, 2.69 and 2.97 ug ml).

Conclusion:

With the advent of modern medicine, it has become possible to find a cure for improbable diseases. However, synthetic antimicrobial or antibacterial drugs are now falling short of curing any microbial or bacterial infections owing the growing immunity against these drugs. It has hence, become the need of the hour to use natural medicines or extract effective bioactive compounds from plant species to combat the increase in drug resistance seen in bacteria. It was proven, on numerous occasions that, Mangroves have an abundance of bioactive compounds which are rich in antibacterial and antioxidant properties. *Sesuvium portulacastrum*, which is one such mangrove, also has shown the presence of bioactive compounds such as alkaloids, saponins, anthraquinones, tannins etc., which might prove beneficial. Significant reduction in bacterial growth of *Klebsiella pneumoniae* and other such bacteria will make this mangrove irreplaceable as a bactericidal drug. The DPPH and FRAP assays have also proven that *S. portulaastrum*, has significant antioxidant properties. Therefore, further studies and experimentation on this little explored mangrove might result in the discovery of a novel drug which can cure bacterial and other life-threatening diseases.

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Table 1: Phytochemical screening of *S. portulacastrum* leaf extracts

Name of test	**Acetone extract**	**Di-Ethyl ether extract**	**Petroleum Ether extract**	**Aqueous extract**
Alkaloids	+	-	-	+
Tannins	-	+	-	+
Saponins	+	+	-	+
Flavonoids	+	-	-	-
Glycosides	-	-	+	+
Terpenoids	-	-	-	-
Steroids	-	-	+	+
Phenols	-	+	-	+
Proteins	+	+	-	-
Anthraquinones	+	-	-	+

Reducing Sugars	-	-	-	+

'+' indicates presence and '-'indicates absence

Table 2: The antibacterial activity of the aqueous leaf extract of *S. portulacastrum*

Test organism	Zone of inhibition (mm)
Bacillus thuringenesis	0.5±0.02
Staphylococcus aureus	0.8±0.6
Klebsiella pneumonia	1.2±0.4
Pseudomonas aeruginosa	0.7±0.08

Results were represented as mean ± standard deviation; n=3.

Table 3: Percentage of DPPH radical scavenging activity of *S. portulacstrum aqueous* leaf extract.

Concentration (µg/ml)	Test extract
31.25	4.08 ±0.1
62.5	19.0 ±0.6
125	38.1 ±0.9
250	48.2 ±0.5
500	78.4±0.5
IC50	**289.92µl/ml**

Results are represented as mean± standard deviation; n=3

Table 4: Ferric reducing antioxidant power ability of *S. portulacstrum aqueous* leaf extract

Concentration (µg/ml)	Aqueous extract
15.62	9.33 ±0.11
31.25	20.33 ±0.14
62.5	27.16 ±1.16
125	34.66 ±0.18
250	44.16 ±0.21

Results are represented as mean± standard deviation; n=3

Research Article

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Purification and characterisation of phytochemicals extracted from *Rhizophora mucronata*: Their efficacy against *Pseudomonas aeruginosa* infection in *Catla catla*

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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 ± 0.1, 1.1 ± 0.3, 0.9 ± 0.1, and 1.2 ± 0.7 mm), *Staphylococcus aureus* (1.2 ± 0.1, 2.3 ± 0.5, 3.2 ± 1.2, and 4.4 ± 1.7 mm), *Klebsiella pneumoniae* (0.5 ± 0.3, 1.7 ± 0.4, 3.9 ± 1.4, and 3.9 ± 0.9 mm), and *Pseudomonas aeruginosa* (0.2 ± 0.1, 2.5 ± 0.6, 3.7 ± 0.5, and 4.2 ± 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% ± 0.5%) compared to remaining extracts (73.77% ± 2.5%, 69.99% ± 0.4%, and 65.24% ± 1.8%) at a concentration of 500 $\mu g \cdot mL^{-1}$. 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 ± 2.2 mm and 80.9% ± 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 $\mu g \cdot mL^{-1}$) 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 $\mu g \cdot mL^{-1}$). 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:

$$\% \text{ DPPH radical scavenging activity} = [(A_0 - A_1)/A_0] \times 100 \quad (1)$$

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 $FeCl_3$ 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 $\mu L \cdot mL^{-1}$) 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 $\mu g \cdot mL^{-1}$ [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 µm 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 µL 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 $0.5\ mL \cdot min^{-1}$. The electrospray 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 $8–10\ L \cdot min^{-1}$ [12].

2.8 *In vivo* studies

2.8.1 Experimental design and confirmatory tests for *P. aeruginosa*

Catla catla fingerlings of 8 ± 0.5 cm length and 10 ± 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°C ± 2°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* ($25\ mg \cdot g^{-1}$ of body weight), and partially purified extract of *R. mucronata* ($10\ mg \cdot g^{-1}$ 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 ± 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) 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.

Table 1: Phytochemical screening of various solvent extracts of *Rhizophora mucronata*

Phytochemicals	*Rhizophora mucronata*			
	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.

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 ± 0.1 to 1.2 ± 0.1 mm, whereas DEE was measured in the range from 1.1 ± 0.3 to 2.5 ± 0.6 mm. Similarly, the bactericidal activity of EE ranged from 0.9 ± 0.1 to 3.9 ± 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 ± 1.7 mm), *Bacillus subtilis* (1.2 ± 0.7 mm), *Klebsiella pneumoniae* (3.9 ± 0.9 mm), and *Pseudomonas aeruginosa* (4.2 ± 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*

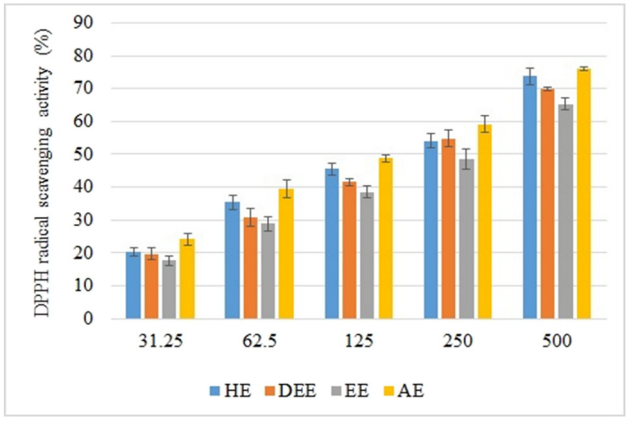
Using the DPPH radical scavenging and FRAP tests, the antioxidant activities of all four *R. mucronata* leaf extracts were studied at varied dosages. The DPPH radical scavenging activity was measured at concentrations ranging from 31.25 to 500 $\mu g \cdot mL^{-1}$. The ability to scavenge radicals improves 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 $\mu g \cdot mL^{-1}$.

FRAP assay for *R. mucronata* extracts has revealed excellent antioxidant potential ranging from 81.25 ± 1.2

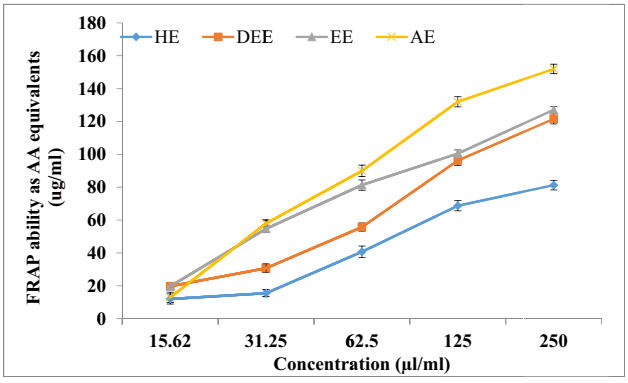
Table 2: Antibacterial activity (mm) of crude *R. mucronata* leaf extract

Test organism	Amikacin	HE (mm)	DEE (mm)	EE (mm)	AE (mm)
Bacillus subtilis	1.9 ± 0.6	0.4 ± 0.1	1.1 ± 0.3	0.9 ± 0.1	1.2 ± 0.7
Staphylococcus aureus	5.1 ± 0.4	1.2 ± 0.1	2.3 ± 0.5	3.2 ± 1.2	4.4 ± 1.7
Klebsiella pneumonia	4.6 ± 0.01	0.5 ± 0.3	1.7 ± 0.4	3.9 ± 1.4	3.9 ± 0.9
Pseudomonas aeruginosa	4.3 ± 0.9	0.2 ± 0.1	2.5 ± 0.6	3.7 ± 0.5	4.2 ± 1.3

Results are expressed as mean value ± standard deviation; $n = 3$.



(a)



(b)

Figure 1: DPPH radical scavenging activity (a) and ferric reducing antioxidant power ability (b) of *R. mucronata* leaf extracts.

to 152.3 ± 2.3 µg of ascorbic acid equivalents (AAE) per mL at 250 µL·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 µL·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 ± 2.1 µg AAE per mL of conversion. Whereas at the same concentration, DEE and EE were able to reduce 19.75 ± 2.3 µg AAE per mL. But at high concentration, i.e., 250 µL·mL^{-1} concentration these recorded lower value than AE.

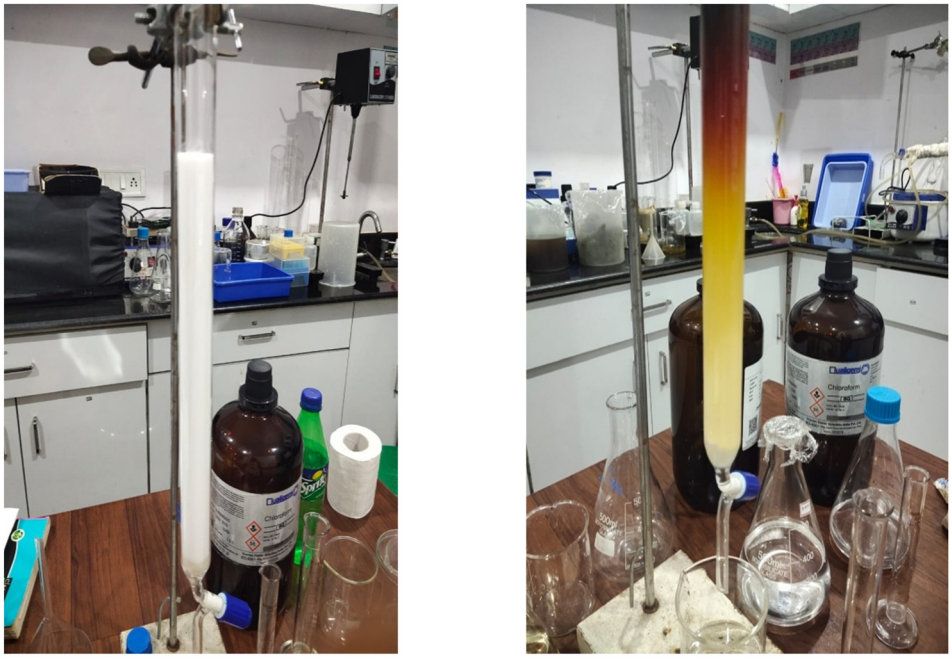
3.4 Purification of AE of *R. mucronata*

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 polled 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 ± 2.2 mm and 80.9% ± 2.3%, respectively. Comparatively, AR1 showed activities increased threefold than the other fractions.



(a) (b)

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*-caffeoyltryramine (*m/z* 120.87), quercetin 3-*O*-*β*-D-glucoside (*m/z* 180.89), angelicoidenol-2-*O*-*β*-D-apiofuranosyl-(1 → 6)-*β*-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*-*β*-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 (%)
n-Hexane:chloroform (100:0)	1–4	A	White	—	—
n-Hexane:chloroform (90:10)	5	B	Green	2.6 ± 0.5	
n-Hexane:chloroform (60:40)	6–7	C	Light green	—	—
n-Hexane:chloroform (40:60)	8–10	D	Orange	—	51.6 ± 2.8
n-Hexane:chloroform (20:80)	11–12	E	Pale orange	3.1 ± 0.9	—
n-Hexane:chloroform (0:100)	13–15	F	Yellowish orange	—	23.8 ± 3.1
Chloroform:methanol (80:20)	16–18	G	Yellow	7.5 ± 1.3	67.9 ± 2.4
Chloroform:methanol (50:50)	19–22	H	Dark yellow	1.8 ± 0.7	47.9 ± 1.7
Chloroform:methanol (0:100)	23–26	I	Honey	—	11.5 ± 0.9

Results expressed as mean value ± 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	*P. aeruginosa* (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 multidrug 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*

m/z	Compounds in crude extract	Compounds in partially purified extract	Structure
148.89	*N*-*p*-Coumaroyltyramine	*N*-*p*-Coumaroyltyramine	$C_{17}H_{17}NO_3$
120.87	*N*-trans-caffeoyltryramine	—	$C_{17}H_{17}NO_4$
180.89	Quercetin 3-*O*-*β*-D-glucoside	—	$C_{21}H_{19}O_{12}$
355.08	Angelicoidenol-2-*O*-*β*-D-apiofuranosyl-(1 → 6)-*β*-D-glucopyranoside	—	$C_{16}H_{28}O_7$
237.05	Lotusine	—	$C_{19}H_{24}NO_3$
429.12	Kaempferol-*O*-hexose-*O*-deoxyhexose	—	$C_{27}H_{30}O_{16}$
132.93	Luteolin	Luteolin	$C_{15}H_{10}O_6$
149.90	Syringaresinol-4-*O*-*β*-D-glucopyranoside	—	$C_{28}H_{36}O_{13}$
150.98	Taxifolin	—	$C_{15}H_{12}O_7$
298.97	Hispidulin	—	$C_{16}H_{12}O_6$
783.70	Ellagitannin	—	$C_{44}H_{32}O_{27}$

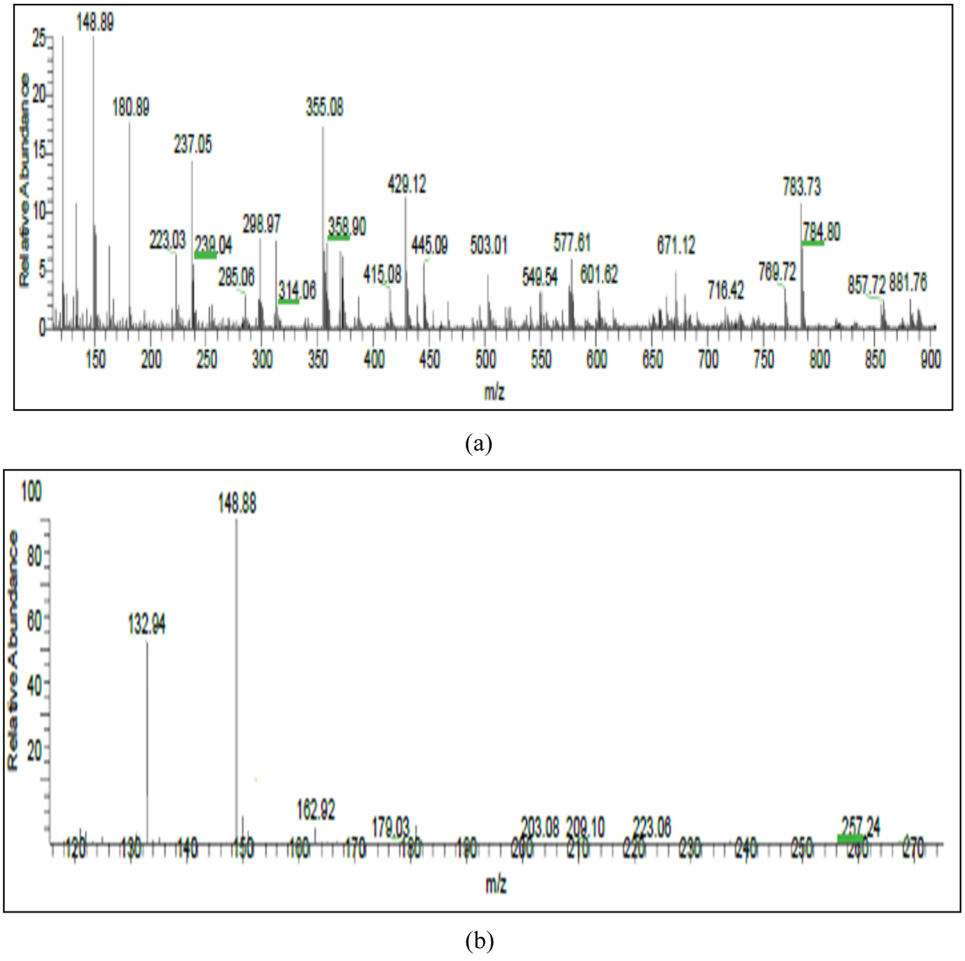


Figure 3: Phytochemicals profiling of (a) crude and (b) partially purified leaf extracts of *R. mucronata*.

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*

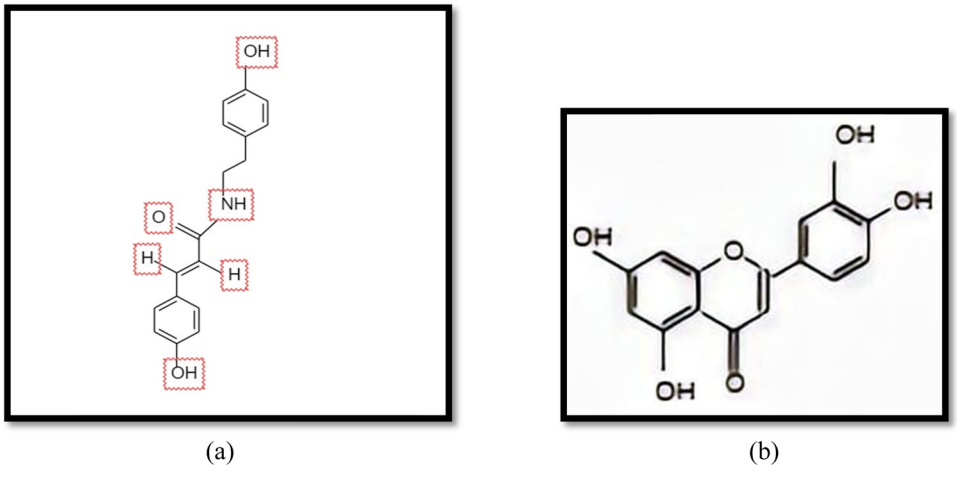


Figure 4: Structures of (a) *N-p*-coumaroyltyramine and (b) luteolin.

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 $g{\cdot}mL^{-1}$, 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 $g{\cdot}mL^{-1}$. 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 $\mu g{\cdot}mL^{-1}$ 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^{3+}) to ferrous (Fe^{2+}) and the reaction is supported with 2,4,6-tripyridyl-*s*-triazine [11]. The experimental solution turns into violet-blue colour upon Fe^{2+} 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 $\mu L{\cdot}mL^{-1}$ 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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The Impact of Human Capital Accounting on Organizational Performance

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Aim/purpose:- The aim of the present descriptive research study is to examine the impact of human resource accounting on organizational performance. The comparative assessment of valuation of human capital and employee-organizational performance is essential in the competitive scenario. The valuation of human capital include: the costs associated with recruitment and selection, performance appraisal, training and development, compensation, salaries and welfare facilities provided to the employees. **Outcome:-**The outcome of the research witnessed that there are many aspects which depends up on the HRA like: performance enhancement, stake holders decision, long-run survival, skill & knowledge enhancement are depends up on the valuation of human capital in the organization. **Research Design/Methodology/Approach:-** Developed a structured closed ended questionnaire to collect the opinion from various respondents with respect to opinion on HRA and its impact on organizational performance. Applied descriptive statistics which Include, Mean, SD and Analysis of Variance (ANOVA) to assess the variation in the opinion of respondents. **Generalizability:-** The outcome of the research can be generalized where need arises to assess the employee-organizational performance based on the valuation of human capital in the organization. **Novelty:-** The relationship between the human capital valuation and the employee-organizational performance assessment is novel in nature. **Type of the research:-** It is a descriptive research design.

Key words:- Human Resource Accounting, HRA, HR Accounting, Accounting, Human Resource Management, etc

Introduction:-

In earlier days companies are not trying to disclose the human resource related investments in the balance and profit and loss account of the statements. In fact, the productive investments on: land and buildings, plant and machinery, furniture and fixtures, raw-material purchase

will become unproductive investments without considering the investments on human resource management. Therefore, there is a strong association between, the investments on real assets and human assets. The financial analysts recognized the importance of HRA from last 50 years only and research on HRA started from 1960 onwards[1]. The American Accounting society defined the HRA identifying, assessing the data about the HRA and communicating the results to the management [2]. The HRA information will facilitates a lot especially to the stakeholders of the organization for effective decision-making [3]. The stake holders of the business concern like: investors, creditors, share-holders and other allied parties will have clear idea about the organizational performance. However, the real assets of the businesses requires reinvestments on productive assets, need to enhance the value of human resources by investing more by fulfilling the requirements of human resources such investments are related to recruitment, training and development costs[4]. The human resource information system explains, what happen regarding human resource investments over a period of time. It also includes the investments on human resources and associated performance [5]. Therefore, the database of human resource management which includes costs and productivity facilitates for further analysis which impact the short-run and long-run of the firm. In few organizations the human resource accounting confined to training and development only [6]. Therefore, a statutory body is essential to regulate the practices of human resource accounting in MNC's in the contemporary scenario.

Literature Review:-

The prosperity of the organization may depend up on the implementation of HRA practices in the organization. Stated that the negative affect of earnings in the share market is due to the view of incorrect balance sheet published by them in which the expenditure made by human asset has not been recorded clearly[7]. Developed various models to assess the HRA in the organizations [8]. The success rate of the company relies up on the company's annual report where disclosing the investments on human resource assets[9]. The organizational performance and profit of the firm relies up on the investments made up on human resources and shown on balance sheet of the company [10]. The enterprise performance and productivity the knowledge management of human resources at various levels of the organization which include: Top level, Middle level and lower level [11]. Therefore, the human resources of an organization treated as wealth of the organization. There is no regularity regarding the projection of human resource accounting details in the financial statements of the company [12]. The HRA will show many evidences like: the cost per employee, wealth created by investing on human resources, the ratio between salaries paid and the revenue generated and the profit created by the individuals in the organization are the various aspects can be studied by assessing the human resource accounting. The success rate of any industry relies up the quality of human resources possesses and its effective utilization. Need to treat the core human intellectuality as a core economic resource of an organization [13]. To evaluate the performances of human resources the human resource accounting is the best way of measurement. In fact, the outcome of the HRA will facilitates the management for effective decision-making [14]. In fact, there is no specific parameter to assess the value of human resources in the organization. Among, the various resources of an organization, the human resource assets are very much essential [15]. The Human resources occupies pivotal role in

every organization as each and every process of the organization controlled by the human resources [16]. Irrespective of the department like: Marketing, production, finance and logistics, in all segments of the business the human resources are very much essential and assessment of human resource valuation plays a crucial role. The related study the human resource accounting facilitates to evaluate the value of human resources and calculate the investments made on training and development of individual employees which is directly related to employee and organizational performance in the present context. A study on human capital accounting and financial statement of an organization have shown the significant positive relationship as higher the investments on human capital which leads higher financial performance of an organization [17]. As per the survey 98.4% of the respondents in a survey opined that, human resource accounting information should be capitalized and should be treated as asset of an organization rather showing in the profit and loss account of the organization [18]. The human resource accounting helps to optimize the value of human resources in the organization [19].

Objectives:-

1. To study the literature of human resource accounting and its impact on organizational performance.
2. To know the opinion of respondents with respect to HRA and its impact on organizational performance
3. To evaluate the opinions of the respondents with respect to demographical variables.

Need & Importance of the Study:-

The HRA is the best source for managerial decisions to achieve its organizational goals. Showing the human resource information system in the financial statements of the organization, will be helpful to predict the future course of actions [20]. The intellectual human capital is essential to achieve organizational goals and objectives in the contemporary context [21]. Therefore, it is evident from the literature that, human resource valuation is essential for effective decision-making and long-run survival of the firm. Even, the stakeholders of the organization should be fully aware of, the human resource information for long lasting relationship with the firm.

Scope of the Study:-

The present study aims to investigate the importance of human resource valuation with respect to organizational performance. The survey has been done with a sample size from various respondents. The research it is restricted to assess the opinion on HRA and its impact on organizational performance.

Statement of the Problem:-

The title entitled to "The Impact of Human Resource Accounting on Organizational Performance", which examines the impact of HRA on employee-organizational performance in the contemporary context.

Hypothesis:-

H_0(1): There is no significant association between the Capital Invested on Human Resources and employee-organizational performance with respect to educational qualification of the respondents.

H_0(2): There is no significant association between HRA details facilitates a lot to the external stake holders to take decision-making related to investments and to assess the value of a firm with respect to educational qualification of the respondents.

H_0(3): There is no significant association between the return on investments from productive expenses like: land and buildings, machinery and infrastructure will be possible only through investments on human capital with respect to educational qualification

H_0(4): There is no significant association between the Financial Position, performance of a company and survival in long-run and strengthening the skills of individuals will happen thro3h the investments made on human capital with respect to educational qualification of respondents.

Research Methodology & Design:-

Data Sources:- Taken the advantage of both primary and secondary data sources to assess the concept in all directions developed a structured closed ended questionnaire to collect opinion from various respondents and literature through published articles and reports.

Sampling Technique:- Applied the convenience sampling from the non-probability sampling technique. Collected data from the available respondents.

Sample Size:- Taken a sample of 50 samples from the various respondents which include both male and female and assessment has done based on educational qualification of respondents.

Statistical Techniques:- Applied the descriptive statistics which include: Chi-Square Assessment using Pearson chi-square test.

Sampling Unit/Frame:- The respondents include graduates and post graduates as they are competent to answer the questionnaire.

Data Analysis and Interpretation:-

Table 1: Capital Invested on Human Resources and employee-organizational performance (Vs) Educational Performance

Human Capital Accounting compares the capital invested on human resources and employee-organizational performance * Educational Qualification Cross tabulation			Educational Qualification		
			Under Graduates	Post Graduates	Total
Human Capital Accounting compares the capital invested on human resources and employee-organizational performance	Strongly Disagree	Count	1	6	7
		% of Total	2.0%	12.0%	14.0%
	Disagree	Count	0	2	2
		% of Total	0.0%	4.0%	4.0%
	Neither Agree nor Disagree	Count	2	6	8
		% of Total	4.0%	12.0%	16.0%
	Agree	Count	6	19	25
		% of Total	12.0%	38.0%	50.0%
	Strongly Agree	Count	3	5	8
		% of Total	6.0%	10.0%	16.0%
Total		Count	12	38	50
		% of Total	24.0%	76.0%	100.0%

Data Sources: Field Survey *In significant at 5%*

From the above table.1 reveals that out of 50 sample respondents the majority 25 sample respondents agreed that human capital accounting compares the capital invested on human resources and employee-organizational performance. Among these respondents the majority 19 (38%) of the respondents are belongs to post graduates and the rest 6(12%) of the respondents are belongs to under graduates followed by 16% of the respondents are strongly agreed that human capital accounting compares the capital invested on human resources and employee-organizational performance. Among these respondents the majority 5 (10%) of the respondents are belongs to post graduates and the rest 3(6%) of the respondents are belongs to under graduates Therefore, the majority 66% of the respondents agreed that human capital accounting plays a key role to assess the organizational and employee performance.

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	1.797[a]	4	.773
Likelihood Ratio	2.230	4	.694
Linear-by-Linear Association	1.154	1	.283
N of Valid Cases	50		

The above internal table reveals that there is no significant difference between educational qualification of respondents with respect to opinion on human capital accounting and employee-organizational performance. The p-value which is .773 is greater than .05. Therefore, the distribution is insignificant. Therefore, there is no significant difference between opinion on human capital accounting compares the capital invested on human resources and employee-organizational performance with respect to education qualification of the respondents.

Table 2: The external stake holders can take decision-making related to investments and to assess the value of a firm (Vs) educational Qualification

The HRA details facilitates a lot to the external stake holders to take decision-making related to investments and to assess the value of a firm (Vs) educational Qualification			Under Graduates	Post Graduates	Total
The HRA details facilitates a lot to the external stake holders to take decision-making related to investments and to assess the value of a firm	Strongly Disagree	Count	0	4	4
		% of Total	0.0%	8.0%	8.0%
	Disagree	Count	2	5	7
		% of Total	4.0%	10.0%	14.0%
	Neither Agree nor Disagree	Count	1	4	5
		% of Total	2.0%	8.0%	10.0%
	Agree	Count	6	18	24
		% of Total	12.0%	36.0%	48.0%
	Strongly Agree	Count	3	7	10
		% of Total	6.0%	14.0%	20.0%
Total		Count	12	38	50
		% of Total	24.0%	76.0%	100.0%

Data Sources: Field Survey *In significant at 5%*

From the above table.2 reveals that out of 50 sample respondents the majority 24 sample respondents agreed that The HRA details facilitates a lot to the external stake holders to take decision-making related to investments and to assess the value of a firm. Among these respondents the majority 18 (36%) of the respondents are belongs to post graduates and the rest 6(12%) of the respondents are belongs to under graduates followed by 20% of the respondents are strongly agreed that The HRA details facilitates a lot to the external stake holders to take decision-making related to investments and to assess the value of a firm. Among these respondents the majority 7(14%) of the respondents are belongs to post graduates and the rest 3(6%) of the respondents are belongs to under graduates Therefore, the majority 68% of the respondents agreed that The HRA details facilitates a lot to the external stake holders to take decision-making related to investments and to assess the value of a firm.

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	1.598[a]	4	.809
Likelihood Ratio	2.519	4	.641
Linear-by-Linear Association	.708	1	.400
N of Valid Cases	50		

The above internal table reveals that there is no significant difference between educational qualification of respondents with respect to opinion on The HRA details facilitates a lot to the external stake holders to take decision-making related to investments and to assess the value of a firm. The p-value which is .809 is greater than .05. Therefore, the distribution is insignificant. Therefore, there is no significant difference between opinion on The HRA details facilitates a lot to the external stake holders to take decision-making related to investments and to assess the value of a firm with respect to education qualification of the respondents.

Table 3: The return on investments from productive expenses like: land and buildings, machinery and infrastructure will be possible only through investments on human capital (vs) Educational Qualification

The return on investments from productive expenses like: land and buildings, machinery and infrastructure will be possible only through investments on human capital * Educational Qualification Cross tabulation			Educational Qualification		Total
			Under Graduates	Post Graduates	
The return on investments from productive expenses like: land and buildings, machinery and infrastructure will be possible only thro3h	Strongly Disagree	Count	0	3	3
		% of Total	0.0%	6.0%	6.0%
	Disagree	Count	2	3	5
		% of Total	4.0%	6.0%	10.0%
	Neither Agree nor Disagree	Count	1	6	7
		% of Total	2.0%	12.0%	14.0%
	Agree	Count	7	19	26
		% of Total	14.0%	38.0%	52.0%

investments on human capital	Strongly Agree	Count	2	7	9
		% of Total	4.0%	14.0%	18.0%
Total		Count	12	38	50
		% of Total	24.0%	76.0%	100.0%

Data Sources: Field Survey *In significant at 5%*

From the above table.3 reveals that out of 50 sample respondents the majority 26 sample respondents agreed that the return on investments from productive expenses like: land and buildings, machinery and infrastructure will be possible only through investments on human capital. Among these respondents the majority 19 (38%) of the respondents are belongs to post graduates and the rest 7(14%) of the respondents are belongs to under graduates followed by 18% of the respondents are strongly agreed that the return on investments from productive expenses like: land and buildings, machinery and infrastructure will be possible only through investments on human capital. Among these respondents the majority 7 (14%) of the respondents are belongs to post graduates and the rest 2(4%) of the respondents are belongs to under graduates Therefore, the majority 70% of the respondents agreed that the return on investments from productive expenses like: land and buildings, machinery and infrastructure will be possible only through investments on human capital.

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	2.149[a]	4	.708
Likelihood Ratio	2.812	4	.590
Linear-by-Linear Association	.110	1	.741
N of Valid Cases	50		

The above internal table reveals that there is no significant difference between educational qualification of respondents with respect to opinion on the return on investments from productive expenses like: land and buildings, machinery and infrastructure will be possible only through investments on human capital. The p-value which is .708 is greater than .05. Therefore, the distribution is insignificant. Therefore, there is no significant difference between opinion on the return on investments from productive expenses like: land and buildings, machinery and infrastructure will be possible only through investments on human capital with respect to education qualification of the respondents.

Table 4: The Financial Position, performance of a company and survival in long-run and strengthening the skills of individuals will happen through the investments made on human capital (Vs) Educational Qualification

Crosstab			
The Financial Position, performance of a company and survival in long-run and strengthening the skills of individuals will happen through the investments made on human capital (Vs) Educational Qualification	Educational Qualification		
	Under Graduates	Post Graduates	Total

The Financial Position, performance of a company and survival in long-run and strengthening the skills of individuals will happen through the investments made on human capital	Strongly Disagree	Count	0	3	3
		% of Total	0.0%	6.0%	6.0%
	Disagree	Count	0	3	3
		% of Total	0.0%	6.0%	6.0%
	Neither Agree nor Disagree	Count	2	9	11
		% of Total	4.0%	18.0%	22.0%
	Agree	Count	8	17	25
		% of Total	16.0%	34.0%	50.0%
	Strongly Agree	Count	2	6	8
		% of Total	4.0%	12.0%	16.0%
Total		Count	12	38	50
		% of Total	24.0%	76.0%	100.0 %

Data Sources: Field Survey *In significant at 5%*

From the above table.4 reveals that out of 50 sample respondents the majority 25 sample respondents agreed that the financial Position, performance of a company and survival in long-run and strengthening the skills of individuals will happen through the investments made on human capital. Among these respondents the majority 17 (34%) of the respondents are belongs to post graduates and the rest 8(16%) of the respondents are belongs to under graduates followed by 16% of the respondents are strongly agreed that The Financial Position, performance of a company and survival in long-run and strengthening the skills of individuals will happen through the investments made on human capital. Among these respondents the majority 6 (12%) of the respondents are belongs to post graduates and the rest 2(4%) of the respondents are belongs to under graduates Therefore, the majority 66% of the respondents agreed that The Financial Position, performance of a company and survival in long-run and strengthening the skills of individuals will happen through the investments made on human capital.

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	2.980[a]	4	.561
Likelihood Ratio	4.336	4	.362
Linear-by-Linear Association	1.946	1	.163
N of Valid Cases	50		

The above internal table reveals that there is no significant difference between educational qualification of respondents with respect to opinion on The Financial Position, performance of a company and survival in long-run and strengthening the skills of individuals will happen through the investments made on human capital. The p-value which is .561 is greater than .05. Therefore, the distribution is insignificant. Therefore, there is no significant difference between opinion on The Financial Position, performance of a company and survival in long-

run and strengthening the skills of individuals will happen through the investments made on human capital with respect to education qualification of the respondents.

Table 5: The employee skill, know-how, knowledge and performance of individuals depends up on the investments made on human capital (Vs) Educational Qualification

The employee skill, know-how, knowledge and performance of individuals depends up on the investments made on human capital (Vs) Educational Qualification			Educational Qualification		
			Under Graduates	Post Graduates	Total
The employee skill, know-how, knowledge and performance of individuals depends up on the investments made on human capital	Strongly Disagree	Count	0	3	3
		% of Total	0.0%	6.0%	6.0%
	Disagree	Count	0	1	1
		% of Total	0.0%	2.0%	2.0%
	Neither Agree nor Disagree	Count	4	4	8
		% of Total	8.0%	8.0%	16.0%
	Agree	Count	4	17	21
		% of Total	8.0%	34.0%	42.0%
	Strongly Agree	Count	4	13	17
		% of Total	8.0%	26.0%	34.0%
Total		Count	12	38	50
		% of Total	24.0%	76.0%	100.0%

Data Sources: Field Survey *In significant at 5%*

From the above table.5 reveals that out of 50 sample respondents the majority 21 sample respondents agreed that the employee skill, know-how, knowledge and performance of individuals depends up on the investments made on human capital. Among these respondents the majority 17 (34%) of the respondents are belongs to post graduates and the rest 4(8%) of the respondents are belongs to under graduates followed by 34% of the respondents are strongly agreed that the employee skill, know-how, knowledge and performance of individuals depends up on the investments made on human capital. Among these respondents the majority 13 (26%) of the respondents are belongs to post graduates and the rest 4(8%) of the respondents are belongs to under graduates Therefore, the majority 76% of the respondents agreed that the employee skill, know-how, knowledge and performance of individuals depends up on the investments made on human capital.

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	4.513[a]	4	.341
Likelihood Ratio	5.017	4	.286
Linear-by-Linear Association	.022	1	.882
N of Valid Cases	50		

The above internal table reveals that there is no significant difference between educational qualification of respondents with respect to opinion on the employee skill, know-how, knowledge and performance of individuals depends up on the investments made on human capital. The p-value which is .341 is greater than .05. Therefore, the distribution is insignificant. Therefore, there is no significant difference between opinion on the employee skill, know-how, knowledge and performance of individuals depends up on the investments made on human capital with respect to education qualification of the respondents.

Table 6: The cost of recruitment, training, performance appraisal, skill enhancement programs, promotions are the major aspects of human resource accounting (Vs) Educational Qualification

The cost of recruitment, training, performance appraisal, skill enhancement programs, promotions are the major aspects of human resource accounting (Vs) Educational Qualification			Educational Qualification		
			Under Graduates	Post Graduates	Total
The cost of recruitment, training, performance appraisal, skill enhancement programs, promotions are the major aspects of human resource accounting	Strongly Disagree	Count	1	2	3
		% of Total	2.0%	4.0%	6.0%
	Disagree	Count	0	2	2
		% of Total	0.0%	4.0%	4.0%
	Neither Agree nor Disagree	Count	2	8	10
		% of Total	4.0%	16.0%	20.0%
	Agree	Count	4	17	21
		% of Total	8.0%	34.0%	42.0%
	Strongly Agree	Count	5	9	14
		% of Total	10.0%	18.0%	28.0%
Total		Count	12	38	50
		% of Total	24.0%	76.0%	100.0%

Data Sources: Field Survey *In significant at 5%*

From the above table.6 reveals that out of 50 sample respondents the majority 21 sample respondents agreed that the cost of recruitment, training, performance appraisal, skill enhancement programs, promotions are the major aspects of human resource accounting. Among these respondents the majority 17 (34%) of the respondents are belongs to post graduates and the rest 4(8%) of the respondents are belongs to under graduates followed by 28% of the respondents are strongly agreed that the cost of recruitment, training, performance appraisal, skill enhancement programs, promotions are the major aspects of human resource accounting. Among these respondents the majority 9 (18%) of the respondents are belongs to post graduates and the rest 5(10%) of the respondents are belongs to under graduates Therefore, the majority 70% of the respondents agreed that the cost of recruitment, training, performance appraisal, skill enhancement programs, promotions are the major aspects of human resource accounting.

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	2.198[a]	4	.699
Likelihood Ratio	2.581	4	.630
Linear-by-Linear Association	.437	1	.509
N of Valid Cases	50		

The above internal table reveals that there is no significant difference between educational qualification of respondents with respect to opinion on the cost of recruitment, training, performance appraisal, skill enhancement programs, promotions are the major aspects of human resource accounting. The p-value which is .699 is greater than .05. Therefore, the distribution is insignificant. Therefore, there is no significant difference between opinion on the cost of recruitment, training, performance appraisal, skill enhancement programs, promotions are the major aspects of human resource accounting with respect to education qualification of the respondents.

Table 7: The Degree of employee participation, involvement and performance associated with the human capital valuation (Vs) educational Qualification

The Degree of employee participation, involvement and performance associated with the human capital valuation (Vs) educational Qualification			Educational Qualification		
			Under Graduates	Post Graduates	Total
The Degree of employee participation, involvement and performance associated with the human capital valuation	Strongly Disagree	Count	1	4	5
		% of Total	2.0%	8.0%	10.0%
	Disagree	Count	2	0	2
		% of Total	4.0%	0.0%	4.0%
	Neither Agree nor Disagree	Count	1	7	8
		% of Total	2.0%	14.0%	16.0%
	Agree	Count	7	18	25
		% of Total	14.0%	36.0%	50.0%
	Strongly Agree	Count	1	9	10
		% of Total	2.0%	18.0%	20.0%
Total		Count	12	38	50
		% of Total	24.0%	76.0%	100.0%

Data Sources: Field Survey *In significant at 5%*

From the above table.7 reveals that out of 50 sample respondents the majority 25 sample respondents agreed that The Degree of employee participation, involvement and performance associated with the human capital valuation. Among these respondents the majority 18 (36%) of the respondents are belongs to post graduates and the rest 7 (14%) of the respondents are belongs to under graduates followed by 20% of the respondents are strongly agreed that The Degree of employee participation, involvement and performance associated with the human

capital valuation. Among these respondents the majority 9 (18%) of the respondents are belongs to post graduates and the rest 1 (2%) of the respondents are belongs to under graduates Therefore, the majority 70% of the respondents agreed that The Degree of employee participation, involvement and performance associated with the human capital valuation.

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	8.251[a]	4	.083
Likelihood Ratio	7.926	4	.094
Linear-by-Linear Association	.702	1	.402
N of Valid Cases	50		

The above internal table reveals that there is no significant difference between educational qualification of respondents with respect to opinion on The Degree of employee participation, involvement and performance associated with the human capital valuation. The p-value which is .083 is greater than .05. Therefore, the distribution is insignificant. Therefore, there is no significant difference between opinion on The Degree of employee participation, involvement and performance associated with the human capital valuation with respect to education qualification of the respondents.

Table 8: The Employee Morality, commitment, recognition, dedication are highly associated with the human resource valuation in the organization (Vs) Educational Qualification

Crosstab					
The Employee Morality, commitment, recognition, dedication are highly associated with the human resource valuation in the organization (Vs) Educational Qualification			Educational Qualification		
			Under Graduates	Post Graduates	Total
The Employee Morality, commitment, recognition, dedication are highly associated with the human resource valuation in the organization	Strongly Disagree	Count	0	3	3
		% of Total	0.0%	6.0%	6.0%
	Disagree	Count	1	3	4
		% of Total	2.0%	6.0%	8.0%
	Neither Agree nor Disagree	Count	1	5	6
		% of Total	2.0%	10.0%	12.0%
	Agree	Count	8	18	26
		% of Total	16.0%	36.0%	52.0%
	Strongly Agree	Count	2	9	11
		% of Total	4.0%	18.0%	22.0%
Total		Count	12	38	50
		% of Total	24.0%	76.0%	100.0%

Data Sources: Field Survey *In significant at 5%*

From the above table.8 reveals that out of 50 sample respondents the majority 26 sample respondents agreed that The Employee Morality, commitment, recognition, dedication is highly associated with the human resource valuation in the organization. Among these respondents the majority 18 (36%) of the respondents are belongs to post graduates and the rest 8 (16%) of the respondents are belongs to under graduates followed by 22% of the respondents are strongly agreed that The Employee Morality, commitment, recognition, dedication is highly associated with the human resource valuation in the organization. Among these respondents the majority 9 (18%) of the respondents are belongs to post graduates and the rest 2 (4%) of the respondents are belongs to under graduates Therefore, the majority 74% of the respondents agreed that The Employee Morality, commitment, recognition, dedication are highly associated with the human resource valuation in the organization.

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	1.984[a]	4	.739
Likelihood Ratio	2.675	4	.614
Linear-by-Linear Association	.332	1	.564
N of Valid Cases	50		

The above internal table reveals that there is no significant difference between educational qualification of respondents with respect to opinion on The Employee Morality, commitment, recognition, dedication are highly associated with the human resource valuation in the organization. The p-value which is .739 is greater than .05. Therefore, the distribution is insignificant. Therefore, there is no significant difference between opinion on The Employee Morality, commitment, recognition, dedication are highly associated with the human resource valuation in the organization with respect to education qualification of the respondents.

Table 9: In the overall, human resource valuation strongly associated with the individual and organizational performance (Vs) Educational Qualification

Crosstab					
In the overall, human resource valuation strongly associated with the individual and organizational performance (Vs) Educational Qualification			Educational Qualification		
			Under Graduates	Post Graduates	Total
In the overall, human resource valuation strongly associated with the individual and organizational performance	Strongly Disagree	Count	1	2	3
		% of Total	2.0%	4.0%	6.0%
	Disagree	Count	1	5	6
		% of Total	2.0%	10.0%	12.0%
	Neither Agree nor Disagree	Count	3	5	8
		% of Total	6.0%	10.0%	16.0%
	Agree	Count	4	14	18
		% of Total	8.0%	28.0%	36.0%
	Strongly Agree	Count	3	12	15
		% of Total	6.0%	24.0%	30.0%
Total		Count	12	38	50

	% of Total	24.0%	76.0%	100.0%

Data Sources: Field Survey *In significant at 5%*

From the above table.9 reveals that out of 50 sample respondents the majority 18 sample respondents agreed that In the overall, human resource valuation strongly associated with the individual and organizational performance. Among these respondents the majority 14 (28%) of the respondents are belongs to post graduates and the rest 4 (8%) of the respondents are belongs to under graduates followed by 30% of the respondents are strongly agreed that In the overall, human resource valuation strongly associated with the individual and organizational performance. Among these respondents the majority 12 (24%) of the respondents are belongs to post graduates and the rest 3 (6%) of the respondents are belongs to under graduates Therefore, the majority 66% of the respondents agreed that In the overall, human resource valuation strongly associated with the individual and organizational performance.

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	1.282[a]	4	.864
Likelihood Ratio	1.216	4	.876
Linear-by-Linear Association	.206	1	.650
N of Valid Cases	50		

The above internal table reveals that there is no significant difference between educational qualification of respondents with respect to opinion on in the overall, human resource valuation strongly associated with the individual and organizational performance. The p-value which is .864 is greater than .05. Therefore, the distribution is insignificant. Therefore, there is no significant difference between opinion on in the overall, human resource valuation strongly associated with the individual and organizational performance with respect to education qualification of the respondents.

Findings:-

- The majority of the under graduate sand post graduates agreed that human capital accounting compares the capital invested on human resources and employee-organizational performance.
- The majority of the respondents agreed that The HRA details facilitates a lot to the external stake holders to take decision-making related to investments and to assess the value of a firm.
- The majority of the sample respondents agreed that the return on investments from productive expenses like: land and buildings, machinery and infrastructure will be possible only through investments on human capital.
- The majority 25 sample respondents agreed that the financial Position, performance of a company and survival in long-run and strengthening the skills of individuals will happen through the investments made on human capital.

- The Majority of the respondents agreed in the overall, human resource valuation strongly associated with the individual and organizational performance.

Conclusion:-

Hence, it can be conclude that the human resource accounting facilitates a lot to conduct a comparative study between the investments made on human resources and associated returns. It will also help to the stake holders of the business to take decision-makings further the HRA motivates, stimulates and drives individuals towards organizational goals. In the overall, the human resource accoutring assessment is very much essential for the prosperity of the organization.

Scope of future Research:-

The research can be further extended by taking the advantage of structural equation modelling algorithm by developing constructs which include mediating and moderating variables. The SEM analysis on HRA will explains the goodness of fit index among various constructs further a comparative study also can be done by taking selected MNC's.

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The Impact of Employee Welfare facilities on Employee-Organizational Performance

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Abstract:-

Aim/Purpose:- The aim of the present descriptive research study is to investigate the impact of employee welfare facilities on employee-organizational performance. The two different types of welfare facilities like: statutory and Non-statutory welfare facilities which motivates the employees to perform well in the organization. The welfare facilities intend to safeguard the health, safety and security to individual employees. Outcome:- The outcome of the research proved that the various welfare facilities like: medical, retirement and workplace benefits are essential for high employee job satisfaction. Research Design and Methodology:- Developed a structured closed ended questionnaire to collect the data from various respondents of undergraduates and post graduates to assess the impact of employee welfare facilities and its impact on performance. Statistical tools:- It is a descriptive research design applied Analysis of Variance (ANOVA) to assess the variance in the data with respect to the respondents opinion on employee welfare facilities and its impact. Social Relevance:- The outcome of the research will facilitate to safeguard the employees from socio-economic problems in the society. Generalizability:- The evidences of the research can be generalized where employee and organizational performance depends up on the welfare facilities provided by the organization. Novelty:- It is a novel research, the content of the research may not be copied from any sources and it is new. Type of the Research:- It is a descriptive research design applied various statistical operations like: mean SD and Variance.

Key Words:- employee welfare, statutory welfare, non-statutory welfare, labour welfare, welfare measures, etc.,

Introduction:-

The employee welfare facilities include showing any sort of monetary and non-monetary benefits provided by the employer to the employees of the organization for better living and protecting the present and future of employees. The employee welfare facilities which are very much essential for

high employee job satisfaction which leads to higher productivity. There are various employee welfare facilities like: medical facilities, retirement benefits, educational benefits, housing benefits, superannuation benefits and providing loans to the employees are the various welfare facilities can be provided to individual employees in the organization. The welfare facilities which enhances the credibility and morale of employees on the employers and which creates peace and harmony at workplace. In fact, the state and central government role is utmost important to implement better policies and procedures to implement various statutory and non-statutory welfare facilities. There are various central government Acts like: Factories Act, 1948 and the Industrial disputes Act, 1947 payment of wages Act, 1936 and workmen's compensation Act, 1923 and other various types of Acts are very much essential to protect the employees. In fact, the employee welfare facilities will raise the overall expenditure; it will create a kind of trust in the minds of employees that the management is much concern about the welfare of employees which boost the organizational productivity. The aim of the present research study was to know the impact of employee welfare facilities on the organizational and employee performance in the organization. Taken 50 sample size from the graduates and post graduates to assess the opinion on welfare facilities and its impact on performance.

Review of Literature:-

In the article labour in Indian coal industry witnessed that the coal miners are not getting adequate welfare facilities [1]. According to Labor welfare of India, there are some measures given by the Government to the Public and Private Sector Companies. There is a difference between Private and Public sector companies in Welfare facilities provided to their employees [2]. As per" Labor Welfare and Industrial Peace in India" states that the discomfort of the employees due to failure of the welfare facilities in the industries [3]. As Per Legislation on Basis of Workmen's Compensation Act 1923, there is a great need for taking care of the welfare and social security to the employees, but they revealed that the provision of welfare facilities to be exercised, and the victims of mine accidents are paid very less compensation[4]. Due to some reasons after Independence Government of Uttar Pradesh not providing sufficient Social and welfare services to the below poverty line[5]. As per "A Socio-Economic Survey of the Workers in the Coal Mines of India" told there is a terrible conditions of coal workers that there are high indebtedness, low wages and poor welfare facilities are responsible for the poor socio-economic conditions of miners[6]. As per "Living Conditions of Colliery Workers in Jharia seen the absence of welfare facilities, there is a dire condition of workers and employees in jharia coal fields and should provide employee welfare facilities to the workers [7]. Based on "Dimensions of Welfare" welfare has improved in post 1973 there is a revolution of operating philosophy in industries [8]. Labor welfare divided into two types those are extra-mural and intra-mural as well as statutory and non-statutory and it undertaken by the employers, Govt., trade unions or voluntary organizations [9]. Welfare plays an important key role to satisfy an organization employee and it also improve quality of work life, productivity of the organization [10]. Welfare is the psychological bond to employee of an organization, which depends on the degree of employee involvement, employee loyalty, and belief in the values of the organization [11]. In this research employee awareness towards various statutory and non-statutory welfare measures and level of satisfaction among employees at both with respective to various welfare measures [12]. An investigation of welfare is study on Workers towards Labor welfare measures in the corporate sector[13]

Need & Importance:-

It is the prime responsibility of every organization to protect the employees from socio-economic conditions. Those facilities which are necessities to individuals will fall under the statutory welfare facilities and the next category of benefits will fall under the non-statutory welfare facilities and the type-II welfare facilities are not compulsory. There are various welfare facilities like: clean drinking water, neat and clean washrooms, proper lighting, leaves, insurance, safety and security are the

various statutory welfare practices provided to their employees and the list of non-statutory welfare facilities include: employee health care, employee recreation, employee tours and other sort of maternity and other sort of benefits.

Objectives of the Study:-

1. To study the various welfare facilities of employees in the organization.
2. To know the opinion of respondents with respect to welfare facilities and the organizational and employee performance.
3. To suggest the best employee welfare practices which motivates the individuals towards organizational performance

Scope of the study:-

The scope of the present research is restricted to study the employee welfare practices and its impact on organizational and employee performance in the organization. The scope with respect to opinion extraction limited to various graduate and post graduates in the selected state.

Statement of the Problem:-

The title entitled to "The Impact of Employee Welfare facilities on Employee-Organizational Performance", which examines the impact of employee welfare facilities on organizational productivity.

Hypothesis:-

H_a(1): There is a significant positive relationship various statutory employee welfare practices and the organizational performance.
H_a(2): There is a significant positive relationship various non-statutory employee welfare practices and the organizational performance.

Research Methodology & Design:-

It is a descriptive research study which includes Mean, SD and assessment of Analysis of Variance with respect to educational qualification and opinion on various welfare practices which include statutory and non-statutory

a. **Data Sources:-** The studies include both primary and secondary data sources. The primary data sources collected through the structured questionnaire and the secondary data sources the literature review on employee welfare practices
b. **Sampling Technique:-** Applied simple random sampling technique to collect the opinion of the respondents on random basis. Developed a Google sheet to collect the opinion of respondents in this regard.
c. **Sample Size:-** Taken 50 samples on random basis which include both male and female and they are belongs to graduates and post graduates.
d. **Sampling Unit:-** The sampling unit comprises of the graduates and post graduate student to collect the opinion on the impact of labour welfare practices on employee-organizational performance in the contemporary scenario
e. **Statistical Technique:-** Applied the concept of descriptive research design which include Mean, SD and Analysis of Variance and there is a scope to apply the inferential statistics also.
f. **Statistical Software:-** The statistical package for social sciences and MS-Excel were used to assess the data with respect to demographical factors.

g. **Reliability:-** The Cronebach's Alpha test is used to assess the reliability of the collected data. The data has shown more than 80% accuracy

Data Analysis and Interpretation:-

1. Employee Motivation (Vs) Welfare facilities:- The employee motivation and welfare facilities are strongly associated with each other as management provides a welfare facility which motivates individuals to work for organizational objectives and goals. The following Table.1 reveals the relationship between the employee motivation and welfare facilities.

Table.1: The employee welfare facilities which motivate employees to work diligently at workplace (Vs) Educational Qualification

	Educational Qualification	N	Mean	Std. Deviation	F-Value	Sig.Value (2-Sided)
The employee welfare facilities which motivate employees to work diligently at workplace	Under graduation	5	3.40	.500	3.701	.040
	Post Graduation	45	4.02	1.177		
	Total	50	3.92	1.158		

Sources: Filed Survey Significant at 5%

From the above Table.1 explains about the opinion on employee welfare facilities which motivates employee to work diligently at work with respect to educational qualification of respondents. The Mean value of post graduates shows positive opinion that (Mean=4.02 and SD=1.177 which is agreed) followed by the undergraduates (Mean=3.40 and SD=0.500). Therefore, the majority of the respondents agreed that the employee welfare facility which motivates individual employees to work diligently at work place. The F-Value is 3.701 and the (p>.040 which is significant at 5%). Therefore, there is no significant difference between educational qualifications of the respondents with respect to opinion on employee welfare facilities which motivates employees to work diligently at workplace. Therefore, the null hypothesis is accepted.

2. The Socio-Economic Conditions (Vs) Employee Welfare:- The socio-economic conditions of individual employee can be improved by providing the statutory and non-statutory welfare facilities to individual employees.

Table.2: The various employee welfare facilities facilitates to safeguard employees from economic evils in future (Vs) Educational Qualification

	Educational Qualification	N	Mean	Std. Deviation	F-Value	Sig.Value (2-Sided)
The various employee welfare facilities facilitates to safeguard employees from economic evils in future	Under graduation	5	3.20	.447	1.225	.274
	Post Graduation	45	3.71	1.014		
	Total	50	3.66	.982		

Sources: Filed Survey Significant at 5%

From the above Table.2 explains about the opinion on employee welfare facilities to safeguard employees from economic evils in future with respect to educational qualification of respondents.

The Mean value of post graduates shows positive opinion that (Mean=3.71 and SD=1.014 which is agreed) followed by the undergraduates (Mean=3.20 and SD=0.447). Therefore, the majority of the respondents agreed that the employee welfare facility to safeguard employees from economic evils in future. The F-Value is 1.225 and the (p>.274 which is significant at 5%). Therefore, there is no significant difference between educational qualifications of the respondents with respect to opinion on employee welfare facilities to safeguard employees from economic evils in future. Therefore, the null hypothesis is accepted.

3. Employee Performance (Vs) Employee welfare:- The employee welfare facilities and the performance of an employee highly correlated with each other as higher the welfare facilities which enhances the individual performance in the organization

Table.3 The employee welfare facilities facilitates to enhance the employee Performance in the organization (Vs) Educational Qualification

	Educational Qualification	N	Mean	Std. Deviation	F-Value	Sig.Value (2-Sided)
The employee welfare facilities facilitates to enhance the employee performance in the organization	Under graduation	5	3.40	.894	.305	.583
	Post Graduation	45	3.71	1.218		
	Total	50	3.68	1.186		

Sources: Filed Survey Significant at 5%

From the above Table.3 explains about the opinion on employee welfare facilities to enhance the Employee Performance in the organization with respect to educational qualification of respondents. The Mean value of post graduates shows positive opinion that (Mean=3.71 and SD=1.218 which is agreed) followed by the undergraduates (Mean=3.40 and SD=0.894). Therefore, the majority of the respondents agreed that the employee welfare facility to enhance the employee Performance in the organization. The F-Value is 0.305 and the (p>.583 which is significant at 5%). Therefore, there is a significant difference between educational qualifications of the respondents with respect to opinion on employee welfare facilities to enhance the employee Performance in the organization. Therefore, the null hypothesis is accepted.

Table.4: The employee living standards can be strengthen with the help of employee welfare facilities (Vs) Educational Qualification

	Educational Qualification	N	Mean	Std. Deviation	F-Value	Sig.Value (2-Sided)
The employee living standards can be strengthen with the help of employee welfare facilities	Under graduation	5	3.60	.894	.134	.716
	Post Graduation	45	3.78	1.042		
	Total	50	3.76	1.021		

Sources: Filed Survey Significant at 5%

From the above Table.4 explains about the opinion on employee living standards can be strengthen with the help of employee welfare facilities with respect to educational qualification of respondents. The Mean value of post graduates shows positive opinion that (Mean=3.78 and SD=1.042 which is agreed) followed by the undergraduates (Mean=3.60 and SD=0.894). Therefore, the majority of the respondents agreed employees living standards can be strengthen with the help of employee welfare

facilities. The F-Value is 0.134 and the (p>.716 which is significant at 5%). Therefore, there is no significant difference between educational qualifications of the respondents with respect to opinion on employee living standards can be strengthen with the help of employee welfare facilities. Therefore, the null hypothesis is accepted.

Table.5 there are various social security benefits, health associated benefits and other sort of benefits can be provided through the employee welfare Schemes (vs) Educational Qualification

	Educational Qualification	N	Mean	Std. Deviation	F-Value	Sig.Value (2-Sided)
There are various social security benefits, health associated benefits and other sort of benefits can be provided through the employee welfare schemes	Under graduation	5	3.80	.447	.008	.928
	Post Graduation	45	3.76	1.069		
	Total	50	3.76	1.021		

Sources: Filed Survey Significant at 5%

From the above Table.5 explains about the opinion on there are various social security benefits, health associated benefits and other sort of benefits can be provided through the employee welfare Schemes with respect to educational qualification of respondents. The Mean value of post graduates shows positive opinion that (Mean=3.76 and SD=1.069 which is agreed) followed by the undergraduates (Mean=3.80 and SD=0.447). Therefore, the majority of the respondents agreed that the there are various social security benefits, health associated benefits and other sort of benefits can be provided through the employee welfare Schemes. The F-Value is 0.008 and the (p>.928 which is significant at 5%). Therefore, there is no significant difference between educational qualifications of the respondents with respect to opinion on the various social security benefits, health associated benefits and other sort of benefits can be provided through the employee welfare Schemes. Therefore, the null hypothesis is accepted.

Table.6 The employee welfare schemes play a key role to strength the socio-economic Conditions of the people (Vs) Educational Qualification

	Educational Qualification	N	Mean	Std. Deviation	F-Value	Sig.Value (2-Sided)
The employee welfare schemes plays a key role to strength the socio-economic conditions of the people	Under graduation	5	2.40	.894	7.009	.011
	Post Graduation	45	3.80	1.140		
	Total	50	3.66	1.189		

Sources: Filed Survey Significant at 5%

From the above Table.6 explains about the opinion on the employee welfare schemes play a key role to strength the socio-economic Conditions of the people with respect to educational qualification of respondents. The Mean value of post graduates shows positive opinion that (Mean=3.80 and SD=1.140 which is agreed) followed by the undergraduates (Mean=2.40 and SD=0.894). Therefore, the majority of the respondents agreed that the employee welfare schemes

play a key role to strength the socio-economic Conditions of the people. The F-Value is 7.009 and the (p>.011 which is significant at 5%). Therefore, there is a significant difference between educational qualifications of the respondents with respect to opinion on the employee welfare schemes play a key role to strength the socio-economic Conditions of the people. Therefore, the null hypothesis is rejected.

Table.7 The employee and organizational productivity purely depends up on the welfare facilities provided by the organization (Vs) Educational Qualification

	Educational Qualification	N	Mean	Std. Deviation	F-Value	Sig.Value (2-Sided)
The employee and organizational productivity purely depends up on the welfare facilities provided by the organization	Under graduation	5	3.80	.447	.157	.693
	Post Graduation	45	3.62	.984		
	Total	50	3.64	.942		

Sources: Filed Survey Significant at 5%

From the above Table.7 explains about the opinion on the employee welfare schemes play a key role to strength the socio-economic Conditions of the people with respect to educational qualification of respondents. The Mean value of post graduates shows positive opinion that (Mean=3.62 and SD=0.984 which is agreed) followed by the undergraduates (Mean=3.80 and SD=0.447). Therefore, the majority of the respondents agreed that the employee welfare schemes play a key role to strength the socio-economic Conditions of the people. The F-Value is 0.157 and the (p>.693 which is significant at 5%). Therefore, there is no significant difference between educational qualifications of the respondents with respect to opinion on the employee welfare schemes play a key role to strength the socio-economic Conditions of the people. Therefore, the null hypothesis is accepted.

Table.8 The achievement of organizational objectives, goals and targets purely depends Up on the welfare facilities provided by the organization to their employees (Vs) Educational Qualification

	Educational Qualification	N	Mean	Std. Deviation	F-Value	Sig.Value (2-Sided)
The achievement of organizational objectives, goals and targets purely depends up on the welfare facilities provided by the organization to their employees	Under graduation	5	4.80	.447	6.232	.016
	Post Graduation	45	3.58	1.076		
	Total	50	3.70	1.093		

Sources: Filed Survey Significant at 5%

From the above Table.8 explains about the opinion on the achievement of organizational objectives, goals and targets purely depends up on the welfare facilities provided by the organization to their

employees of respondents. The Mean value of post graduates shows positive opinion that (Mean=3.58 and SD=1.076 which is agreed) followed by the undergraduates (Mean=4.80 and SD=0.447). Therefore, the majority of the respondents agreed that the achievement of organizational objectives, goals and targets purely depends up on the welfare facilities provided by the organization to their employees. The F-Value is 6.232 and the (p>.016 which is significant at 5%). Therefore, there is a significant difference between educational qualifications of the respondents with respect to opinion on the achievement of organizational objectives, goals and targets purely depends up on the welfare facilities provided by the organization to their employees. Therefore, the null hypothesis is accepted.

Table.9 Gaining the employee morality depends on the promises made and fulfilled by the management with respect to welfare facilities in the organization (Vs) Educational Qualification

	Educational Qualification	N	Mean	Std. Deviation	F-Value	Sig.Value (2-Sided)
Gaining the employee morality depends on the promises made and fulfilled by the management with respect to welfare facilities in the organization	Under graduation	5	4.20	.447	.965	.331
	Post Graduation	45	3.69	1.145		
	Total	50	3.74	1.103		

Sources: Filed Survey Significant at 5%

From the above Table.9 explains about the opinion on Gaining the employee morality depends on the promises made and fulfilled by the management with respect to welfare facilities in the organization with respect to educational qualification of respondents. The Mean value of post graduates shows positive opinion that (Mean=3.69 and SD=1.145 which is agreed) followed by the undergraduates (Mean=4.20 and SD=0.447). Therefore, the majority of the respondents agreed that the Gaining the employee morality depends on the promises made and fulfilled by the management with respect to welfare facilities in the organization. The F-Value is 0.965 and the (p>.331 which is significant at 5%). Therefore, there is no significant difference between educational qualifications of the respondents with respect to opinion on Gaining the employee morality depends on the promises made and fulfilled by the management with respect to welfare facilities in the organization. Therefore, the null hypothesis is accepted.

Table.10 The usage of employee intelligence, efficiency, intellectuality may be depends up on the welfare facilities provided by the organization (Vs) Educational Qualification

	Educational Qualification	N	Mean	Std. Deviation	F-Value	Sig.Value (2-Sided)
The usage of employee intelligence,	Under graduation	5	3.80	.447	.208	.651

efficiency, intellectuality may be depends up on the welfare facilities provided by the organization	Post Graduation	45	3.60	.963		
	Total	50	3.62	.923		

Sources: Filed Survey Significant at 5%

From the above Table.10 explains about the opinion on the usage of employee intelligence, efficiency, intellectuality may be depends up on the welfare facilities provided by the organization with respect to educational qualification of respondents. The Mean value of post graduates shows positive opinion that (Mean=3.60 and SD=0.963 which is agreed) followed by the undergraduates (Mean=3.80 and SD=0.447). Therefore, the majority of the respondents agreed that the usage of employee intelligence, efficiency, intellectuality may be depends up on the welfare facilities provided by the organization. The F-Value is 0.208 and the (p>.651 which is significant at 5%). Therefore, there is a significant difference between educational qualifications of the respondents with respect to opinion on the usage of employee intelligence, efficiency, intellectuality may be depends up on the welfare facilities provided by the organization. Therefore, the null hypothesis is accepted.

Table.11 On the overall the employee job satisfaction, stress management, organization long-run survival, employee security, mental health and physical health purely depends up on the various statutory and non-statutory welfare facilities provided by the organization (Vs) Educational Qualification

	Educational Qualification	N	Mean	Std. Deviation	F-Value	Sig.Value (2-Sided)
On the overall the employee job satisfaction, stress management, organization long-run survival, employee security, mental health and physical health purely depends up on the various statutory and non-statutory welfare facilities provided by the organization	Under graduation	5	3.00	.000	.676	.415
	Post Graduation	45	3.44	1.198		
	Total	50	3.40	1.143		

Sources: Filed Survey Significant at 5%

From the above Table.11 explains about the opinion on the overall the employee job satisfaction, stress management, organization long-run survival, employee security, mental health and physical health purely depends up on the various statutory and non-statutory welfare facilities provided by the organization with respect to educational qualification of respondents. The Mean value of post graduates shows positive opinion that (Mean=3.44 and SD=1.198 which is agreed) followed by the

undergraduates (Mean=3.00 and SD=0.000). Therefore, the majority of the respondents agreed that the On the overall the employee job satisfaction, stress management, organization long-run survival, employee security, mental health and physical health purely depends up on the various statutory and non-statutory welfare facilities provided by the organization. The F-Value is 0.676 and the (p>.415 which is significant at 5%). Therefore, there is a significant difference between educational qualifications of the respondents with respect to opinion on the overall the employee job satisfaction, stress management, organization long-run survival, employee security, mental health and physical health purely depends up on the various statutory and non-statutory welfare facilities provided by the organization. Therefore, the null hypothesis is accepted.

Findings:-

- In the overall opinion the employee welfare practices which facilitates the employee job satisfaction, stress management, organization long-run survival, employee security, mental health and physical health purely depends up on the various statutory and non-statutory welfare facilities provided by the organization.
- As the mean value 3.80, which depict that the usage of employee intelligence, efficiency intellectuality may be depends up on the welfare facilities provided by the organization.
- As the Mean value 4.20 witnessed that Gaining the employee morality depends on the promises made and fulfilled by the management with respect to welfare facilities in the organization
- It is also witnessed from the analysis that the majority 4.80 reveals that the achievement of organizational objectives, goals and targets purely depends up on the welfare facilities provided by the organization to their employees.
- It is witnessed from the mean value 3.80 that the employee and organizational productivity purely depends up on the welfare facilities provided by the organization.
- The Majority from post graduates also agreed that the employee welfare schemes play a key role to strength the socio-economic conditions of the people.
- As the Mean value 3.78, the employee living standards can be strengthen with the help of employee welfare facilities.

Conclusion:-

Hence, it can be conclude that the employee welfare facilities which are very much essential to motivate individuals which facilitates to enhance the performance of employees and to develop to socio-economic status of individuals in the present condition. Therefore, the role of various statutory and non-statutory welfare facilities explains the importance of welfare facilities in the contemporary context.

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Optimality Conditions and Saddle Point Criteria for Fractional Interval-Valued Optimization Problem via Convexificator

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Abstract. In this work, we use the notion of convexificators to discuss optimality conditions for a fractional interval-valued optimization problem. We illustrate the sufficient optimality conditions established in the paper by the example of a nonconvex fractional interval-valued optimization problem with the help of generalized invex functions. Further, we study saddle point criteria of a Lagrange function defined for a fractional interval-valued optimization problem.

Keywords: Convexificator; Fractional problem; LU optimal solution; Lagrange functions; Saddle point.

1. Introduction

In the last two decades, a large number of research has been devoted for solving fractional programming problems. This follows from the fact that optimization problems with the objective function of ratio of two functions have a wide range

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of applications in engineering and economics, game theory, and many more (cf. [12, 18, 22]).

Interval-valued optimization problem is used to tackle interval uncertainity that appears in many real world mathematical problems. For example, it is applied to solve the fixed-charge transportation problem [15], chemical engineering problem [17] and municipal solid waste management [20], etc. Interval-valued programming problem was first studied by Ben-Israel and Robers [2]. Wu [21] formulated four kinds of interval-valued optimization problems and discussed optimality conditions. Further, they also established duality results to relate the primal and dual problems. Singh et al. [16] proposed a theoretical and practical solution method for a multiobjective interval-valued programming problem. In the recent past, many mathematicians have shown their interest to study different types of interval-valued programming problems [1, 3, 11, 14, 19].

The notion of convexificators introduced by Demyanov [6] and extended further by Jeyakumar and Luc [10]. Convexificators can be viewed as weaker versions of the notion of subdifferentials as they are in general closed sets unlike the well-known subdifferentials which are convex and compact sets. In literature, a lot of research has been carried out for convexificators regarding its theoretical properties (see, e.g. [5, 7, 8, 11, 13] and the references therein). Recently, making use of these notions, Karush-Kuhn-Tucker necessary optimality conditions for local weak efficient solutions were established by Hejazi and Nobakhtian [9] for a multiobjective fractional programming problem. Also, Hejazi and Nobakhtian [9] gave some constraint qualifications and subsequently they discussed relationship between these constraint qualifications.

In this paper, by using the idea of convexificators, we study optimality conditions for a fractional interval-valued optimization problem. Further, we establish equivalence between the saddle point and LU optimal solution of the fractional interval-valued optimization problem involving generalized invex functions.

2. Preliminaries

In this section, we give a number of basic definitions and lemmas which will be used in the paper. Let R^n be the n-dimensional Euclidean space and R^n_+ be its non-negative orthant. Throughout this paper, we shall be concerned with Banach spaces. Let X^* be topological dual of a given Banach space X with the canonical dual pairing $\langle ., . \rangle$. Let X and Y be Banach spaces and we denote by $L(X, Y)$ the set of continuous linear mappings between X and Y.

Let $f : X \to R \cup \{+\infty\}$, be an extended real-valued function. Then

$$f^-(x, d) = \lim_{t \to 0+} \inf \frac{f(x + td) - f(x)}{t},$$

$$f^+(x, d) = \lim_{t \to 0+} \sup \frac{f(x + td) - f(x)}{t}$$

denote, respectively, the lower and upper Dini directional derivatives of f at

$x \in X$ in the direction of d.

Now, we begin with the definition of convexificator given by Jeyakumar and Luc [10].

Definition 2.1. *A function $f : X \to R \cup \{+\infty\}$ is said to have a convexificator $\partial^* f(x)$ at x if $\partial^* f(x) \subset X^*$ is weak* closed and*

$$f^+(x,d) \geq \inf_{x^* \in \partial^* f(x)} \langle x^*, d\rangle \text{ and } f^-(x,d) \leq \sup_{x^* \in \partial^* f(x)} \langle x^*, d\rangle\,,\ \forall d \in X.$$

Along the lines of Gadhi [8], we now give the definitions of generalized invex functions by using the concept of convexificators. Assume that $f : X \to R$ admits a convexificator $\partial^* f(\bar{x}) \subset L(X,R)$ at $\bar{x} \in X$.

Definition 2.2. *A function $f : X \to R$ is said to be $(\eta, \partial^* f)$-invex at $\bar{x} \in X$ if there exists $\eta : X \times X \to X$ such that,*

$$f(x) - f(\bar{x}) \geq \langle \xi, \eta(x,\bar{x})\rangle\,,\ \textit{for all } \xi \in \partial^* f(\bar{x}) \textit{ and } x \in X.$$

If strict inequality holds in above definition for $x \neq \bar{x}$, then f is said to be strict $(\eta, \partial^* f)$-invex at $\bar{x}$.

Definition 2.3. *A function $f : X \to R$ is said to be $(\eta, \partial^* f)$-pseudoinvex at $\bar{x} \in X$ if there exists $\eta : X \times X \to X$ such that,*

$$f(x) < f(\bar{x}) \Rightarrow \langle \xi, \eta(x,\bar{x})\rangle < 0,\ \textit{for all } \xi \in \partial^* f(\bar{x}) \textit{ and } x \in X,$$

equivalently

$$\langle \xi, \eta(x,\bar{x})\rangle \geq 0 \Rightarrow f(x) \geq f(\bar{x}),\ \textit{for all } \xi \in \partial^* f(\bar{x}) \textit{ and } x \in X.$$

Definition 2.4. *A function $f : X \to R$ is said to be strict $(\eta, \partial^* f)$-pseudoinvex at $\bar{x} \in X$ if there exists $\eta : X \times X \to X$ such that,*

$$f(x) \leq f(\bar{x}) \Rightarrow \langle \xi, \eta(x,\bar{x})\rangle < 0,\ \textit{for all } \xi \in \partial^* f(\bar{x}) \textit{ and } x \in X,$$

equivalently

$$\langle \xi, \eta(x,\bar{x})\rangle \geq 0 \Rightarrow f(x) > f(\bar{x}),\ \textit{for all } \xi \in \partial^* f(\bar{x}) \textit{ and } x \in X.$$

Definition 2.5. *A function $f : X \to R$ is said to be $(\eta, \partial^* f)$-quasiinvex at $\bar{x} \in X$ if there exists $\eta : X \times X \to X$ such that,*

$$f(x) \leq f(\bar{x}) \Rightarrow \langle \xi, \eta(x,\bar{x}\rangle \leq 0,\ \textit{for all } \xi \in \partial^* f(\bar{x}) \textit{ and } x \in X,$$

equivalently

$$\langle \xi, \eta(x, \bar{x}\rangle > 0 \Rightarrow f(x) > f(\bar{x}),\ \textit{for all } \xi \in \partial^* f(\bar{x})\ \textit{and } x \in X.$$

In order to proceed further, we need the following fundamental concepts of interval mathematics:

Let $\dfrac{\mathbb{A}}{\mathbb{B}} = \left[\dfrac{\alpha_1^L}{\gamma_1^L}, \dfrac{\alpha_1^U}{\gamma_1^U}\right]$ and $\dfrac{\mathbb{C}}{\mathbb{D}} = \left[\dfrac{\alpha_2^L}{\gamma_2^L}, \dfrac{\alpha_2^U}{\gamma_2^U}\right]$ be two fractional closed intervals with $\dfrac{\alpha_1^L}{\gamma_1^L} \leq \dfrac{\alpha_1^U}{\gamma_1^U}$ and $\dfrac{\alpha_2^L}{\gamma_2^L} \leq \dfrac{\alpha_2^U}{\gamma_2^U}$, $\gamma_1^L, \gamma_1^U, \gamma_2^L, \gamma_2^U \neq 0$.

(i) $\dfrac{\mathbb{A}}{\mathbb{B}} + \dfrac{\mathbb{C}}{\mathbb{D}} = \left[\dfrac{\alpha_1^L}{\gamma_1^L} + \dfrac{\alpha_2^L}{\gamma_2^L}, \dfrac{\alpha_1^U}{\gamma_1^U} + \dfrac{\alpha_2^U}{\gamma_2^U}\right]$,

(ii) $\dfrac{-\mathbb{A}}{\mathbb{B}} = \left[\dfrac{-\alpha_1^U}{\gamma_1^U}, \dfrac{-\alpha_1^L}{\gamma_1^L}\right]$,

(iii) $\dfrac{\mathbb{A}}{\mathbb{B}} - \dfrac{\mathbb{C}}{\mathbb{D}} = \dfrac{\mathbb{A}}{\mathbb{B}} + \left(\dfrac{-\mathbb{C}}{\mathbb{D}}\right) = \left[\dfrac{\alpha_1^L}{\gamma_1^L} - \dfrac{\alpha_2^U}{\gamma_2^U}, \dfrac{\alpha_1^U}{\gamma_1^U} - \dfrac{\alpha_2^L}{\gamma_2^L}\right]$,

(iv) $\beta\left(\dfrac{\mathbb{A}}{\mathbb{B}}\right) = \begin{cases} \left[\dfrac{\alpha_1^L}{\gamma_1^L}, \dfrac{\alpha_1^U}{\gamma_1^U}\right], & \text{if } \beta \geq 0, \\ \left[\dfrac{\alpha_1^U}{\gamma_1^U}, \dfrac{\alpha_1^L}{\gamma_1^L}\right], & \text{if } \beta < 0. \end{cases}$

An order relation $\leq_{LU}$ between two intervals $\dfrac{\mathbb{A}}{\mathbb{B}}$ and $\dfrac{\mathbb{C}}{\mathbb{D}}$ are defined as

(i) $\dfrac{\mathbb{A}}{\mathbb{B}} \leq_{LU} \dfrac{\mathbb{C}}{\mathbb{D}}$ iff $\dfrac{\alpha_1^L}{\gamma_1^L} \leq \dfrac{\alpha_2^L}{\gamma_2^L}$ and $\dfrac{\alpha_1^U}{\gamma_1^U} \leq \dfrac{\alpha_2^U}{\gamma_2^U}$.

(ii) $\dfrac{\mathbb{A}}{\mathbb{B}} < \dfrac{\mathbb{C}}{\mathbb{D}}$ iff $\dfrac{\mathbb{A}}{\mathbb{B}} \leq \dfrac{\mathbb{C}}{\mathbb{D}}$ and $\dfrac{\mathbb{A}}{\mathbb{B}} \neq \dfrac{\mathbb{C}}{\mathbb{D}}$, equivalently

$$\begin{cases} \dfrac{\alpha_1^L}{\gamma_1^L} < \dfrac{\alpha_2^L}{\gamma_2^L} \\ \dfrac{\alpha_1^U}{\gamma_1^U} \leq \dfrac{\alpha_2^U}{\gamma_2^U} \end{cases}, \text{ or } \begin{cases} \dfrac{\alpha_1^L}{\gamma_1^L} \leq \dfrac{\alpha_2^L}{\gamma_2^L} \\ \dfrac{\alpha_1^U}{\gamma_1^U} < \dfrac{\alpha_2^U}{\gamma_2^U} \end{cases}, \text{ or } \begin{cases} \dfrac{\alpha_1^L}{\gamma_1^L} < \dfrac{\alpha_2^L}{\gamma_2^L} \\ \dfrac{\alpha_1^U}{\gamma_1^U} < \dfrac{\alpha_2^U}{\gamma_2^U}. \end{cases}$$

Consider the following non-differentiable fractional interval-valued optimization problem:

$$\begin{aligned} &\min \left[\frac{f^L(x), f^U(x)}{g^L(x), g^U(x)}\right] \\ &\text{subject to} \\ &h_i(x) \leq 0, i = 1, 2, ..., m, \\ &x \in X, \end{aligned}$$

which further reduces to the problem

$$\begin{aligned}&\min \left[\frac{f^L(x)}{g^U(x)}, \frac{f^U(x)}{g^L(x)}\right]\\&\text{subject to}\\&h_i(x) \leq 0, i = 1, 2, ..., m,\\&x \in X,\end{aligned}$$

where $f^L(x),\ f^U(x) \geq 0,\ g^L(x),\ g^U(x) > 0$, and $h_i,\ i = 1, 2, \ldots, m$ are continuous functions on X. Set $f^L = p^L,\ g^U = q^L,\ f^U = p^U,\ g^L = q^U$. Then, the above problem reduces to

$$\begin{aligned}\text{(NFIVP)} \qquad &\min \left[\frac{p^L}{q^L}(x), \frac{p^U}{q^U}(x)\right]\\&\text{subject to}\\&h_i(x) \leq 0, i = 1, 2, ..., m,\\&x \in X.\end{aligned}$$

Let $\mathbb{F}$ be the feasible set for the problem (NFIVP).

Definition 2.6. [21] *A feasible point $\bar{x}$ is said to be a LU optimal solution for* (NFIVP) *if and only if there exists no feasible point x such that*

$$\left[\frac{p^L}{q^L}(x), \frac{p^U}{q^U}(x)\right] <_{LU} \left[\frac{p^L}{q^L}(\bar{x}), \frac{p^U}{q^U}(\bar{x})\right].$$

3. Optimality Conditions

For the given feasible solution $\bar{x}$, consider two fractional problems as given below:

$$\begin{aligned}\text{(FP1)} \qquad &\min \phi^L(x) = \frac{p^L}{q^L}(x) \qquad\qquad \text{(FP2)} \qquad \min \phi^U(x) = \frac{p^U}{q^U}(x)\\&\text{subject to} \qquad\qquad\qquad\qquad\quad \text{subject to}\\&h_i(x) \leq 0,\ i = 1, 2, \ldots, m, \qquad\quad h_i(x) \leq 0,\ i = 1, 2, \ldots, m,\\&\frac{p^U}{q^U}(x) \leq \frac{p^U}{q^U}(\bar{x}), \qquad\qquad \frac{p^L}{q^L}(x) \leq \frac{p^L}{q^L}(\bar{x}),\\&x \in X. \qquad\qquad\qquad\qquad\quad x \in X.\end{aligned}$$

The following result gives the relationship between (NFIVP) and (FP1) and (FP2).

Lemma 3.1. [4] *If $\bar{x}$ is a LU optimal solution for the problem (NFIVP) if and only if $\bar{x}$ is an optimal solution for the problems (FP1) and (FP2).*

Lemma 3.2. [4] *$\bar{x}$ is a LU optimum of the problem (NFIVP) if and only if $\bar{x}$ minimizes $\frac{p^L}{q^L}(x)$ on the following constraint set*

$$N = \left\{ x \in X | \frac{p^U}{q^U}(x) \leq \frac{p^U}{q^U}(\bar{x}), h_i(x) \leq 0, i = 1, 2, \ldots, m \right\}.$$

Considered the following single-objective fractional problem:

$$\begin{aligned} \text{(D)} \qquad & \min \phi(x) = \frac{p_1}{q_1}(x) \\ & \text{subject to} \\ & \ell_i(x) \leq 0, i = 1, 2, ..., m, \\ & x \in X, \end{aligned}$$

where p_1, q_1 and ℓ_i, $i = 1, 2, ..., m$ are continuous functions on X such that $p_1(x) \geq 0$ and $q_1(x) > 0$, for all $x \in X$.

On the lines of Theorem 6 of Gadhi [8], we state the following theorem for the problem (D):

Theorem 3.3. *Suppose that $\bar{x}$ is an optimal solution of the problem (D) and a suitable constraint qualification is satisfied at $\bar{x}$. Assume that p_1, q_1 and ℓ_i, $i = 1, 2, ..., m$ are continuous and admit bounded convexificators $\partial^* p_1(\bar{x})$, $\partial^* q_1(\bar{x})$ and $\partial^* \ell_i(\bar{x})$, $i = 1, 2, ..., m$ at $\bar{x}$ respectively and that $\partial^* p_1(\bar{x})$, $\partial^* q_1(\bar{x})$ and $\partial^* \ell_i(\bar{x})$, $i = 1, 2, ..., m$ are upper semicontinuous at $\bar{x}$, then there exist $\lambda > 0$, and $\mu \in R^m_+$ such that*

$$0 \in \lambda \left(\partial^* p_1(\bar{x}) - \phi(\bar{x}) \partial^* q_1(\bar{x}) \right) + \sum_{i=1}^{m} \mu_i \partial^* \ell_i(\bar{x}), \tag{1}$$

$$\mu_i \ell_i(\bar{x}) = 0, j = 1, 2, ..., m, \tag{2}$$

$$\mu_i \geq 0 \text{ and } \ell_i(\bar{x}) \leq 0, \ i = 1, 2, ..., m. \tag{3}$$

Theorem 3.4. (Karush-Kuhn-Tucker Necessary Optimality Conditions) *Suppose that $\bar{x}$ is a LU optimal solution of the problem* (NFIVP) *and a suitable constraint qualification is satisfied at $\bar{x}$. Assume that p^L, q^L, p^U, q^U and h_i, $i = 1, 2, ..., m$ are continuous and admit bounded convexificators $\partial^* p^L(\bar{x})$, $\partial^* q^L(\bar{x})$, $\partial^* p^U(\bar{x})$, $\partial^* q^U(\bar{x})$ and $\partial^* h_i(\bar{x})$, $i = 1, 2, ..., m$ at $\bar{x}$ respectively and that $\partial^* p^L(\bar{x})$, $\partial^* q^L(\bar{x})$, $\partial^* p^L(\bar{x})$, $\partial^* q^L(\bar{x})$, and $\partial^* h_i(\bar{x})$, $i = 1, 2, ..., m$ are upper semicontinuous at $\bar{x}$, then there exist $\lambda^L > 0$, $\lambda^U > 0$ and $\mu \in R^m_+$ such that*

$$\begin{aligned} 0 \in & \lambda^L \left(\partial^* p^L(\bar{x}) - \phi^L(\bar{x}) \partial^* q^L(\bar{x}) \right) \\ & + \lambda^U \left(\partial^* p^U(\bar{x}) - \phi^U(\bar{x}) \partial^* q^U(\bar{x}) \right) + \sum_{i=1}^{m} \mu_i \partial^* h_i(\bar{x}), \end{aligned} \tag{4}$$

$$\mu_i h_i(\bar{x}) = 0, j = 1, 2, ..., m, \tag{5}$$

$$\mu_i \geq 0 \text{ and } h_i(\bar{x}) \leq 0, \ i = 1, 2, ..., m. \tag{6}$$

Proof. By assumption, $\bar{x}$ is a LU optimal solution for the problem (NFIVP), and a suitable constraint qualification is satisfied at $\bar{x}$. Since $\bar{x}$ is an LU optimal solution, by Lemma 3.1, $\bar{x}$ is also a optimal solution for the problems (FP1) and (FP2). Hence, by Lemma 3.2, at $\bar{x}$ the minimum value of $\frac{p^L}{q^L}(x)$ is obtained on the constraint set

$$N_L = \left\{ x \in X | \frac{p^U}{q^U}(x) \leq \frac{p^U}{q^U}(\bar{x}), h_i(x) \leq 0, i = 1, 2, ..., m \right\},$$

and the minimum value of $\frac{p^U}{q^U}(x)$ is obtained at $\bar{x}$ on the constraint set

$$N_U = \left\{ x \in X | \frac{p^L}{q^L}(x) \leq \frac{p^L}{q^L}(\bar{x}), h_i(x) \leq 0, i = 1, 2, ..., m \right\}.$$

By Theorem 3.3, it follows that there exist $\lambda^{LL} > 0, \lambda^{LU} > 0, \mu^L \in R_+^m$ and $\lambda^{UL} > 0, \lambda^{UU} > 0, \mu^U \in R_+^m$ such that

$$\begin{aligned} 0 \in \lambda^{LL} \left(\partial^* p^L(\bar{x}) - \phi^L(\bar{x}) \partial^* q^L(\bar{x}) \right) \\ + \lambda^{LU} \left(\partial^* p^U(\bar{x}) - \phi^U(\bar{x}) \partial^* q^U(\bar{x}) \right) + \sum_{i=1}^{m} \mu_i^L \partial^* h_i(\bar{x}), \end{aligned} \tag{7}$$

$$\mu_i^L h_i(\bar{x}) = 0, j = 1, 2, ..., m, \tag{8}$$

$$\mu_i^L \geq 0 \text{ and } h_i(\bar{x}) \leq 0, \ i = 1, 2, ..., m. \tag{9}$$

and

$$\begin{aligned} 0 \in \lambda^{UL} \left(\partial^* p^L(\bar{x}) - \phi^L(\bar{x}) \partial^* q^L(\bar{x}) \right) \\ + \lambda^{UU} \left(\partial^* p^U(\bar{x}) - \phi^U(\bar{x}) \partial^* q^U(\bar{x}) \right) + \sum_{i=1}^{m} \mu_i^U \partial^* h_i(\bar{x}), \end{aligned} \tag{10}$$

$$\mu_i^U h_i(\bar{x}) = 0, j = 1, 2, ..., m, \tag{11}$$

$$\mu_i^U \geq 0 \text{ and } h_i(\bar{x}) \leq 0, \ i = 1, 2, ..., m. \tag{12}$$

From (7) to (12), we have

$$\begin{aligned} 0 \in [\lambda^{LL} + \lambda^{UL}] \left(\partial^* p^L(\bar{x}) - \phi^L(\bar{x}) \partial^* q^L(\bar{x}) \right) \\ + [\lambda^{LU} + \lambda^{UU}] \left(\partial^* p^U(\bar{x}) - \phi^U(\bar{x}) \partial^* q^U(\bar{x}) \right) + \sum_{i=1}^{m} [\mu_i^L + \mu_i^U] \partial^* h_i(\bar{x}), \end{aligned} \tag{13}$$

$$[\mu_i^L + \mu_i^U] h_i(\bar{x}) = 0, j = 1, 2, ..., m, \tag{14}$$

$$[\mu_i^L + \mu_i^U] \geq 0 \text{ and } h_i(\bar{x}) \leq 0, \ i = 1, 2, ..., m. \tag{15}$$

Let us denote $\lambda^{LL} + \lambda^{UL} = \lambda^L$, $\lambda^{LU} + \lambda^{UU} = \lambda^U$ and $\mu^L + \mu^U = \mu$. Thus, from

(13)-(15), it yields

$$0 \in \lambda^L \left(\partial^* p^L(\bar{x}) - \phi^L(\bar{x})\partial^* q^L(\bar{x})\right)$$
$$+\lambda^U \left(\partial^* p^U(\bar{x}) - \phi^U(\bar{x})\partial^* q^U(\bar{x})\right) + \sum_{i=1}^{m} \mu_i \partial^* h_i(\bar{x}),$$
$$\mu_i h_i(\bar{x}) = 0, j = 1, 2, ..., m,$$
$$\mu_i \geq 0 \text{ and } h_i(\bar{x}) \leq 0,\ i = 1, 2, ..., m.$$

This completes the proof. ∎

Theorem 3.5. (Sufficient Optimality Conditions) *Suppose that $\bar{x}$ is a feasible solution of* (NFIVP) *and there exist $\lambda^L > 0, \lambda^U > 0, \mu \in R_+^m$ such that* (4)–(6) *are satisfied at $\bar{x}$. Also, assume that*

(i) *$p^L(.) - \phi^L(\bar{x})q^L(.)$ and $p^U(.) - \phi^U(\bar{x})q^U(.)$ are respectively $(\eta, \partial^* p^L - \phi^L(\bar{x})\partial^* q^L)$-invex and $(\eta, \partial^* p^U - \phi^U(\bar{x})\partial^* q^U)$-invex at $\bar{x}$,*

(ii) *$\mu_i h_i$, for $i = 1, 2, ..., m$, is $(\eta, \partial^* h_i(.))$-invex at $\bar{x}$.*

Then $\bar{x}$ is a LU optimal solution for (NFIVP).

Proof. By assumption, (4)-(6) are satisfied at $\bar{x}$ with Lagrange multipliers $\lambda^L > 0, \lambda^U > 0, \mu \in R_+^m$. As it follows from (4), there exist $\xi^L \in \partial^* p^L(\bar{x})$, $\nu^L \in \partial^* q^L(\bar{x})$, $\xi^U \in \partial^* p^U(\bar{x})$, $\nu^U \in \partial^* q^U(\bar{x})$, and $\zeta_i \in \partial^* h_i(\bar{x}),\ i = 1, 2, ..., m$, such that

$$\lambda^L \left[\xi^L - \phi^L(\bar{x})\nu^L\right] + \lambda^U \left[\xi^U - \phi^U(\bar{x})\nu^U\right] + \sum_{i=1}^{m} \mu_i \zeta_i = 0. \tag{16}$$

Suppose contrary to the result, that $\bar{x}$ is not a LU optimal solution for (NFIVP). Hence, by Definition 2.6, there exists a feasible solution x such that

$$\left[\frac{p^L}{q^L}(x), \frac{p^U}{q^U}(x)\right] <_{LU} \left[\frac{p^L}{q^L}(\bar{x}), \frac{p^U}{q^U}(\bar{x})\right]$$

that is $\begin{cases} \frac{p^L}{q^L}(x) < \frac{p^L}{q^L}(\bar{x}) \\ \frac{p^U}{q^U}(x) \leq \frac{p^U}{q^U}(\bar{x}) \end{cases}$, or $\begin{cases} \frac{p^L}{q^L}(x) \leq \frac{p^L}{q^L}(\bar{x}) \\ \frac{p^U}{q^U}(x) < \frac{p^U}{q^U}(\bar{x}) \end{cases}$, or $\begin{cases} \frac{p^L}{q^L}(x) < \frac{p^L}{q^L}(\bar{x}) \\ \frac{p^U}{q^U}(x) < \frac{p^U}{q^U}(\bar{x}) \end{cases}$.

This implies

$$p^L(x) - \phi^L(\bar{x})q^L(x) < p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x}),$$
$$p^U(x) - \phi^U(\bar{x})q^U(x) \leq p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x}),$$

or

$$p^L(x) - \phi^L(\bar{x})q^L(x) \leq p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x}),$$
$$p^U(x) - \phi^U(\bar{x})q^U(x) < p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x}),$$

or

$$p^L(x) - \phi^L(\bar{x})q^L(x) < p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x}),$$
$$p^U(x) - \phi^U(\bar{x})q^U(x) < p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x}).$$

From hypothesis (i), $p^L(.) - \phi^L(\bar{x})q^L(.)$ and $p^U(.) - \phi^U(\bar{x})q^U(.)$ are respectively $(\eta, \partial^* p^L - \phi^L(\bar{x})\partial^* q^L)$-invex and $(\eta, \partial^* p^U - \phi^U(\bar{x})\partial^* q^U)$-invex at $\bar{x}$ and therefore, there exists $\eta : X \times X \to X$ such that

$$\left\langle \left[\xi^L - \phi^L(\bar{x})\nu^L\right], \eta(x, \bar{x}) \right\rangle \leq 0, \text{ for all } \xi^L \in \partial^* p^L(\bar{x}), \text{ and } \nu^L \in \partial^* q^L(\bar{x}),$$

$$\left\langle \left[\xi^U - \phi^U(\bar{x})\nu^U\right], \eta(x, \bar{x}) \right\rangle < 0, \text{ for all } \xi^U \in \partial^* p^U(\bar{x}), \text{ and } \nu^U \in \partial^* q^U(\bar{x}),$$

or

$$\left\langle \left[\xi^L - \phi^L(\bar{x})\nu^L\right], \eta(x, \bar{x}) \right\rangle < 0, \text{ for all } \xi^L \in \partial^* p^L(\bar{x}), \text{ and } \nu^L \in \partial^* q^L(\bar{x}),$$

$$\left\langle \left[\xi^U - \phi^U(\bar{x})\nu^U\right], \eta(x, \bar{x}) \right\rangle \leq 0, \text{ for all } \xi^U \in \partial^* p^U(\bar{x}), \text{ and } \nu^U \in \partial^* q^U(\bar{x}),$$

or

$$\left\langle \left[\xi^L - \phi^L(\bar{x})\nu^L\right], \eta(x, \bar{x}) \right\rangle < 0, \text{ for all } \xi^L \in \partial^* p^L(\bar{x}), \text{ and } \nu^L \in \partial^* q^L(\bar{x}),$$

$$\left\langle \left[\xi^U - \phi^U(\bar{x})\nu^U\right], \eta(x, \bar{x}) \right\rangle < 0, \text{ for all } \xi^U \in \partial^* p^U(\bar{x}), \text{ and } \nu^U \in \partial^* q^U(\bar{x}).$$

From the fact $\lambda^L > 0$, $\lambda^U > 0$ and by above inequalities, we have

$$\left\langle \lambda^L \left[\xi^L - \phi^L(\bar{x})\nu^L\right] + \lambda^U \left[\xi^U - \phi^U(\bar{x})\nu^U\right], \eta(x, \bar{x}) \right\rangle < 0. \tag{17}$$

On the other hand, by using the feasibility of x, $\mu_i \geq 0$, $i = 1, 2, ..., m$ and (5), we obtain

$$\mu_i h_i(x) \leq \mu_i h_i(\bar{x}), i = 1, 2, ..., m,$$

which by hypothesis (ii), we get

$$\langle \mu_i \zeta_i, \eta(x, \bar{x}) \rangle \leq 0, \text{ for all } \zeta_i \in \partial^* h_i(\bar{x}), \ i = 1, 2, ..., m. \tag{18}$$

On adding (17) and (18), we have

$$\left\langle \lambda^L \left[\xi^L - \phi^L(\bar{x})\nu^L\right] + \lambda^U \left[\xi^U - \phi^U(\bar{x})\nu^U\right] + \sum_{i=1}^{m} \mu_i \zeta_i, \eta(x, \bar{x}) \right\rangle < 0,$$

which contradicts (16). Hence, $\bar{x}$ is a LU optimal solution for (NFIVP). ■

In order to illustrate the sufficient optimality conditions established in the Theorem 3.5, we consider the following example:

Example 3.6.

$$\begin{aligned}(\text{IVP1}) \qquad & \min\left[\frac{f_1^L(x), f_1^U(x)}{g_1^L(x), g_1^U(x)}\right] \\ & = \min\left[\frac{2x^2, x^2+1}{-x+4, -x^2+6}\right] \\ & \text{subject to} \\ & h_1(x) = -x+2 \leq 0, x \in X = R.\end{aligned}$$

Now, we rewrite the considered optimization problem in the following manner

$$\begin{aligned}& \min\left[\frac{2x^2}{-x^2+6}, \frac{x^2+1}{-x+4}\right] \\ & \text{subject to} \\ & h_1(x) = -x+2 \leq 0, x \in X = R.\end{aligned}$$

where $\frac{p_1^L}{q_1^L}(x) = \frac{2x^2}{-x^2+6}$, $\frac{p_1^U}{q_1^U}(x) = \frac{x^2+1}{-x+4}$. The feasible set is $\mathbb{F}_1 = \{x : -x+2 \leq 0, x \in S\}$. By simple calculations, for the feasible point $\bar{x} = 2$, we see that $\partial^* p^L(\bar{x}) = \{-8, 8\}$, $\partial^* q^L(\bar{x}) = \{-4, 4\}$, $\partial^* p^U(\bar{x}) = \{-4, 4\}$, $\partial^* q^U(\bar{x}) = \{-1, 1\}$ and $\partial^* h_1(\bar{x}) = \{-1, 1\}$. Also, we see that for the feasible point $\bar{x} = 2$, there exist $\lambda^L > 0, \lambda^U > 0, \mu \in R_+^m$ such that (4)-(6) are satisfied at $\bar{x}$ and it is easy to see that

(i) $p_1^L(.) - \phi_1^L(\bar{x})q_1^L(.)$ and $p_1^U(.) - \phi_1^U(\bar{x})q_1^U(.)$ are respectively $(\eta, \partial^* p_1^L - \phi_1^L(\bar{x})\partial^* q_1^L)$-invex and $(\eta, \partial^* p_1^U - \phi_1^U(\bar{x})\partial^* q_1^U)$-invex at $\bar{x}$,

(ii) μh_1 is $(\eta, \partial^* h_1(.))$-invex at $\bar{x}$.

Therefore, by Theorem 3.5, $\bar{x} = 2$ is a LU optimal solution for (IVP1).

Theorem 3.7. (Sufficient Optimality Conditions) *Suppose that $\bar{x}$ is a feasible solution of* (NFIVP) *and there exist $\lambda^L > 0, \lambda^U > 0, \mu \in R_+^m$ such that* (4)–(6) *are satisfied at $\bar{x}$. Also, assume that*

(i) $\lambda^L[p^L(.) - \phi^L(\bar{x})q^L(.)] + \lambda^U[p^U(.) - \phi^U(\bar{x})q^U(.)]$ *is* $(\eta, \lambda^L[\partial^* p^L - \phi^L(\bar{x})\partial^* q^L] + \lambda^U[\partial^* p^U - \phi^U(\bar{x})\partial^* q^U])$*-pseudoinvex at $\bar{x}$,*

(ii) $\mu_i h_i$*, for $i = 1, 2, ..., m$, is $(\eta, \partial^* h_i(.))$-quasiinvex at $\bar{x}$.*

Then $\bar{x}$ is a LU optimal solution for (NFIVP).

Proof. By assumption, (4)-(6) are satisfied at $\bar{x}$ with Lagrange multipliers $\lambda^L > 0, \lambda^U > 0, \mu \in R_+^m$. As it follows from (4), there exist $\xi^L \in \partial^* p^L(\bar{x})$, $\nu^L \in \partial^* q^L(\bar{x})$, $\xi^U \in \partial^* p^U(\bar{x})$, $\nu^U \in \partial^* q^U(\bar{x})$, and $\zeta_i \in \partial^* h_i(\bar{x})$, $i = 1, 2, ..., m$, such that

$$\lambda^L\left[\xi^L - \phi^L(\bar{x})\nu^L\right] + \lambda^U\left[\xi^U - \phi^U(\bar{x})\nu^U\right] + \sum_{i=1}^{m}\mu_i\zeta_i = 0. \tag{19}$$

Suppose contrary to the result, that $\bar{x}$ is not a LU optimal solution for (NFIVP). Hence, by Definition 2.6, there exist a feasible solution x such that

$$\left[\frac{p^L}{q^L}(x), \frac{p^U}{q^U}(x)\right] <_{LU} \left[\frac{p^L}{q^L}(\bar{x}), \frac{p^U}{q^U}(\bar{x})\right]$$

that is

$$\begin{cases} \frac{p^L}{q^L}(x) < \frac{p^L}{q^L}(\bar{x}) \\ \frac{p^U}{q^U}(x) \leq \frac{p^U}{q^U}(\bar{x}) \end{cases}, \text{ or } \begin{cases} \frac{p^L}{q^L}(x) \leq \frac{p^L}{q^L}(\bar{x}) \\ \frac{p^U}{q^U}(x) < \frac{p^U}{q^U}(\bar{x}) \end{cases}, \text{ or } \begin{cases} \frac{p^L}{q^L}(x) < \frac{p^L}{q^L}(\bar{x}) \\ \frac{p^U}{q^U}(x) < \frac{p^U}{q^U}(\bar{x}) \end{cases}.$$

This implies

$$p^L(x) - \phi^L(\bar{x})q^L(x) < p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x}),$$
$$p^U(x) - \phi^U(\bar{x})q^U(x) \leq p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x}),$$

or

$$p^L(x) - \phi^L(\bar{x})q^L(x) \leq p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x}),$$
$$p^U(x) - \phi^U(\bar{x})q^U(x) < p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x}),$$

or

$$p^L(x) - \phi^L(\bar{x})q^L(x) < p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x}),$$
$$p^U(x) - \phi^U(\bar{x})q^U(x) < p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x}).$$

From the fact $\lambda^L > 0$, $\lambda^U > 0$ and by above inequalities, we have

$$\begin{aligned} &\lambda^L[p^L(x) - \phi^L(\bar{x})q^L(x)] + \lambda^U[p^U(x) - \phi^U(\bar{x})q^U(x)] \\ &< \lambda^L[p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x})] + \lambda^U[p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x})]. \end{aligned}$$

From hypothesis (i), $\lambda^L[p^L(.) - \phi^L(\bar{x})q^L(.)] + \lambda^U[p^U(.) - \phi^U(\bar{x})q^U(.)]$ is $(\eta, \lambda^L[\partial^* p^L - \phi^L(\bar{x})\partial^* q^L] + \lambda^U[\partial^* p^U - \phi^U(\bar{x})\partial^* q^U])$-pseudoinvex at $\bar{x}$ and therefore, there exists $\eta : X \times X \to X$ such that

$$\left\langle \lambda^L\left[\xi^L - \phi^L(\bar{x})\nu^L\right] + \lambda^U\left[\xi^U - \phi^U(\bar{x})\nu^U\right], \eta(x, \bar{x})\right\rangle < 0, \tag{20}$$

for all $\xi^L \in \partial^* p^L(\bar{x}), \nu^L \in \partial^* q^L(\bar{x}), \xi^U \in \partial^* p^U(\bar{x})$, and $\nu^U \in \partial^* q^U(\bar{x})$.

On the other hand, by using the feasibility of x, $\mu_i \geq 0$, $i = 1, 2, ..., m$ and (5), we obtain

$$\mu_i h_i(x) \leq \mu_i h_i(\bar{x}), i = 1, 2, ..., m,$$

which by hypothesis (ii), we get

$$\langle \mu_i \zeta_i, \eta(x, \bar{x})\rangle \leq 0, \text{ for all } \zeta_i \in \partial^* h_i(\bar{x}),\ i = 1, 2, ..., m. \tag{21}$$

On adding (20) and (21), we have

$$\left\langle \lambda^L \left[\xi^L - \phi^L(\bar{x})\nu^L\right] + \lambda^U \left[\xi^U - \phi^U(\bar{x})\nu^U\right] + \sum_{i=1}^{m} \mu_i \zeta_i, \eta(x, \bar{x}) \right\rangle < 0,$$

which contradicts (19). Hence, $\bar{x}$ is a LU optimal solution for (NFIVP). ∎

4. Lagrangian Type Function and Saddle-point Analysis

In this section, for the feasible point $\bar{x} \in \mathbb{F}$, we define the Lagrangian type function for the primal problem (NFIVP) as follows:

$$L(x, \lambda^L, \lambda^U, \mu) = \lambda^L \left(p^L(x) - \phi^L(\bar{x})q^L(x)\right) + \lambda^U \left(p^U(x) - \phi^U(\bar{x})q^U(x)\right) + \sum_{i=1}^{m} \mu_i h_i(x)$$

where $x \in X$, $\lambda^L \geq 0$, $\lambda^U \geq 0$ and $\mu \in R_+^m$. Now, we define a saddle-point of $L(x, \lambda^L, \lambda^U, \mu)$ and subsequently we discuss its relation to the problem (NFIVP).

Definition 4.1. *A point $(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu}) \in X \times R_+ \times R_+ \times R_+^m$ is said to be a saddle point for $L(x, \lambda^L, \lambda^U, \mu)$, if*

(i) $L(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \mu) \leq L(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu})$, *for all* $\mu \in R_+^m$,
(ii) $L(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu}) \leq L(x, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu})$, *for all* $x \in X$.

Theorem 4.2. *Let $\bar{\lambda}^L > 0$, $\bar{\lambda}^U > 0$ and $(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu})$ be a saddle point for $L(x, \lambda^L, \lambda^U, \mu)$. Then $\bar{x}$ is a LU optimal solution to* (NFIVP).

Proof. Suppose contrary to the result, that $\bar{x}$ is not a LU optimal solution for (NFIVP). Hence, by Definition 2.6, there exists a feasible solution x such that

$$\left[\frac{p^L}{q^L}(x), \frac{p^U}{q^U}(x)\right] <_{LU} \left[\frac{p^L}{q^L}(\bar{x}), \frac{p^U}{q^U}(\bar{x})\right]$$

that is

$$\begin{cases} \dfrac{p^L}{q^L}(x) < \dfrac{p^L}{q^L}(\bar{x}) \\ \dfrac{p^U}{q^U}(x) \leq \dfrac{p^U}{q^U}(\bar{x}) \end{cases}, \text{ or } \begin{cases} \dfrac{p^L}{q^L}(x) \leq \dfrac{p^L}{q^L}(\bar{x}) \\ \dfrac{p^U}{q^U}(x) < \dfrac{p^U}{q^U}(\bar{x}) \end{cases}, \text{ or } \begin{cases} \dfrac{p^L}{q^L}(x) < \dfrac{p^L}{q^L}(\bar{x}) \\ \dfrac{p^U}{q^U}(x) < \dfrac{p^U}{q^U}(\bar{x}) \end{cases}$$

This implies

$$p^L(x) - \phi^L(\bar{x})q^L(x) < p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x}),$$

$$p^U(x) - \phi^U(\bar{x})q^U(x) \leq p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x}),$$

or

$$p^L(x) - \phi^L(\bar{x})q^L(x) \leq p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x}),$$
$$p^U(x) - \phi^U(\bar{x})q^U(x) < p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x}),$$

or

$$p^L(x) - \phi^L(\bar{x})q^L(x) < p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x}),$$
$$p^U(x) - \phi^U(\bar{x})q^U(x) < p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x}).$$

By above inequalities and from $\lambda^L > 0$, $\lambda^U > 0$, we have

$$\begin{aligned} &\bar{\lambda}^L[p^L(x) - \phi^L(\bar{x})q^L(x)] + \bar{\lambda}^U[p^U(x) - \phi^U(\bar{x})q^U(x)] \\ &< \bar{\lambda}^L[p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x})] + \bar{\lambda}^U[p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x})]. \end{aligned} \tag{22}$$

Since $(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu})$ is a saddle point for $L(x, \lambda^L, \lambda^U, \mu)$, by Definition 4.1 (i), we get

$$L(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \mu) \leq L(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu}),$$

that is,

$$\sum_{i=1}^{m} \mu_i h_i(\bar{x}) \leq \sum_{i=1}^{m} \bar{\mu}_i h_i(\bar{x}). \tag{23}$$

Taking $(\mu_1, \mu_2, ...\mu_{i-1}, \mu_i, \mu_{i+1}, ..., \mu_m) = (\bar{\mu}_1, \bar{\mu}_2, ...\bar{\mu}_{i-1}, \bar{\mu}_i + 1, \bar{\mu}_{i+1}, ..., \bar{\mu}_m)$ in the above inequality (23), we obtain

$$h_i(\bar{x}) \leq 0, i = 1, 2, ..., m,$$

which shows that $\bar{x}$ is a feasible solution to (NFIVP).

Using $\bar{\mu} \in R_+^m$, above inequality implies

$$\bar{\mu}_i h_i(\bar{x}) \leq 0, i = 1, 2, ..., m. \tag{24}$$

Again taking $\mu_i = 0$, $i = 1, 2, ..., m$, in the inequality (23), we get

$$\bar{\mu}_i h_i(\bar{x}) \geq 0, i = 1, 2, ..., m. \tag{25}$$

From the inequalities (24) and (25), we conclude that

$$\bar{\mu}_i h_i(\bar{x}) = 0, i = 1, 2, ..., m. \tag{26}$$

On other hand, since $(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu})$ is a saddle point for $L(x, \lambda^L, \lambda^U, \mu)$, by Definition 4.1 (ii), we get

$$L(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu}) \leq L(x, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu}),$$

that is

$$\begin{aligned} &\bar{\lambda}^L \left(p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x})\right) + \bar{\lambda}^U \left(p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x})\right) + \sum_{i=1}^{m} \bar{\mu}_i h_i(\bar{x}) \\ &\leq \bar{\lambda}^L \left(p^L(x) - \phi^L(\bar{x})q^L(x)\right) + \bar{\lambda}^U \left(p^U(x) - \phi^U(\bar{x})q^U(x)\right) + \sum_{i=1}^{m} \bar{\mu}_i h_i(x) \end{aligned}$$

Using the feasibilty of x of the problem (NFIVP) together with $\bar{\mu} \in R_+^m$ and (26), above inequality gives

$$\begin{aligned} &\bar{\lambda}^L \left(p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x})\right) + \bar{\lambda}^U \left(p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x})\right) \\ \leq\ &\bar{\lambda}^L \left(p^L(x) - \phi^L(\bar{x})q^L(x)\right) + \bar{\lambda}^U \left(p^U(x) - \phi^U(\bar{x})q^U(x)\right). \end{aligned}$$

This contradicts (22). Hence the proof. ∎

Theorem 4.3. *Let $\bar{x}$ be a LU optimal solution to* (NFIVP) *and assume that there exist $\bar{\lambda}^L > 0, \bar{\lambda}^U > 0, \bar{\mu} \in R_+^m$ such that* (4)–(6) *are satisfied at $\bar{x}$. Also, assume that*

(i) *$p^L(.) - \phi^L(\bar{x})q^L(.)$ and $p^U(.) - \phi^U(\bar{x})q^U(.)$ are respectively $(\eta, \partial^* p^L - \phi^L(\bar{x})\partial^* q^L)$-invex and $(\eta, \partial^* p^U - \phi^U(\bar{x})\partial^* q^U)$-invex at $\bar{x}$.*

(ii) *$\mu_i h_i$, for $i = 1, 2, ..., m$, is $(\eta, \partial^* h_i(.))$-invex at $\bar{x}$.*

Then $(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu})$ is a saddle point for $L(x, \lambda^L, \lambda^U, \mu)$.

Proof. By assumption, (4)–(6) are satisfied at $\bar{x}$ with Lagrange multipliers $\bar{\lambda}^L > 0, \bar{\lambda}^U > 0, \bar{\mu} \in R_+^m$. As it follows from (4), there exist $\xi^L \in \partial^* p^L(\bar{x})$, $\nu^L \in \partial^* q^L(\bar{x})$, $\xi^U \in \partial^* p^U(\bar{x})$, $\nu^U \in \partial^* q^U(\bar{x})$, and $\zeta_i \in \partial^* h_i(\bar{x})$, $i = 1, 2, ..., m$, such that

$$\bar{\lambda}^L \left[\xi^L - \phi^L(\bar{x})\nu^L\right] + \bar{\lambda}^U \left[\xi^U - \phi^U(\bar{x})\nu^U\right] + \sum_{i=1}^{m} \bar{\mu}_i \zeta_i = 0. \tag{27}$$

From the hypothesis (i), $p^L(.) - \phi^L(\bar{x})q^L(.)$ and $p^U(.) - \phi^U(\bar{x})q^U(.)$ are respectively $(\eta, \partial^* p^L - \phi^L(\bar{x})\partial^* q^L)$-invex and $(\eta, \partial^* p^U - \phi^U(\bar{x})\partial^* q^U)$-invex at $\bar{x}$, therefore, there exists $\eta : X \times X \to X$ such that

$$\begin{aligned} &[p^L(x) - \phi^L(\bar{x})q^L(x)] - [p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x})] \\ \geq\ &\left\langle \left[\xi^L - \phi^L(\bar{x})\nu^L\right], \eta(x, \bar{x}) \right\rangle, \text{ for all } \xi^L \in \partial^* p^L(\bar{x}), \text{ and } \nu^L \in \partial^* q^L(\bar{x}), \end{aligned}$$

and

$$\begin{aligned} &[p^U(x) - \phi^U(\bar{x})q^U(x)] - [p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x})] \\ \geq\ &\left\langle \left[\xi^U - \phi^U(\bar{x})\nu^U\right], \eta(x, \bar{x}) \right\rangle, \text{ for all } \xi^U \in \partial^* p^U(\bar{x}), \text{ and } \nu^U \in \partial^* q^U(\bar{x}). \end{aligned}$$

From the fact $\bar{\lambda}^L > 0$, $\bar{\lambda}^U > 0$ and by above inequalities, we have

$$\begin{aligned} &\bar{\lambda}^L[p^L(x) - \phi^L(\bar{x})q^L(x)] - \bar{\lambda}^L[p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x})] \\ \geq\ &\left\langle \bar{\lambda}^L \left[\xi^L - \phi^L(\bar{x})\nu^L\right], \eta(x, \bar{x} \right\rangle, \text{ for all } \xi^L \in \partial^* p^L(\bar{x}), \text{ and } \nu^L \in \partial^* q^L(\bar{x}), \end{aligned} \tag{28}$$

and

$$\begin{aligned} &\bar{\lambda}^U[p^U(x) - \phi^U(\bar{x})q^U(x)] - \bar{\lambda}^U[p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x})] \\ \geq\ &\left\langle \bar{\lambda}^U \left[\xi^U - \phi^U(\bar{x})\nu^U\right], \eta(x, \bar{x}) \right\rangle, \text{ for all } \xi^U \in \partial^* p^U(\bar{x}), \text{ and } \nu^U \in \partial^* q^U(\bar{x}). \end{aligned} \tag{29}$$

From hypothesis (ii), we get

$$\sum_{i=1}^{m} \bar{\mu}_i h_i(x) - \sum_{i=1}^{m} \bar{\mu}_i h_i(\bar{x}) \geq \left\langle \sum_{i=1}^{m} \bar{\mu}_i \zeta_i, \eta(x, \bar{x}) \right\rangle, \tag{30}$$

for all $\zeta_i \in \partial^* h_i(\bar{x}),\ i = 1, 2, ..., m.$

On adding (28)-(30), we have

$$\begin{aligned}
&\bar{\lambda}^L[p^L(x) - \phi^L(\bar{x})q^L(x)] + \bar{\lambda}^U[p^U(x) - \phi^U(\bar{x})q^U(x)] + \sum_{i=1}^{m} \bar{\mu}_i h_i(x) \\
&- \left[\bar{\lambda}^L[p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x})] + \bar{\lambda}^U[p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x})] + \sum_{i=1}^{m} \bar{\mu}_i h_i(\bar{x})\right] \\
&\geq \left\langle \bar{\lambda}^L\left[\xi^L - \phi^L(\bar{x})\nu^L\right] + \bar{\lambda}^U\left[\xi^U - \phi^U(\bar{x})\nu^U\right] + \sum_{i=1}^{m} \bar{\mu}_i \zeta_i,\ \eta(x, \bar{x}) \right\rangle,
\end{aligned}$$

which by (27), yields

$$\begin{aligned}
&\bar{\lambda}^L[p^L(x) - \phi^L(\bar{x})q^L(x)] + \bar{\lambda}^U[p^U(x) - \phi^U(\bar{x})q^U(x)] + \sum_{i=1}^{m} \bar{\mu}_i h_i(x) \\
&\geq \left[\bar{\lambda}^L[p^L(\bar{x}) - \phi^L(\bar{x})q^L(\bar{x})] + \bar{\lambda}^U[p^U(\bar{x}) - \phi^U(\bar{x})q^U(\bar{x})] + \sum_{i=1}^{m} \bar{\mu}_i h_i(\bar{x})\right],
\end{aligned}$$

that is

$$L(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu}) \leq L(x, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu}). \tag{31}$$

On the other hand, using the feasibility of $\bar{x}$ of the problem (NFIVP) and the fact $\mu \in R^m_+$, we have

$$\mu_i h_i(\bar{x}) \leq 0, i = 1, 2, ..., m, \tag{32}$$

By using (32) and the optimality conditions (5), we get

$$L(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \mu) \leq L(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu}). \tag{33}$$

By inequalities (31) and (33) we conclude that $(\bar{x}, \bar{\lambda}^L, \bar{\lambda}^U, \bar{\mu})$ is a saddle point for $L(x, \lambda^L, \lambda^U, \mu)$. Hence the proof. ∎

5. Conclusion

In this paper, with the idea of convexificators, we have discussed optimality conditions and saddle point criteria for a nonconvex fractional interval-valued optimization problem. Also, we provided an example to validate the results of sufficient optimality conditions established in this paper. In our opinion,

the techniques employed in this paper can be extended for proving the similar results for other classes of fractional programming problems with the functions involving are convexificators. This may be the topic of some of our forthcoming papers.

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Effect of Microwave irradiations on germination of *Trigonella foenum-graecum*

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Abstract
The aim of the study was to evaluate the effect of microwave treatment on seed germination and radicle length of fenugreek seeds subjected. Microwaves - electromagnetic radiations have frequencies ranging from 300 MHz to 300 GHz. Microwaves also cause massive electromagnetic pollution. Fenugreek seeds were exposed to three different microwave exposure periods of 0 to 30 seconds. There was a considerable increase in the germination percentage and radicle length after shorter exposure periods to the microwave. However, longer exposure periods reduced the germination percentage and decreased the radicle length. The positive effects of microwaves could be of considerable importance for better crop production.
Keywords: Microwaves, seed germination, fenugreek

I. Introduction

Germination of seed is a fundamental process by which plant species grow from a seed into a plant. Germination commences with the uptake of water by imbibition by the dry seed, followed by embryo expansion. The uptake of water is triphasic with a rapid initial uptake, imbibition phase followed by a plateau phase. A further increase in water uptake phase occurs as the embryo axis elongates and breaks through the covering layers to complete germination (Schopfer P, Plachy C and Frahry G. 2001). Once the radicle and plumule emerge, the shoot starts growing upwards. In the last stage, the cells of the seed become metabolically active, elongate and give rise to the seedling.

Applications of microwave energy in agriculture have increased over the past 50 years. (Vadivambal *et al*., 2007). Microwaves form part of the Electromagnetic spectrum whose frequencies range from 300 MHz TO 300 GHz (Manoj Gupta and WongWai Leong Eugene, 2001). Microwaves are classified into three bands: Ultra High Frequency band (300 MHz to 3 GHz), Super high frequency band (3 to 30 GHz) and Extremely High Frequency band (30 to 300 GHz). Microwaves are used for their ability to heat materials. Few applications are microwave heating, radar detection, electronic warfare, scientific, power transmission, communication, navigation, medical, industrial, weather control. Research has shown that microwaves radiations affect seed germination, plant growth and also plant development. Various studies have been conducted to understand the effect of microwaves on seed germination.

The aim of the present investigation is to understand the effect of microwave radiations on fenugreek seed germination.

II.Materials and Methods

Equipment used

LG Microwave, 1200 W, 230 V, 50 Hz is used for this investigation.

Fig.1. Parts of Microwave

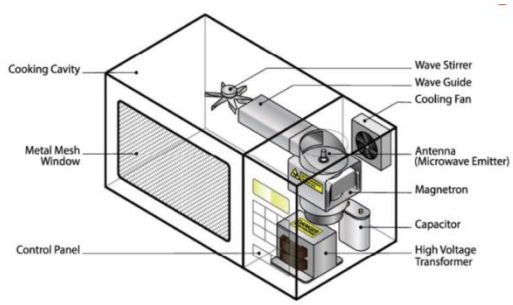
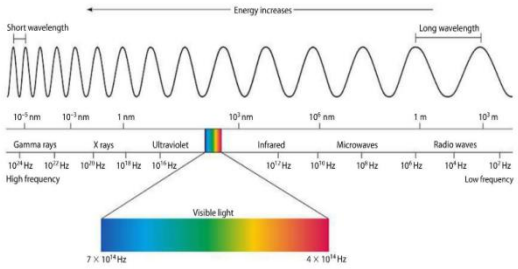


Fig.2. Electromagnetic spectrum



Experimental Design

The seeds of *Trigonella foenum-graecum (*Fenugreek) were used for the present investigation. 100 seeds were taken for each period of exposure. 100 uniform sized seeds were placed in 5 different petri dishes. Each petri dish with the seeds was exposed to microwaves for duration of 10, 20, 30, seconds (1200 Watts). The seeds were soaked for 2 hours and were then evenly spread on the moistened filter papers in the Petri dishes. Water was regularly sprinkled on the filter paper, to ensure that the seeds get sufficient moisture for the imbibition process to begin. All Petri dishes with the seeds were arranged on a tray and covered with cling wrap to retain moisture within the tray. The seeds not exposed to the microwave served as control. Emergence of radicle was considered as germination and the germination count was taken after 72 hours (third day) of placing the seeds on moistened filter paper. Radicle length was measured using a graph paper on the seventh day of the experiment.

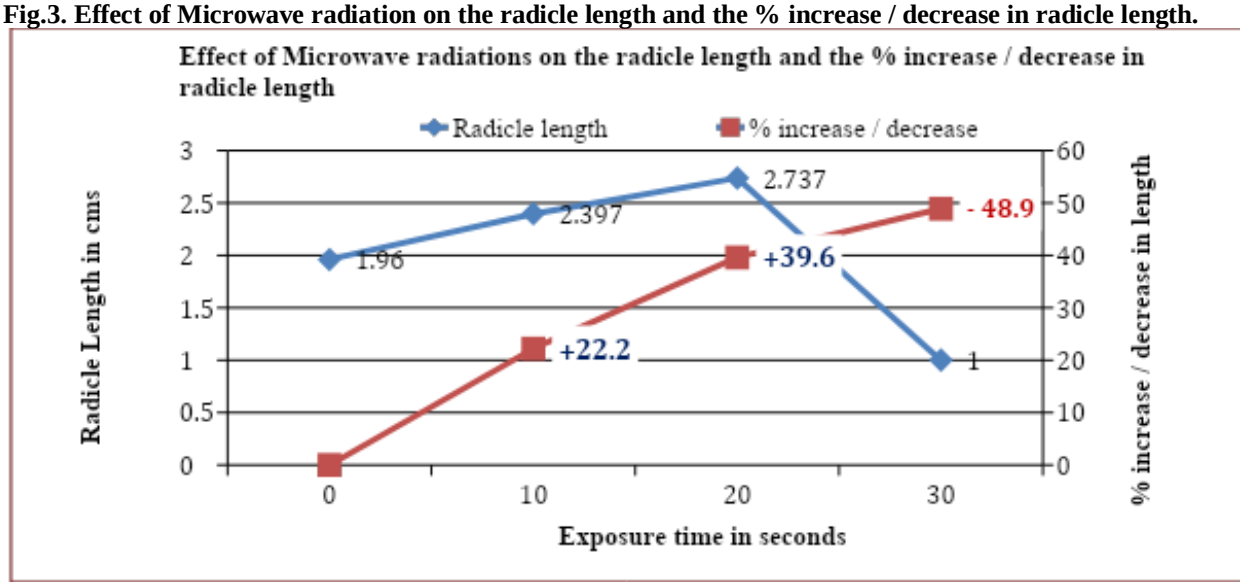
III.Result and Discussion

Table.1. Effect of Microwave radiation on germination percentage and percent enhancement / inhibition of germination in Fenugreek seeds at different exposure time

Exposure time in seconds	% of germination	% Enhancement / Inhibition of germination
Control	42	0
10 sec	42	0 (Same as control)
20 sec	50	+19 (more than control)
30 sec	7	-83 (Less than control)

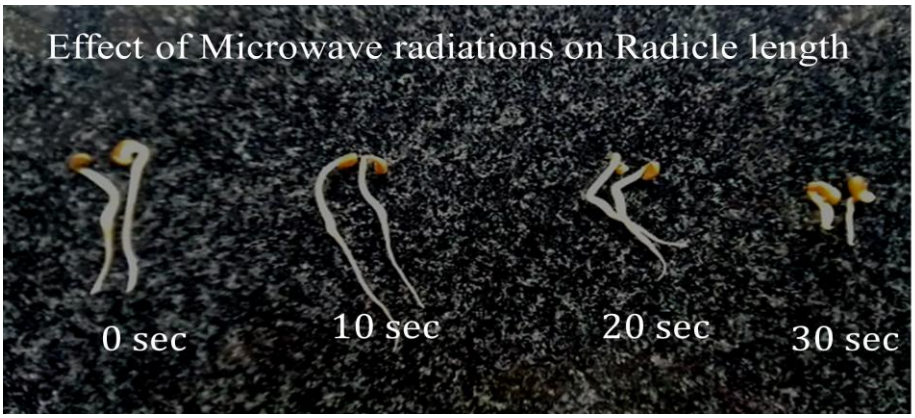
Effect of microwave radiation on germination percentage and percent enhancement / inhibition of germination in Fenugreek seeds at different exposure time is presented in table 1.The seeds exposed to microwave radiations for 20 seconds were found to have germinated more in number when compared to control. An enhancement of 19% was observed in the seeds treated for 20sec. whereas, an inhibition of 83% was observed in the seeds treated for 30 sec.

Fig.3. Effect of Microwave radiation on the radicle length and the % increase / decrease in radicle length.



Effect of Microwave radiations on the radicle length and the %increase or decrease in length is shown in Fig.3. It is observed that seeds exposed to microwave radiations of 10and 20 seconds had longer radicles when compared to control seeds. The increase was 22.2% and 39.4% in seeds exposed to 10 and 20 seconds respectively. However, the seeds exposed to 30 seconds showed a decrease of 48.9% which accounts for nearly 50% inhibition in the radicle length.

Fig.4. Radicle length of Fenugreek seeds exposed to different duration of microwave radiations.



Germination of seeds depends on seed structures and the environmental factors such as temperature that affect the growth potential of the embryo (Koornneef M *et al*., 2002). Germination parameters were dose-dependent. Germination percentage and radicle length were evaluated under different durations of exposure to microwave radiation. Some research has been done on the effects of microwave on seed germination in species such as wheat (*Triticum aestivum*), Bengal gram (*Cicer arietinum*), green gram (*Vigna radiate*), and moth bean (*Vigna aconitefolia*), under microwave conditions. It has been reported that a threshold level of microwave energy and exposure time can improve seed germination and seedling vigour in plant species (Talei *et al*., 2013). (Mohammad Mohsenkhah *et al*., 2018) reported an increase in the plant growth indices of pepper seedlings subjected to microwave radiations. The present investigation has also shown an increase in the germination percentage and radicle length. The overall results suggested that 20 second exposure to microwave frequency as the best exposure to microwave frequency for germination and radicle growth of Fenugreek seeds.

IV.Conclusion

On the basis of the results obtained from the investigation of the effect of microwave irradiation on seed germination. A positive effect of microwave radiation was observed in the seed exposed for 20seconds to radiations. The positive effect of irradiation of microwaves is better expressed at later stages of development, on the 5th day. The investigation is in the preliminary stage and further investigations are needed to understand the effects of microwave irradiations on seeds.

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Arduino based Bi Directional Visitor Counter integrated with automatic power off from control room

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Abstract

The longevity and comfort of human life has increased many fold with the advent of innovations in the fields of sensors and smart devices. A Bidirectional Visitor Counter with an interface to Arduino UNO can be designed to detect the presence /absence of humans .It is portable and can be easily installed at various places. The system can be configured to count the number of people entering and exiting through a corridor/gate at a given time. In educational institutions, it can be used to monitor the number of students attending the class, paying the fees at the counter and students in the laboratory, an auditorium and many such places where there is a need to monitor the crowd. This device is quite economical and applicable in places where the visitors have to be counted and controlled. Depending upon the sensors interruption, system identifies the entry and exit of the visitor and displays the number of visitors present in the laboratory, auditorium or hall. Counting the visitors manually can be time consuming and thus helps in optimal utilization of the available resources and precious time. It enables the educators, managers to plan ahead for the seating capacity of the organizations. Eventually it leads to maximize the efficiency and effectiveness of employees.

Keywords: PIR Sensor, Ultrasonic sensor, Arduino Uno, 32*8 Dot Matrix LED .

1.Introduction

The objective of the present work is to develop an Arduino based Visitor Counter that can be installed in various places such as colleges, schools, shopping malls etc. to determine the number of people visiting or leaving a place. This is to ensure the number of people that are present in a particular place to maintain adequate social distancing. With the increase in the standard of living, there is a continuous need for automatic appliances that would ease the complexities of life. Many times, one needs to monitor the number of people visiting or leaving a particular place such as Hotels, Restaurants, Colleges, Schools, Shopping malls etc. In the present scenario, such a kind of Visitor Counter would help the management to monitor and maintain social distancing. And thereby limit the number of people that can be present in a particular room or space. This project would also help in conserving energy by switching on/off the lights and fans depending on the situation whether anybody is present in a room.

2.Materials and Methods

Components used

Arduino Uno(**SKU648421,Italy**), PIR sensor[2], and 32*8 DOT matrix LED display are integrated and incorporated to observe the counter value.

Assembly of Bi directional counter

Figure 1a shows the circuit of counter along with the components, Two PIR sensors, Arduino Uno and 32 *8 Dot Matrix LED display. It also comprises of the battery holder and a reset button. The circuit design is implemented in Multisim and the Arduino Uno is programmed in embedded C using the Arduino IDE 1.8.13

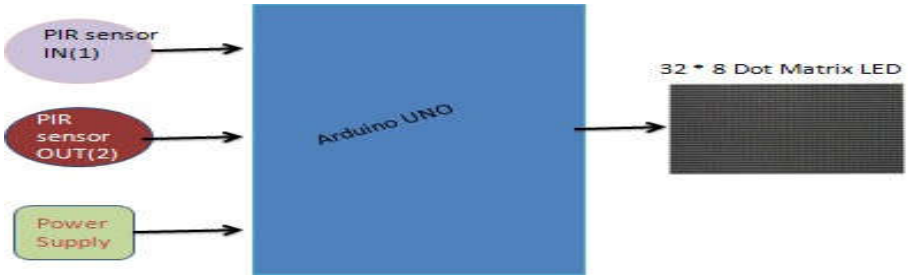

Figure 1a Block diagram of the circuit implemented

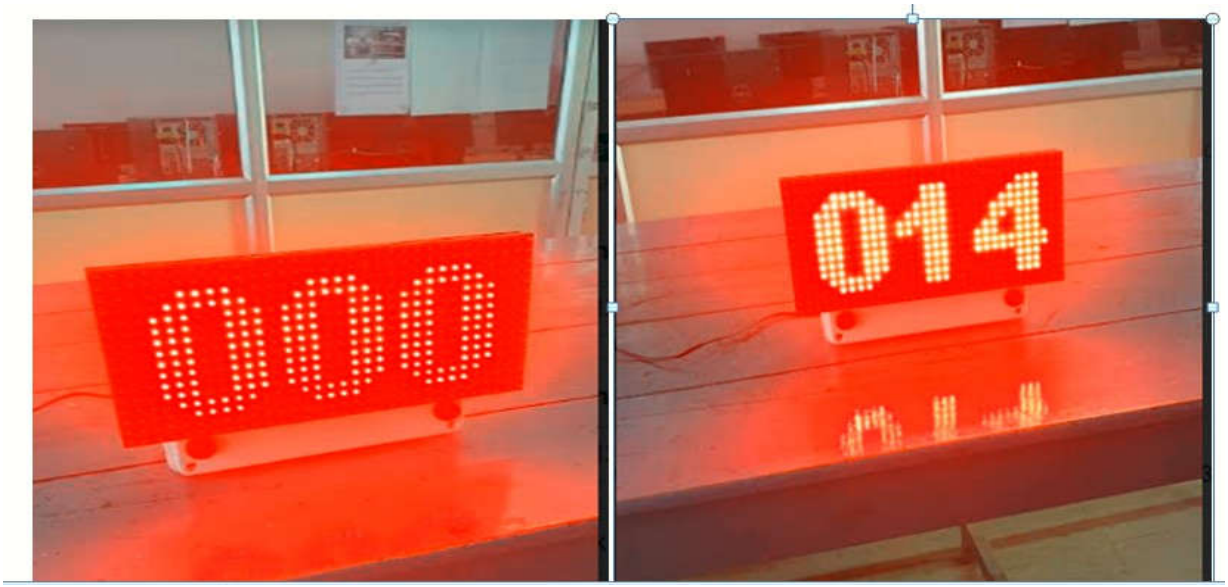

Figure 2 a Bi Directional Counter showing the Number of students in the lab before and after the class

Programming arrangement

The Arduino IDE 1.8.13 software is used to program the microcontroller. Once a person enters the room the PIR sensor receives the signal and the micro controller is programmed for an increment. Another PIR sensor is used to count the number of people leaving the room. The final output is displayed by considering the data from both the IR sensors. The instrument is tested with students from various distances and the range was found to be around 12 to 15 cm which is a good distance for installing at the entrance of the laboratory. The algorithm developed for net number of students entered and left the room which is displayed on the monocolor Dot matrix 32 * 4 LED display

2.4 Hardware considerations

Arduino Uno

An Arduino board [1] historically consists of an Atmel 8-, 16-or 32-bit AVR microcontroller with complementary components that facilitate programming and incorporation into other circuits. An important aspect of the Arduino is its standard connectors, which let users connect the CPU board to a variety of interchangeable add-on modules termed shields. Some shields communicate with the Arduino board directly over various pins, but many shields are individually addressable via an I²C serial bus—so many shields can be stacked and used in parallel. It provides 14 digital I/O pins, six of which can produce pulse width modulated signals, and six analog inputs, which can also be used as six digital I/O pins. This board has a 5-volt linear regulator and a 16 MHz crystal oscillator

Passive Infrared Sensor

A Passive Infrared (PIR) sensor detects changes in infrared (heat) radiation. The PIR works by accumulating thermal energy from the surrounding area[2], which is then processed to produce an output signal proportional to the rate of thermal energy being received at its sensing face. This type of motion detector may be thought of as a heat-seeking device; it will detect any moving object that has a significantly higher temperature than its surroundings or background ‘noise’. In this way, even relatively cool objects can activate the detector if they are moving quickly enough and their emitted heat signature is high enough compared with ambient levels. PIR sensors are passive devices, detecting changes in infrared radiation without generating or radiating any power themselves.

*32 * 8 Dot Matrix LED*

The biggest advantage of the LED display is its efficient and low-energy consumption, which is especially needed for handhelds and chargeable devices such as mobile phones and tablets. An LED display consists of a number of LED panels that, in turn, consist of several LEDs. LEDs have numerous advantages over other light-emitting sources that can be used alternatively. Aside from being power efficient, LEDs produce more brilliance and greater light intensity. LED Display is different from the vacuum fluorescent display used in some consumer electronics such as car stereos, videocassette recorders.

3. Methodology

Arduino enables the users to monitor various kinds of sensors such as IR sensor and motion detectors in real-time. The analog and digital pins on the Arduino board can serve as general purpose input and output pins (GPIO). Usually, The Atmega 328 microcontroller embedded on the Arduino board contains the analog-to-digital converter (ADC), which converts the analog input signal to a number between 0 and 1023. The integer number is always proportional to the amount of the voltage being applied to the analog input. Any sensor operating on 5 volts can be directly connected to the Arduino board. The algorithm checks for a zero condition (Zero condition means no one in the room) and finds it is true then Arduino turn off the bulb by deactivating the relay through transistor. And if zero condition is false then Arduino turns on the light. Two functions for enter and exit are employed. This increment or decrement is displayed on the LED. In order to implement and demonstrate the system developed theoretically, a prototype is created that represents the system. The image of the visitor counter panel designed using Arduino (IDE) with the student count present in the laboratory is shown in the figure 2a.The prototype has been implemented on the board. The triggering is finally done by Arduino gateway. While monitoring sensors in real-time, t he Arduino takes action in real time to control the on/off of the led and controlling the buzzer. In this system it has two section one the transmitter section where the power supply and the light output is given. The other one is the receiver section where light input is taken and implemented on enters sensor circuit and exit sensor circuit

Future work

This project can be made as an automatic, cheap and effective way of limiting the crowd in public places - such as shopping malls, supermarkets, offices - and public transport vehicles, such as buses and trains.

Using a human to limit the number of people in a particular place would not be as effective as using an automatic system due to the lack of staff members in some areas. This prototype can be used in real-life situations by replacing the servo motor with a solid state relay module.

The solid-state relay module will control the motor operating the automatic sliding doors in buildings and vehicles. There will be a slight change in code when replacing the servo motor.

The sensor is integrated o the power supply with fans or tube lights and other such electronic equipment inside the shop or office and by doing so one can automate the device and save electricity as well. It can be checked if there is someone inside the room or shop and then turn ON the lights automatically, and when there is no one inside the controller will automatically turn OFF the loads.

Conclusion

This project can be made as an automatic, cheap and effective way of limiting the crowd in public places - such as shopping malls, supermarkets, offices - and public transport vehicles, such as buses and trains. Using a human to limit the number of people in a particular place would not be as effective as using an automatic system due to the lack of staff members in some areas. This prototype can be used in real-life situations by a servo motor with a solid-state relay module. The solid-state relay module can control the motor operating the automatic sliding doors in buildings and vehicles. There will be a slight change in code when replacing the servo motor.It can also be integrated with fans or tube lights and other such electronic equipment inside the shop or office and by doing so one can automate the device and save electricity as well. It can be checked if there is someone inside the room or shop and then turn ON the lights automatically, and when there is no one inside the controller will automatically turn OFF the loads. A novel architecture for an economic bidirectional Visitor Counter and power controller is proposed and implemented in project. It gives basic idea of how to control the bidirectional visitor counter and room light counter using Arduino Uno and Arduino (IDE). The cost of this technology is very economical. This project uses low cost off the shelf components, and is based on Arduino platform which is an open-source software. This low-cost system is designed to improve the living standard and complexity of visitors counting. It provides accurate data with minimal errors.

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Determination of Percentage of Dilution of Milk by Efficient and Economic Methodology

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ABSTRACT

The critical angle of pure and diluted milk samples were measured with Pulfrich refractometer. The measured data was analysed and extrapolated for increased levels of dilution of milk with ordinary water for assessing the quality of the milk. The experiments were carried out with commercial cow milk that had fat volume concentrations of 1.53. It is observed that the distance corresponding to critical angle varied from 25 cm to 14.5 cm for a particular length of observation as the dilution of the milk moved from nil to 50 %. This method could help the common man to have a rough estimate of milk that is being diluted with water on a day-to-day basis thus avoiding laborious and expensive methods.

Introduction

The quality of raw milk affects all the dairy products indirectly impacting the food safety and human health. Quality testing of the raw milk presents an essential requirement of the contemporary dairy farming. Compositional changes of milk can be used for the precise monitoring of not only milk quality, but also provide valuable information about animal health, well-being and living conditions. Milk quality monitoring are of vital importance for successful dairy farm management and decision-making processes[1]. Traditional techniques based on chromatography–mass spectroscopy are widely used in practice for highly accurate determination of milk composition. They come with high cost, time-consuming and tedious analysisdemanding trained personnel and sample preparation. This renders them unsuitable for routine every day analysis. Alternatives, being used nowadays are spectroscopic and imaging methods, which have been heavily used, in general, for quality estimation[3] and inspection of food and food products for a number of years, and in particular, near-infrared spectroscopy is considered as a very powerful tool that can answer the needs of contemporary dairy industry. Optical measurement techniques can be applied to the inspection of milk quality. The refractive index and the absorption coefficient of the milk can provide information about the concentration of various constituents of the milk. Since milk is a turbid opaque medium, multiple light scattering is present. Nevertheless, light reflection from a milk-glass prism interface can be used to estimate the wavelength-dependent refractive index and the absorption coefficient of milk.

A traditional method of estimating the refractive index of a liquid or the critical angle between glass and liquid interface is based on the use of a Pulfrich Refractometer. It works reliably with transparent liquids because the method relies on accurate detection of the critical angle of total reflection in a liquid-prism interface. The operation of such a device is based on the principle of total internal reflection. The refractive index is defined as the ratio of speed of light in vacuum to the speed of light in another substance. It remains constant for

a given substance at a particular temperature and pressure and hence also the critical angle(fig 1b)The light wave travels with a maximum velocity in vacuum and when it is passed through any other medium it is constantly absorbed and reemitted by atoms of material[2].

Description

The refractive index can be used to estimate the purity of a sample by comparing it with the refractive indices of a pure substance and the concentration of a solute in a solution can be determined by comparing it with the refractive index of a solution to the standard curve.

Refractometers are the devices that are used measure the refractive index of sample.The principle involved in the refractometer is that it measures the extent to which light is refracted when it moves from air to sample. These refractometers are famous for measuring liquids for quick evaluation of concentration of dissolved substances.

There are three types of refractometers (i) Abbes Refractometer (ii) Immersion refractometer (iii) Pulfrich Refractometer

Pulfrich Refractometer- The Pulfrich refractometer is designed in 19th Century and this refractometer is a critical angle refractometer which can measure the values upto fifth decimal place for differential measurements and dispersion determinations[4].

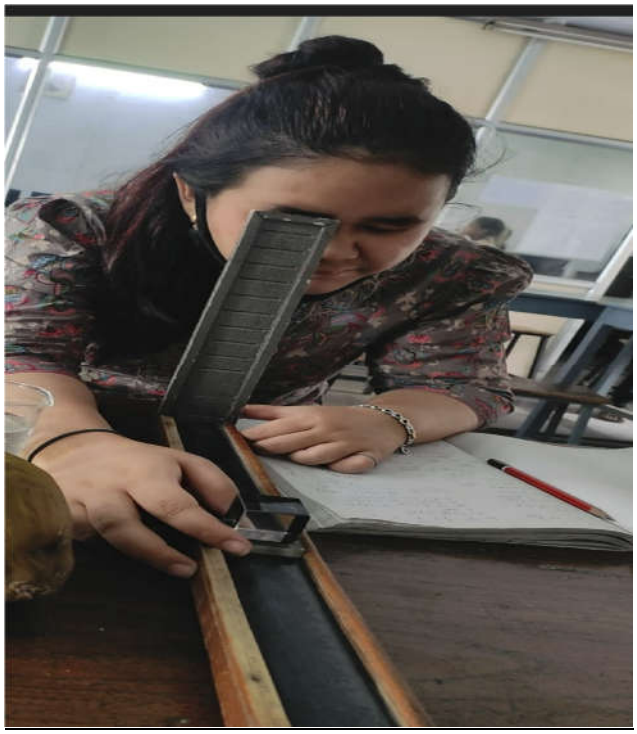

figure 1 a Measurement of total internal reflection using Pulfrich Refractometer

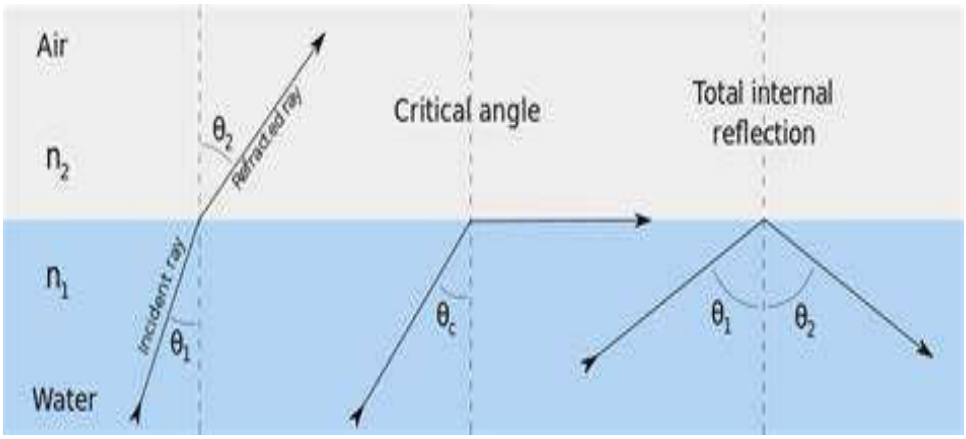

figure 1b Total Internal Reflection from denser to rarer medium

The apparatus consists of a wooden platform fixed with a metal strip containing slits. The wooden platform consists of a movable slab fixed with a cross mark and a glass tube.This will be placed on the slab. Initially the heights of the slit from the platform is measured and noted.The liquid sample whose refractive index is to be determined is poured on the slab and the cube is placed over. The cube along with the slab slowly moved away from the metal strip and the cross mark is observed till some distance.At a particular position cross mark is observed silvery and disappears. This angle corresponds to the critical angle / total internal reflection.At this point the distance of cube is measured.The procedure is repeated by observing the cube/cross from various slits.

Methodology and Observations

The experiment is initially performed for pure milk purchased over the counter from a standard vendor. The distance at which total internal reflection takes place as observed from each slit is noted. The process is repeated by diluting the milk with normal water by 25% and then by 50% subsequently. The process is repeated for 15 times and the results are averaged and tabulated in the following table

Table 1 Observed length in cm of critical angle with vertical distance/order of the slit

No of Slit	Pure milk(cm)	Dilution(25%)(cm)	Dilution(50%)(cm)
1	25	21	14.5
2	20	19	13
3	18.2	17.8	12
4	16	14.5	11
5	14.5	12.5	10
6	13.5	10	9
7	12	9	8
8	10.5	8	6.5

Conclusions

It is quite evident from the observations that the dilution of raw milk has a direct effect on the critical angle which can be measured through a simple set up. This can be standardised by fixing the observation to afixed length on a day-to-day basis and then compare the length to the pure milk value. Any amount of dilution of the milk with water would alter the length at which total internal reflection would occur. This method presents a simple yet efficient way of assessing the quality of the raw milk by the local tea and restaurant owners at a very economical cost.

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ORIGINAL PAPER

Studies on Ablative Performance of Silicone Low-Density Ablative Material

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Abstract
In the present studies, silicon based low-density ablative material has been studied for its ablative performance to protect flight hardware from temperature raises due to aerodynamic forces for a longer duration. The oxyacetylene flame test estimates ablative properties at 100–120 W/m^2 heat flux. The time duration for the flame test is taken 200 s. The material shows low mass and linear ablation rate with high ablative effectiveness. The degradation kinetics of silicon ablator is studied by Thermo Gravimetric Analysis (TGA) method. Non-isothermal TGA is performed in the temperature range of 30 °C to 900 °C at various heating rates, viz. 5, 10, 15, and 20 °C/ min. Knowledge of the kinetic parameters of thermal decomposition is used to predict the degradation kinetics of the material at high heating rates, which the ablator faces during aerospace applications. During ablation, the silicone ablative material consists of a virgin zone, reaction zone, and char zone. These are explained by kinetic parameters determined with the help of free software called Kinetic Calculation.

Keywords Ablation · Thermal degradation · TGA · Kinetic parameters · Oxyacetylene flame test

1 Introduction

Ablation in ablative materials is a complex phenomenon, and much research has been done on this subject [1–6]. Ablation is an effective and reliable process primarily used in flight structures to protect the hardware from high external temperatures. Knowledge of degradation kinetics help in modeling of ablation phenomenon. In ablation, the heat is dissipated through thermochemical degradation reactions. As a result, char is formed on the material's surface, which becomes a barrier between the virgin material and the hyperthermal atmosphere. It prevents direct plume contact with virgin and gives transpiration cooling at the same time. Elastomeric ablative materials are widely used for thermal protection in aerospace applications. Some examples of elastomeric systems are nitrile butadiene rubber, styrene-butadiene rubber, silicone rubber, EPDM, etc. The most used reinforcements are carbon, glass, ceramic fibers, and thermally stable fillers [7]. As ablative materials are critical to the safety of flight structures, it is necessary to investigate their ablation behaviors in advance. In this work, ablative properties and kinetics of degradation of a low-density ablative material based on silicone matrix and hollow glass microspheres as filler [8, 9] are studied by oxyacetylene flame test (OFT) and thermogravimetric analysis (TGA). OFT gives an insight into the ablation rate and ablative effectiveness. The oxyacetylene flame test is simple, cost-effective, and valuable in estimating ablative materials' thermal processes [10, 11]. At the same time, the TGA of materials allows us to calculate hypothetically the time required for the degradation of materials at the desired temperature, which leads to calculating kinetic parameters. These kinetic parameters help analyze

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decomposition rates at various temperatures. The Committee of International Confederation for Thermal Analysis and Calorimetry (ICTAC) offers guidance for obtaining kinetic data for the thermal decomposition of inorganic solids and thermal and thermo-oxidative degradation of polymers and composites. These recommendations for kinetic measurement are focused on thermal analysis methods such as Thermogravimetric analysis (TGA), Differential scanning calorimetry (DSC), and Differential thermal analysis (DTA) [12]. Currently, isoconversional methods are widely used because it assumes that the rate of reaction does not change with the variation of heating rate and depends only on temperature. It involves temperature measurements for the constant degree of conversions at different heating rates [13, 14], and kinetic software is available for calculating kinetic parameters [15–17]. The estimated kinetic parameters activation energy and pre-exponential factor are utilized to generate plausible degradation mechanisms for ablative material.

2 Experimental

2.1 Raw Materials

Sylgard 184, a commercially available Vinyl terminated silicone resin from Dow Corning, is used as the matrix material. It is a two-part system. Part A is low viscous resin (viscosity: 3500–5500 cps), and Part B is a hardener / cross-linking agent. When both parts are mixed in a 10:1 ratio by weight, respectively, it polymerizes with a cured density of 1.03 g/cm^3. The HGMs of K-25 grade from 3 M India Ltd is used as filler. Karstedt's catalyst (from Sigma Aldrich) is also purchased. Primer SS-4155 is purchased from Momentive India Ltd.

2.2 Sample Fabrication for Property Evaluation

Sylgard 184, part A (100 g), and part B (10 g) are mixed in a ratio of 10:1 by weight. 40 g of HGM particulates are reinforced slowly in the resin by mixing with hands. Karstedt's catalyst (0.01 g /10 g of resin) is added to the mixture [9]. The composite mixture is then sprayed on a square type of metal plate (Mild steel) of size $120 \times 120 \times 3.5$ mm^3. The ablative material is coated on the substrate with a 5 mm thickness. The application of ablative material by spraying method is given in our earlier studies [9]. Now castings can cure at room temperature for about 3–4 h. 08 samples are prepared for analysis, of which 07 plates are kept for OFT, and from one plate, the coating is scratched out for TGA analysis.

3 Instrumentation

3.1 Oxyacetylene Flame Test (OFT)

The nozzle is typically placed on the sample's surface at a distance of 40 mm from the sample surface, and the flame impinges precisely at the center of the sample. The heat flux maintained is 100- 120 W/cm^2 through the regulation of flow rates of oxygen and acetylene. This is kept constant throughout the experiments. The test is conducted for 200 s, and the substrate back wall temperature is measured by placing a K-type thermocouple on the back side of the substrate. The schematic diagram of the OFT setup is given below in Fig. 1.

After the test, samples are visually inspected to check char layer formation and ablative layer intactness to the substrate. The charred layers are removed such that unaffected layers are observed. The thickness and mass of the sample are then taken. The ablative parameters—Linear Ablation Rate (LAR), Mass Ablation Rate (MAR), and Ablative Effectiveness (AE) of ablative materials are determined.

3.2 Thermal Properties

The non-isothermal TGA experiments at different heating rates (5, 10, 15 & 20 °C/min) are performed using a thermo gravimetric analyser (TA instrument, model TGA–55). Approximately 10 mg of finely powdered sample is placed in a small platinum crucible for each run under an air atmosphere with a 60 ml/min flow rate. The instrument collects the weight loss variation concerning the time and temperature automatically and is determined through the TA universal analysis software. The experiments are done multiple times under identical conditions to confirm the reproducibility of the results. Thermal stability in the material is studied concerning its thermal degradation behavior as a function of time and temperature.

The open-source software Kinetic Calculation [15] has been used to calculate kinetic parameters and generate kinetic equations of thermal decomposition. This is a user-friendly software that allows calculating kinetic parameters using by Vyazovkin method [18] and Flynn–Wall–Ozawa (FWO) method [19, 20]. The software has a provision for

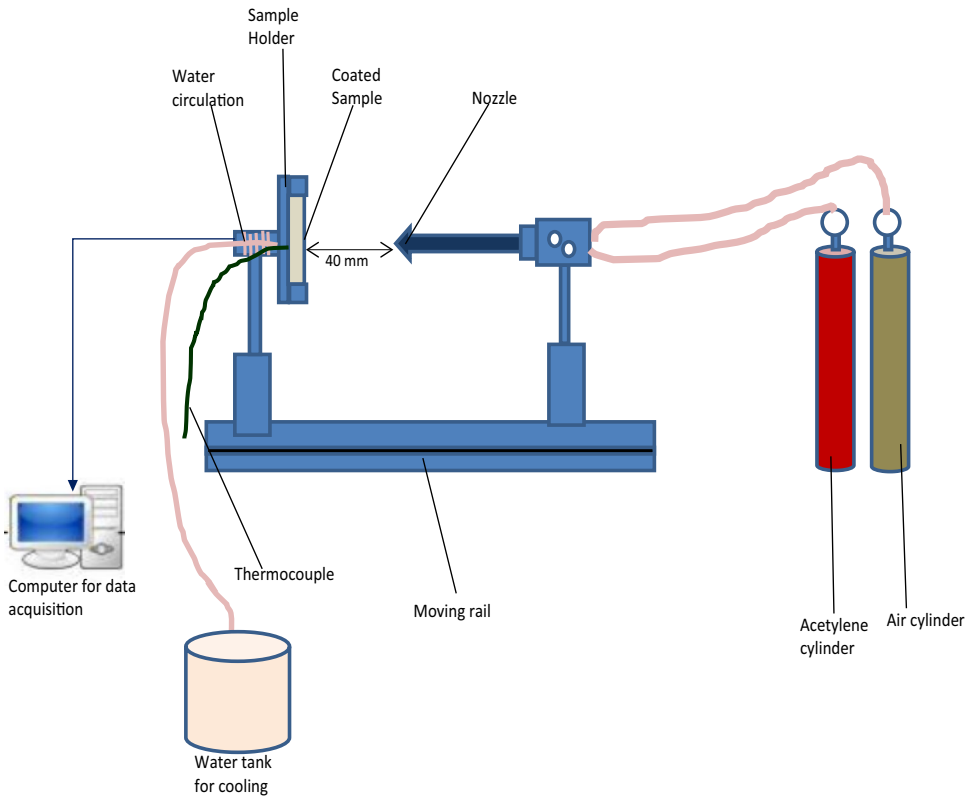


Fig. 1 Schematic diagram of OFT setup

dividing the entire process into multistage decomposition processes and accurately calculating the mechanism's function, which is not dependent on the models. The following equations are used for kinetic parameters:

Vyazovkin method

$$\frac{d\alpha}{dT} = \frac{A}{\beta}.e^{-E_a/RT}.\alpha^m(1-\alpha)^n - \ln(1-\alpha)^p \quad (1)$$

FWO method

$$\ln(\beta) = \ln\left(\frac{AE_a}{Rg(\alpha)}\right) - 5.331 - 1.052\left(\frac{E_a}{RT}\right) \quad (2)$$

4 Results and Discussions

4.1 Thermal Stability

Thermo-oxidative degradation profiles of ablative material are recorded in the temperature range from 30 °C to 1000 °C at varying heating rates of 5, 10, 15 & 20 °C/min, and the results are given in Fig. 2. The shifting of Thermogravimetric (TG) curves of the material to higher degradation temperatures with the increase of heating rate is a general phenomenon for thermally activated processes. The degradation is a multi-step process, and variation in heating rate does not affect the pattern for degradation. In this thermo-oxidative degradation, the weight change of ablative material is minimal up to 350 °C due to the loss of volatile contents present in the material. After that temperature range of 350 °C – 440 °C shows another degradation step. A rapid weight loss in ablative material is started after 440 °C at all four heating rates. This may be considered the primary

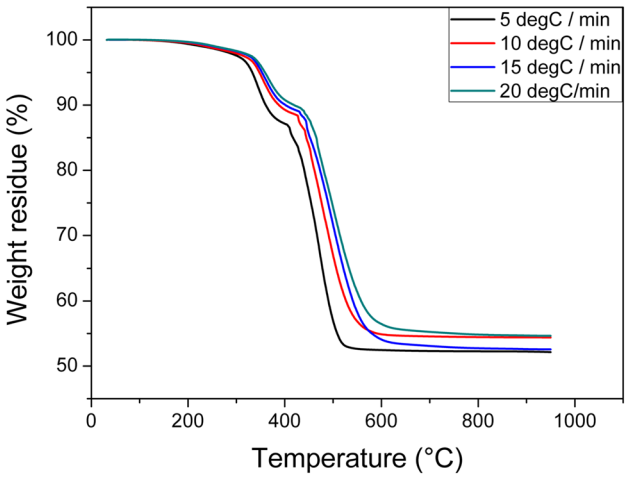


Fig. 2 Thermo-oxidative degradation profiles of ablative material at different heating rates

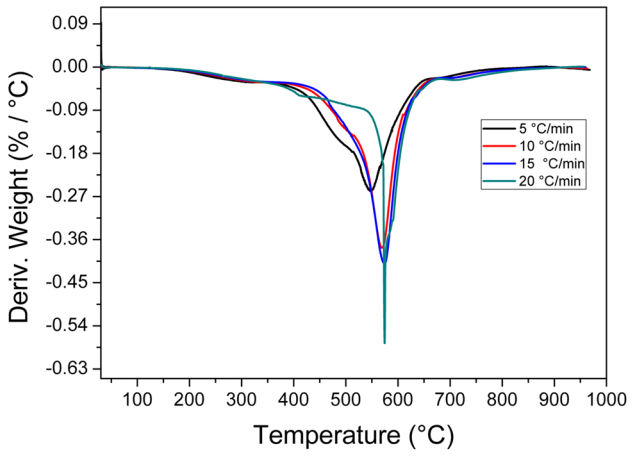


Fig. 3 Derivative TG (DTG) curves of ablative material at different heating rates

and final degradation process. After 630 °C, the weight loss started diminishing slowly, and weight residue was constant.

The char yield at 990 °C is approximately 53% to 57% and is not affected significantly by which heating rates. This residual mass is correlated well with the quantity of inorganic filler used in the ablative compound [21]. The first derivatives of the TG (DTG) curves obtained for ablative material are shown in Fig. 3 at different heating rates.

As the heating rate increases, the onset and end set degradation temperatures are also increasing. It is observed from the DTG plots that below 350 °C, the rate of thermal degradation is low. The maximum degradation rates are narrow in the temperature region of 525 to 571 °C at all heating rates. After the maxima, the decomposition is slow again until it finishes at the final degradation temperature. The volatile/moisture evaporation region and end set of degradation are identical for all heating rates.

Nevertheless, the heating rate affects the maximum and minimum points of DTG curves which are shifted towards higher temperatures as the heating rate increases. This phenomenon can be explained based on heat transfer limitation. At a low heating rate, a large instantaneous heat is provided, and a long time may be required for the purge gas to reach equilibrium with the temperature of the furnace or the sample. In the same temperature region, there is a short reaction time at the higher heating rate, and the temperature needed for the sample to decompose becomes higher. This causes the maximum rate curve to shift to the right [22, 23]. Yang Xue et al. reported similar results of maximum degradation temperature for silica-filled silicone rubber composite [24].

4.2 Kinetic Studies

Kinetic studies are done using Kinetic Calculation software [15] which uses Vyazovkin and FWO methods. In order to verify the results given by the software, the FWO method, as per ASTM E1641, is also used. First, the software is run for the entire temperature range (35 – 986 °C).

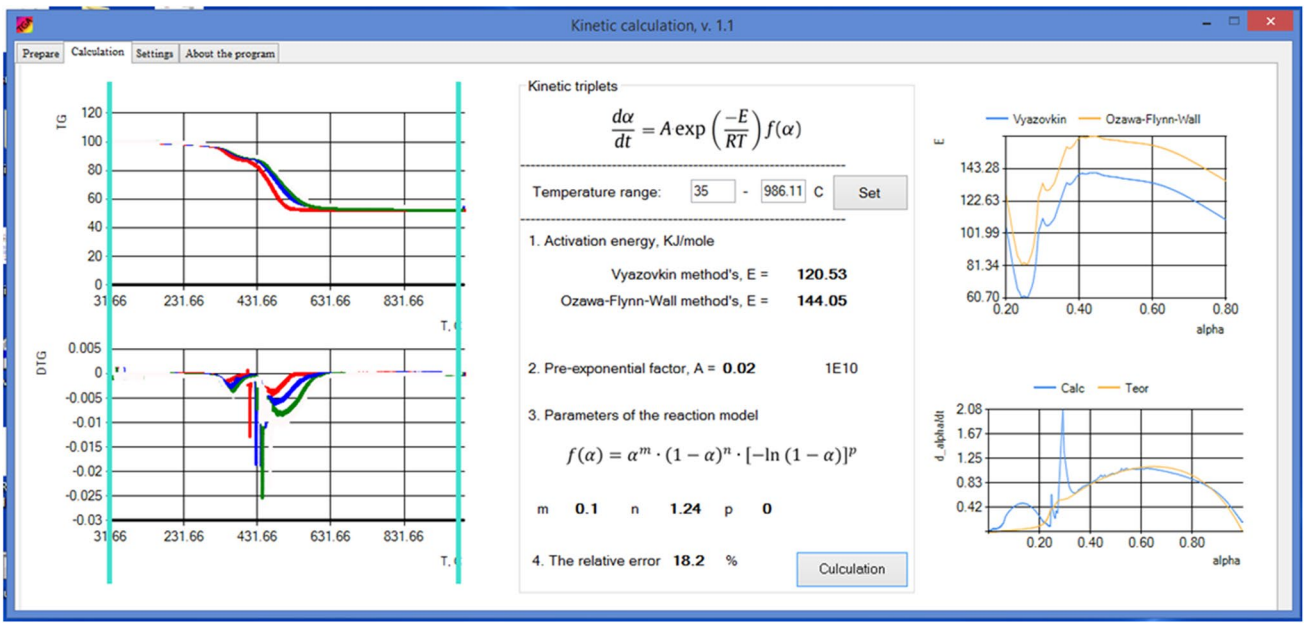


Fig. 4 Kinetic parameter for temperature range 35 – 986 °C

The screenshot of the results is given in Fig. 4. The overall activation energy (E_a) calculated by the Vyazovkin and FWO methods are 120.53 and 144.05, respectively. Ea's dependency on the conversion degree is also shown in the top right corner. This also confirms that the thermo –oxidative degradation is not a single-step process. The bottom right corner plot shows the correlation between experimental and calculated data. The relative error (18.2%) is greater than 10%, indicating a multi-step degradation mechanism [25].

The kinetic equation for the whole process can be written as

$$\frac{d\alpha}{dT} = \frac{2\times 10^8}{\beta}.e^{-120530/RT}.\alpha^{0.1}(1-\alpha)^{1.24} \quad (3)$$

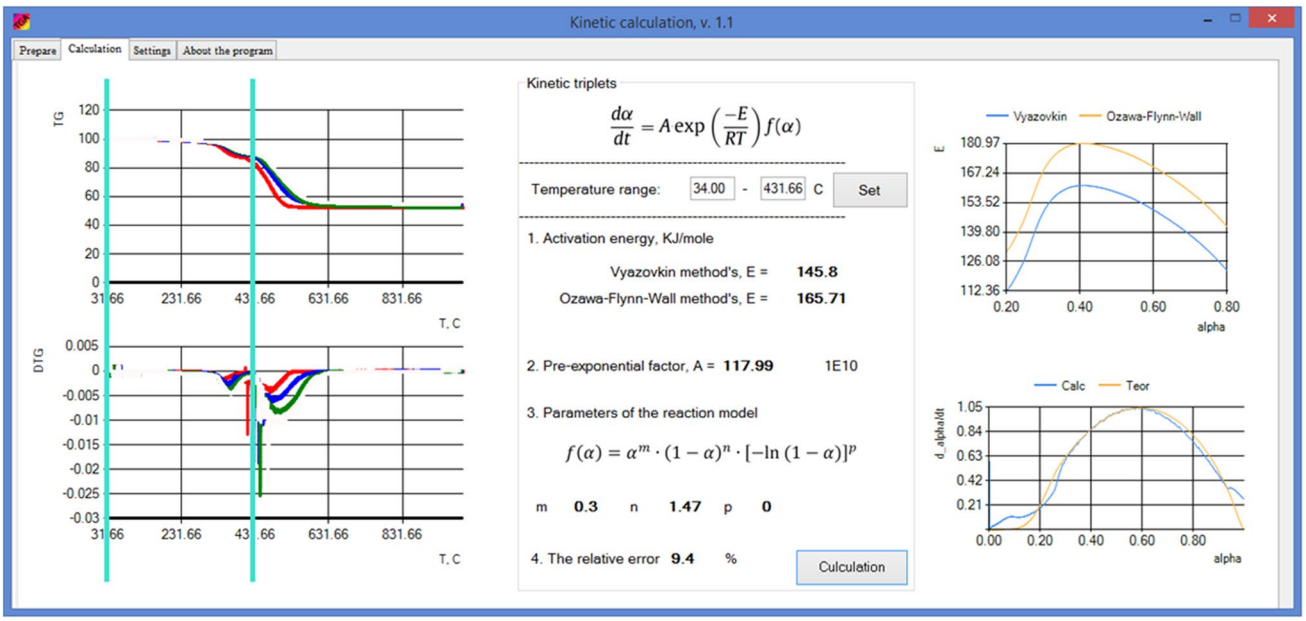


Fig. 5 Kinetic parameter for temperature range 34 – 431 °C

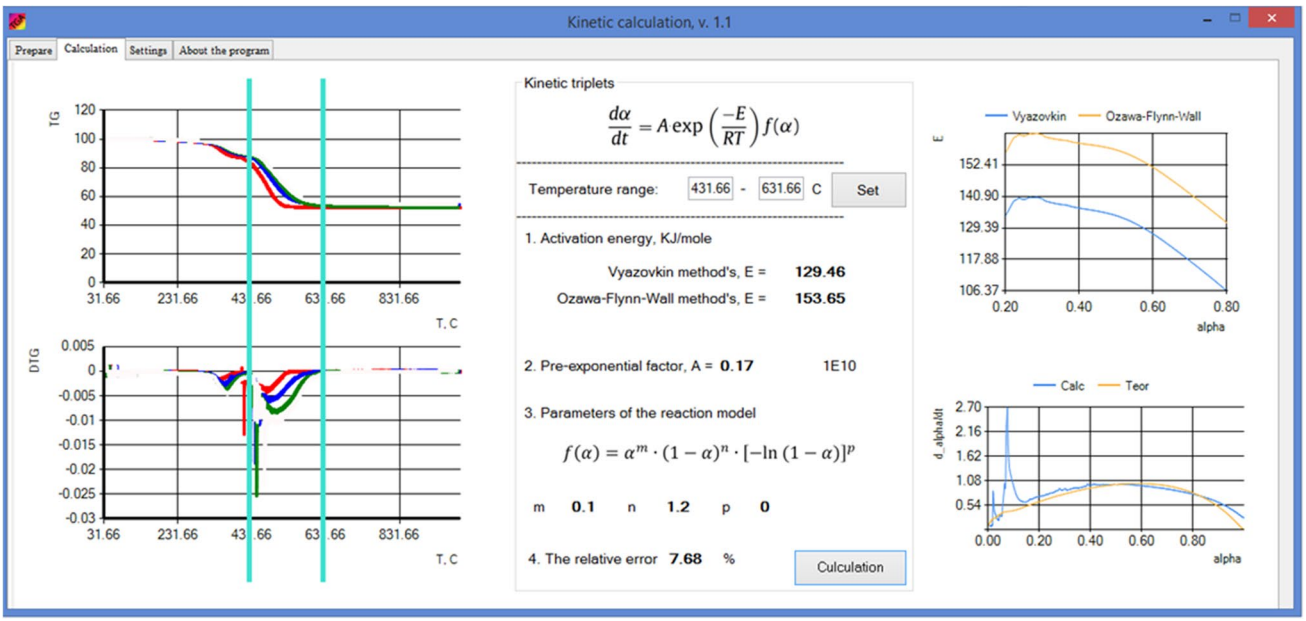


Fig. 6 Kinetic parameter for temperature range 431 – 631 °C

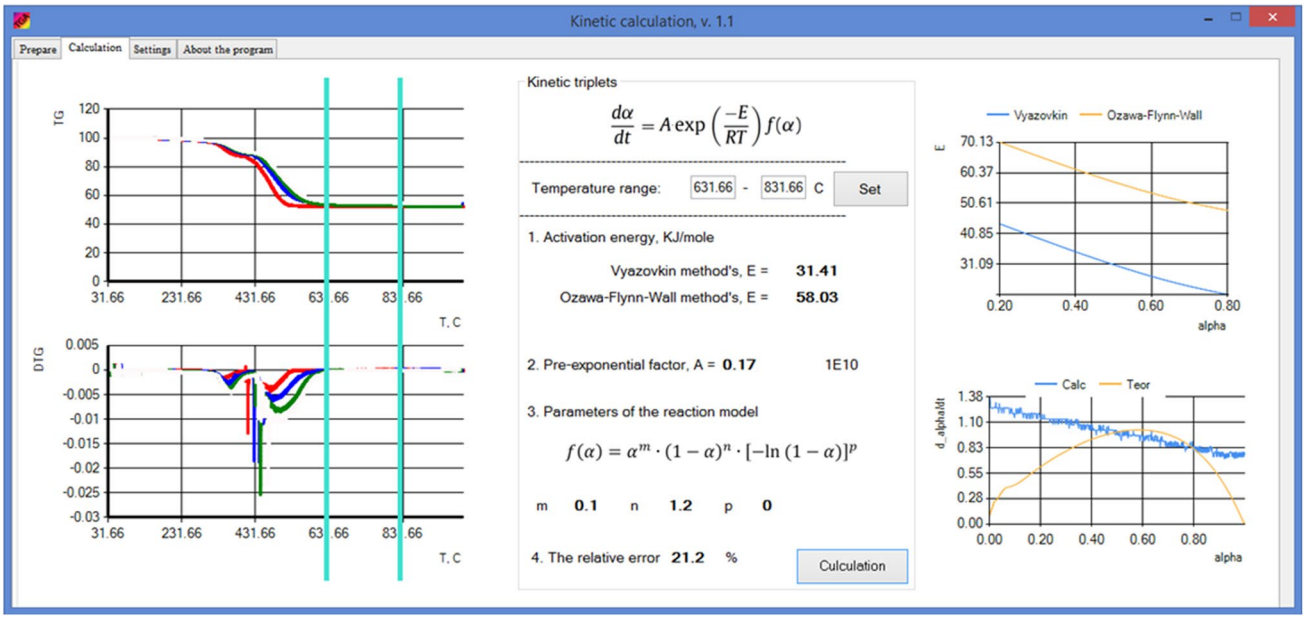


Fig. 7 Kinetic parameter for temperature range 631 – 831 °C

Now the software is run in three different temperature ranges 34–431 °C, 431–631 °C, and 631–831 °C. The results are shown in Figs. 5, 6, and 7. In Figs. 5 and 6, the relative error is less than 10%, indicating that these steps are single-step mechanisms. However, in Fig. 7, the relative error is more than 10%, and activation energy is low compared to earlier steps. It indicates the ending of the degradation process with multiple steps.

The kinetic equations for all the steps can be written as.

Temperature range 34 °C—431 °C

$$\frac{d\alpha}{dT} = \frac{117.99 \times 10^{10}}{\beta}.e^{-145800/RT}.\alpha^{0.3}(1-\alpha)^{1.47} \tag{4}$$

Temperature range 431 °C—631 °C

$$\frac{d\alpha}{dT} = \frac{1.7 \times 10^{9}}{\beta}.e^{-129460/RT}.\alpha^{0.1}(1-\alpha)^{1.2} \tag{5}$$

Temperature range 631 °C – 831 °C

$$\frac{d\alpha}{dT} = \frac{1.7 \times 10^{9}}{\beta}.e^{-31410/RT}.\alpha^{0.1}(1-\alpha)^{1.2} \tag{6}$$

The activation energy is also calculated as per ASTM E1641 (FWO method) at various conversion degrees. Here the graph has been plotted between the log of the heating rate and the inverse of temperature. The slope of the graph

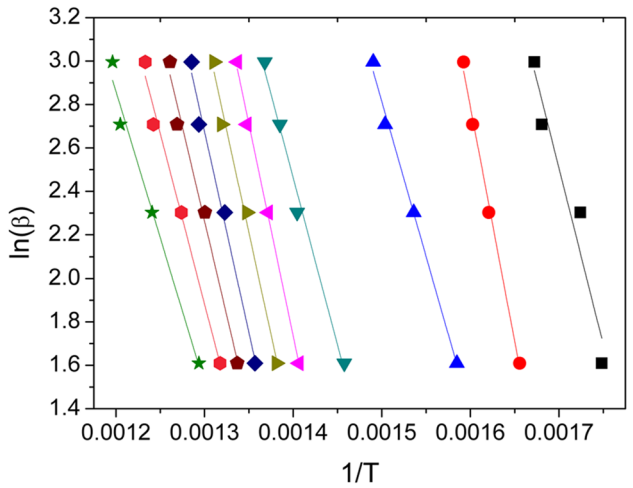


Fig. 8 Log of heating rate vs. inverse of the temperature plot

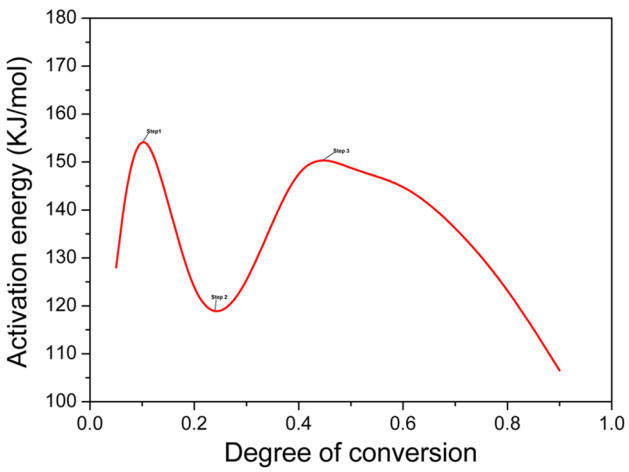


Fig. 9 Activation energy with the degree of conversion

Table 1 Oxyacetylene test results of ablative material

Properties	Value	Formula
Linear Ablation rate	0.039 mm/sec	$LAR = \frac{d_i - d_f}{t}$
Mass Ablation rate	0.050 g/sec	$MAR = \frac{m_i - m_f}{t}$
Back Wall Temperature	104 °C at the end of 200 s	
Ablative effectiveness (J/g)	9.05×10^4 J/g	$AE = \frac{H_f \times t}{\rho \times l}$

calculates the Ea for each degree of conversion. The results are shown in Figs. 8 and 9.

This also gives similar results as from software and confirms multistep thermo-oxidative degradation of ablative material.

4.3 Ablation Studies

The oxyacetylene flame test is done on three samples, and the averages of the three results are given in Table 1.

where,

d_i is the initial thickness of the sample in mm

d_f is the final thickness of the sample after the test in mm and

t is the time of the test in seconds

m_i is the initial mass of the sample in grams,

m_f is the final mass of the sample after the test in gram

H_f is the heat flux applied to the sample in W/cm^2 (J/sec-cm^2)

ρ is the density of the sample in g/cm^3

l is ablation or char depth in cm

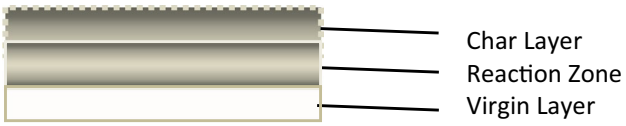


Fig. 11 Schematic diagram of ablative material after ablation

The low linear and mass ablation rate and high ablative effectiveness show that the material has good properties. Figure 10a is coated sample before the test. The SEM images were taken before and after the oxyacetylene flame test (Fig. 10b and c). The test results show before the test (Fig. 10b) that the HGMs are primarily intact and dispersed in the resin, but the HGMs are not intact. They are broken and eroded (Fig. 10c).

4.4 Thermal Decomposition Mechanism

After the ablation, the material consists of the char layer, the reaction zone, and the virgin layer, as shown in Fig. 11 below.

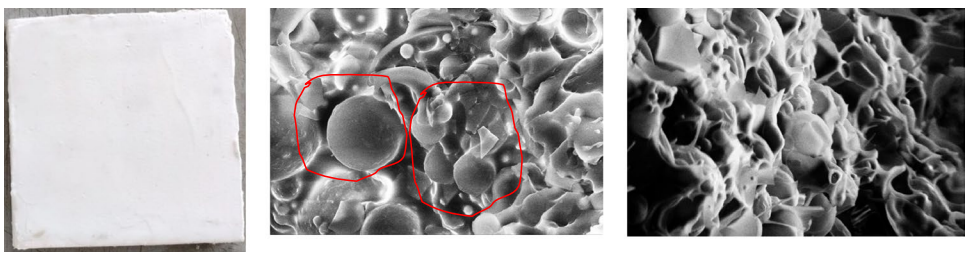
The char layer generates porosity, and hot gases are released after the material's decomposition. The char contains mainly silicon dioxide, which is confirmed by FTIR and EDX analysis of char. The FTIR spectra (Fig. 12) show absorption peaks of stretching at 1261 cm^{-1} and 1075 cm^{-1}, bending at 795 cm^{-1} and rocking at 590 cm^{-1} wave number of the Si–O-Si bonds in SiO_2 [26].

The EDX analysis also shows more silica content than carbon compared to virgin material. (Fig. 13 and Table 2).

The thermo-oxidative degradation occurs in the reaction zone, and its mechanism has been elaborately reported in the literature. It follows the Andrinov mechanism for releasing volatile oxidation products and forming silicon dioxide, as mentioned in Fig. 14 [27, 28].

The significant events in the thermo- oxidative degradation of vinyl silicones are the first initial wt loss due

Fig. 10 Coated sample and SEM images of ablative material



(a) Coated Sample (b) Before the test (c) After the test

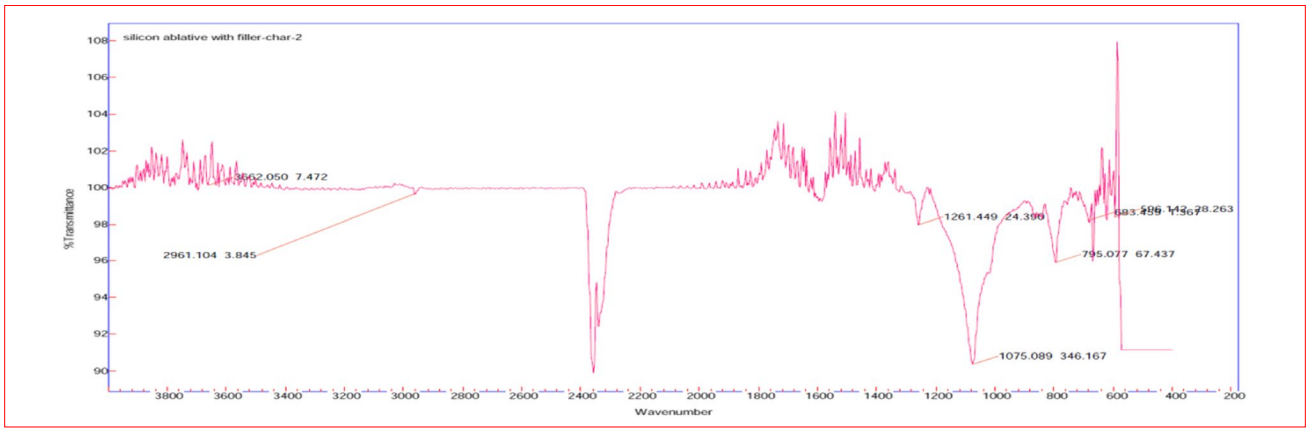


Fig. 12 FTIR spectrum of char after ablation

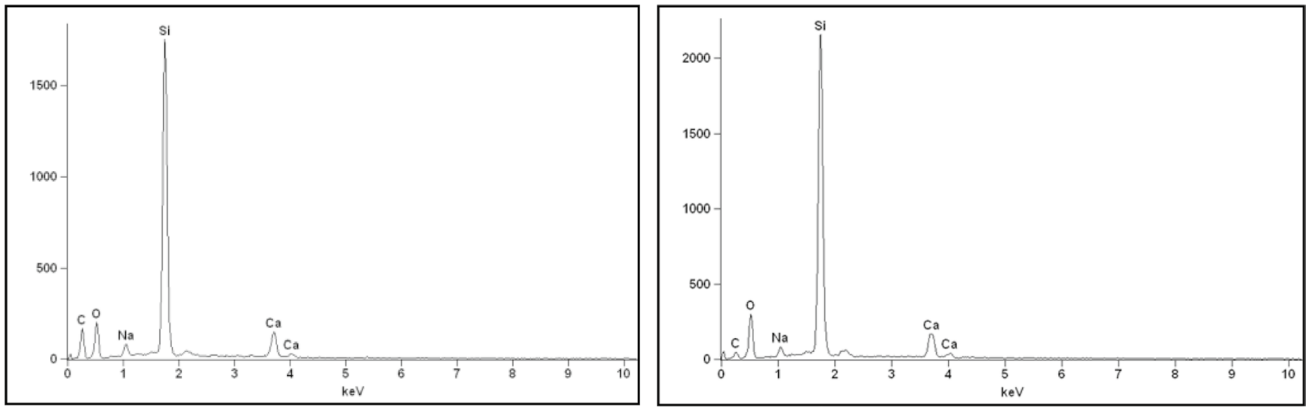


Fig. 13 EDX spectra of ablative material before and after ablation

Table 2 Elemental composition of Ablative material

Type of atoms	Elemental Composition %	
	Before Ablation	After Ablation
C K	34.69	10.03
O K	31.33	43.37
Na K	2.03	2.28
Si K	27.74	37.98
Ca K	4.21	6.34

to solvent removal. The second stage, decomposition at a temperature range of 350 to 440 °C where weight loss is approximately 12%, may be assigned to the vinyl group's breakage and side chains. The third-stage decomposition (i.e., in the temperature range of 440 to 630 °C) is due to the dimethyl siloxane unit of the silicone, where weight loss is about 30%. This decomposition happens in the reaction zone, and the weight residue is constant due to the formation of a hard silica char layer. The relatively low activation energy also indicates that side chain

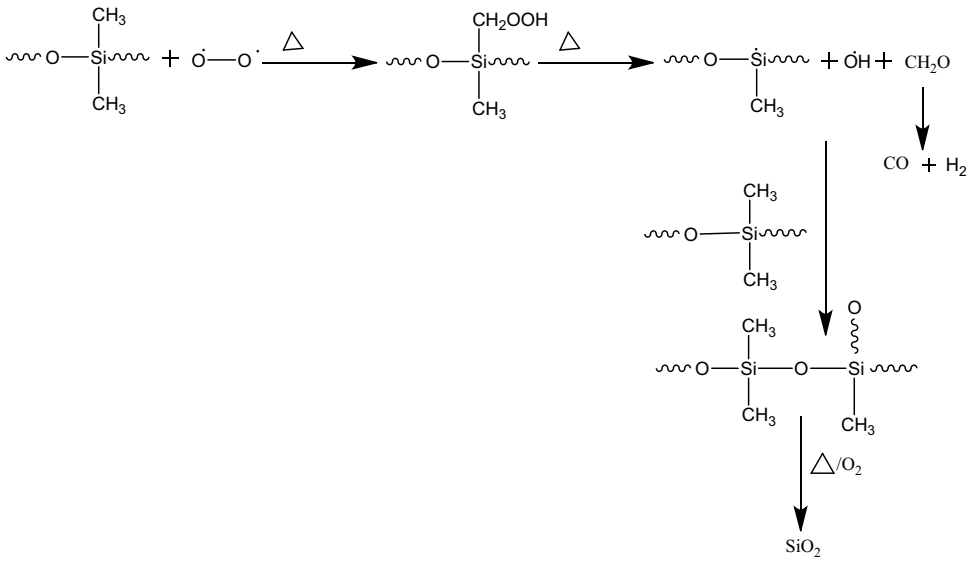


Fig. 14 Thermo-oxidative degradation mechanism of polysiloxane

$$SiO_2 + C \longrightarrow SiO + CO + 147000 \text{ calories}$$
$$SiO_2 + 2C \longrightarrow Si + 2CO + 157000 \text{ calories}$$
$$SiO_2 + 3C \longrightarrow SiC + 2CO + 137000 \text{ calories}$$
$$2SiO_2 + SiC \longrightarrow 3SiO + CO + 304000 \text{ calories}$$

Fig. 15 Reactions between silica char and carbon residue

cleavage occurs in thermo-oxidative degradation because -Si–O- has high bond energy. The reaction between carbon residue and silica is reported at the 1250–1750 °C temperature range [29]. These endothermic reactions (Fig. 15) may occur in the char layer and contribute to transpiration cooling [30].

5 Conclusion

A simple oxyacetylene flame test studies a silicone low-density ablative material's ablative properties for a lengthy application and kinetic parameters are determined using open-source software. The ablative properties show low linear and mass ablation rates with high ablative effectiveness. So the material is a good candidate for thermal protection of flight hardware in long-duration applications. It is tried here to predict the kinetic model for degradation by thermo gravimetric analysis. The kinetic degradation model is in good agreement with the experimental values shown by the software. The dependency of activation energy with degree conversion also shows the multistep degradation process, and a similar conclusion has been reported in the literature.

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Data Availability No further data and materials are available.

Declarations

This article does not contain any studies with human or animal subjects.

Competing interests The authors declare no competing interests.

Research involving Human Participants and Animals This article does not contain any studies with human or animal subjects.

Statement on the Welfare of Animals This article does not contain any studies with human participants or animals performed by any authors.

Consent to Participate I, Naresh Kumar Katari, on behalf of all authors, hereby declare that we participated in the study and development of this manuscript.

Consent for Publication I have read the final version and consent for the article to be published in your esteemed journal.

Conflicts of Interest The authors declare that no known conflicts of interest are associated with this publication.

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Determination of Percentage of Dilution of Milk by Efficient and Economic Methodology

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ABSTRACT

The critical angle of pure and diluted milk samples were measured with Pulfrich refractometer. The measured data was analysed and extrapolated for increased levels of dilution of milk with ordinary water for assessing the quality of the milk. The experiments were carried out with commercial cow milk that had fat volume concentrations of 1.53. It is observed that the distance corresponding to critical angle varied from 25 cm to 14.5 cm for a particular length of observation as the dilution of the milk moved from nil to 50 %. This method could help the common man to have a rough estimate of milk that is being diluted with water on a day-to-day basis thus avoiding laborious and expensive methods.

Introduction

The quality of raw milk affects all the dairy products indirectly impacting the food safety and human health. Quality testing of the raw milk presents an essential requirement of the contemporary dairy farming. Compositional changes of milk can be used for the precise monitoring of not only milk quality, but also provide valuable information about animal health, well-being and living conditions. Milk quality monitoring are of vital importance for successful dairy farm management and decision-making processes[1]. Traditional techniques based on chromatography–mass spectroscopy are widely used in practice for highly accurate determination of milk composition. They come with high cost, time-consuming and tedious analysisdemanding trained personnel and sample preparation. This renders them unsuitable for routine every day analysis. Alternatives, being used nowadays are spectroscopic and imaging methods, which have been heavily used, in general, for quality estimation[3] and inspection of food and food products for a number of years, and in particular, near-infrared spectroscopy is considered as a very powerful tool that can answer the needs of contemporary dairy industry. Optical measurement techniques can be applied to the inspection of milk quality. The refractive index and the absorption coefficient of the milk can provide information about the concentration of various constituents of the milk. Since milk is a turbid opaque medium, multiple light scattering is present. Nevertheless, light reflection from a milk-glass prism interface can be used to estimate the wavelength-dependent refractive index and the absorption coefficient of milk.

A traditional method of estimating the refractive index of a liquid or the critical angle between glass and liquid interface is based on the use of a Pulfrich Refractometer. It works reliably with transparent liquids because the method relies on accurate detection of the critical angle of total reflection in a liquid-prism interface. The operation of such a device is based on the principle of total internal reflection. The refractive index is defined as the ratio of speed of light in vacuum to the speed of light in another substance. It remains constant for

a given substance at a particular temperature and pressure and hence also the critical angle(fig 1b)The light wave travels with a maximum velocity in vacuum and when it is passed through any other medium it is constantly absorbed and reemitted by atoms of material[2].

Description

The refractive index can be used to estimate the purity of a sample by comparing it with the refractive indices of a pure substance and the concentration of a solute in a solution can be determined by comparing it with the refractive index of a solution to the standard curve.

Refractometers are the devices that are used measure the refractive index of sample.The principle involved in the refractometer is that it measures the extent to which light is refracted when it moves from air to sample. These refractometers are famous for measuring liquids for quick evaluation of concentration of dissolved substances.

There are three types of refractometers (i) Abbes Refractometer (ii) Immersion refractometer (iii) Pulfrich Refractometer

Pulfrich Refractometer- The Pulfrich refractometer is designed in 19th Century and this refractometer is a critical angle refractometer which can measure the values upto fifth decimal place for differential measurements and dispersion determinations[4].

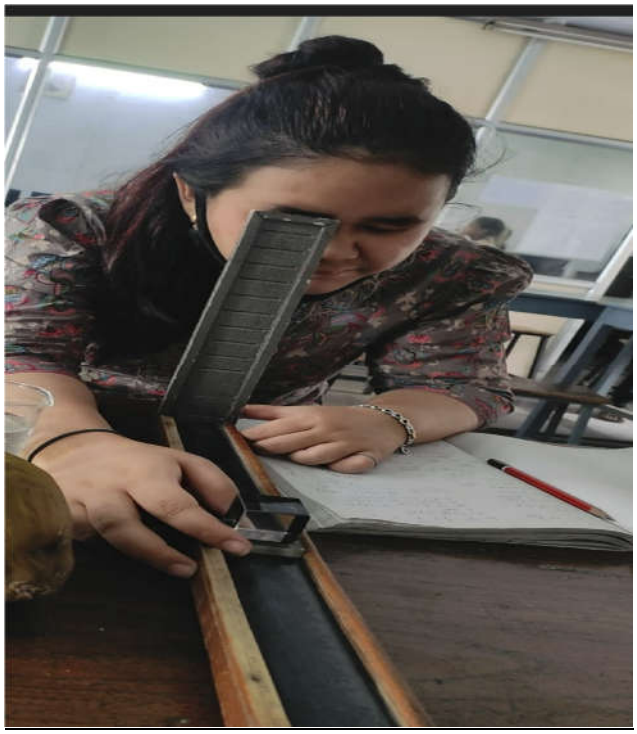

figure 1 a Measurement of total internal reflection using Pulfrich Refractometer

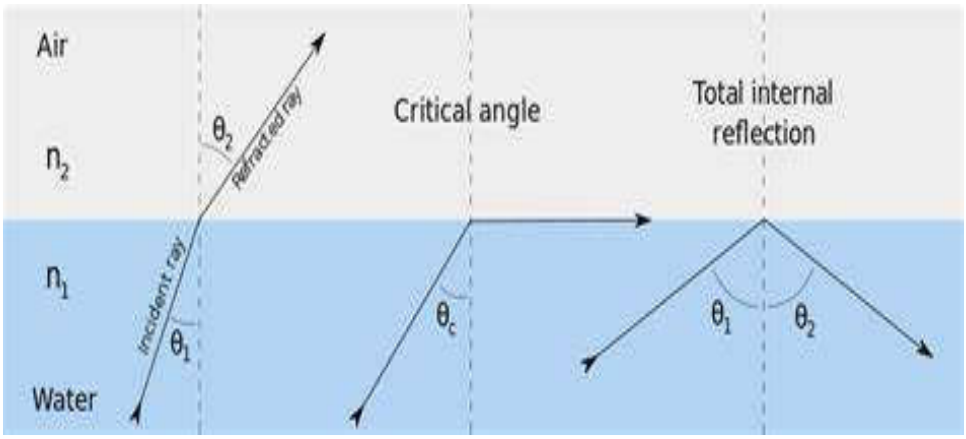

figure 1b Total Internal Reflection from denser to rarer medium

The apparatus consists of a wooden platform fixed with a metal strip containing slits. The wooden platform consists of a movable slab fixed with a cross mark and a glass tube.This will be placed on the slab. Initially the heights of the slit from the platform is measured and noted.The liquid sample whose refractive index is to be determined is poured on the slab and the cube is placed over. The cube along with the slab slowly moved away from the metal strip and the cross mark is observed till some distance.At a particular position cross mark is observed silvery and disappears. This angle corresponds to the critical angle / total internal reflection.At this point the distance of cube is measured.The procedure is repeated by observing the cube/cross from various slits.

Methodology and Observations

The experiment is initially performed for pure milk purchased over the counter from a standard vendor. The distance at which total internal reflection takes place as observed from each slit is noted. The process is repeated by diluting the milk with normal water by 25% and then by 50% subsequently. The process is repeated for 15 times and the results are averaged and tabulated in the following table

Table 1 Observed length in cm of critical angle with vertical distance/order of the slit

No of Slit	Pure milk(cm)	Dilution(25%)(cm)	Dilution(50%)(cm)
1	25	21	14.5
2	20	19	13
3	18.2	17.8	12
4	16	14.5	11
5	14.5	12.5	10
6	13.5	10	9
7	12	9	8
8	10.5	8	6.5

Conclusions

It is quite evident from the observations that the dilution of raw milk has a direct effect on the critical angle which can be measured through a simple set up. This can be standardised by fixing the observation to afixed length on a day-to-day basis and then compare the length to the pure milk value. Any amount of dilution of the milk with water would alter the length at which total internal reflection would occur. This method presents a simple yet efficient way of assessing the quality of the raw milk by the local tea and restaurant owners at a very economical cost.

Acknowledgements

The authors sincerely acknowledge the Department of Physics and Electronics, St.Ann's College for Women, Hyderabad for providing all the laboratory equipment required for the present work**.**

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Determination of Vitamin-C Content in Selected Vegetables

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Abstract

Vitamin C scientific name is Ascorbic acid and it is water soluble compound. It is an essential working micronutrient and a key element for metabolism of almost all living organism. It plays a role in protecting immune system against deficiencies, cardiovascular disease, eye disease, and even in skin wrinkling also. Vitamin C rich food, also play a key role in the production of collagen, a protein found in bones, tissues and blood vessels. In this experiment we estimated the ascorbic acid content from selected vegetables and were collected from a local market and the ascorbic acid content in these vegetables were determined by volumetric method. In these method we used DCIP + Sodium Bicarbonate solution, and taken in burette and titrate it against the vegetable juice the end point is pink color. The results obtained are represented as means standard deviation. Ascorbic acid content was high in cabbage (***Brassica oleracea***) I.e.166mg/100g and less in spinach (***Spinacia oleracea***) 33.3mg/100g these results clearly suggest that a wide variety of vegetables can be consumed to meet the daily requirements of vitamin c in a cost effective manner.

KEY WORDS: Vitamin C, ascorbic acid, DCIP dye, sulphuric acid, oxalic acid, *Brassica oleracea*, *Spinacia oleracea.*

Introduction

Vitamin C is water soluble vitamin which has strong reducing action and it has an important co-enzyme used for hydroxylation reaction. [Bendich A., Machlin L.J., Scandurra O., Burton G.W., Wayner D.D.M. ,1986]. It has both reducing form (ascorbic acid) and oxidized form (dehydroascorbic acid) it is widely used as food additive with many functional roles based on its oxidation-reduction properties, functional roles include nutrition food additive, antioxidant, reducing agents, stabilizer, modifier, color stabilizer.[Gulcin, İ , *Arch Toxicol* **94**, 651–715, 2020]

Vitamin C is abundant which work in one-electron reactions, and it is also an essential micronutrient, and a key element for metabolism of all living organisms. [May, J.M. , 2012] In humans, vitamin C produce numerous functions, such as an antioxidant, cofactor for mono-oxygenase and dioxygenases. [Carole L. Linster, Emile Van Schaftingen , 06 December 2006]. Vitamin C also influences iron metabolism through the stimulation of ferritin synthesis which inhabits ferritin degradation. [M W Hentze and L C Kühn , august 06 – 1996]
Vitamin C, act as cofactor for peptidyl-glycine alpha-amidating monooxygenase, it is also involved in biosynthesis of signal peptides, such as oxytocin, vasopressin, cholecystokinin, and calcitonin [S. T. Prigge, R. E. Mains, B. A. Eipper & L. M. Amzel 2000]. Vitamin C contributes correct formation of collagen by post-translational modifications of procollagen.[Lars Ellgaard, Nicholas McCaul, Anna Chatsisvili, Ineke Braakman , 2016]. Particularly, vitamin C acts as a cofactor for reaction which catalyzed by prolyl 3-hydroxylase, prolyl 4-hydroxylase, and lysyl hydroxylase, which also involved in the hydroxylation of lysine and proline and permit the formation of stable structure of the collagen.[SJ Padayatty, M Levine First published: 25 January]

The best way to obtain vitamin C in diet, by plant food which represents the primary source of vitamin.[Paul Knekt, head of laboratory, Ritva Jarvinen, lecturer**b**, Antti Reunanen, head of laboratory**a**, Jouni Maatela, chief physician , Published 24 February 1996]. Synthetic vitamin C is chemically indistinguishable from plant derived vitamins, fruits and vegetables have different micronutrients and phytochemicals that can affect its bioavailability.
[V.AbinashT.RahulM.G.AntonirajJ.A.MosesC.Anandharamakrishnan , 29 January 2021]

Nowadays, vitamin C level is very low which are present in main crops, and as a consequence human does not received proper amount of intake vitamin C in their diet· [Giuseppina P. P. Lima, Fabio Vianello , 17 December 2010]. Thus the plant which provide proper amount of vitamin C as food is represent as an important goal for human health. [*Int. J. Mol. Sci.* **2015]**

Significant part of accessible glucose is used in vitamin C production, which re-present as a high concentration in the plants. [Glen L. Wheeler, Mark A. Jones & Nicholas Smirnoff 1998]. Vitamin C was found in all cell compartments, including the apoplast which reach concentration of 20 mm in the chloroplasts. [Nicholas Smirnoff & Glen L. Wheeler , 2010]. However, vitamin C content is significantly differs among plant species and in same species between among diverse cultivars. Moreover, the vitamin C content varies in different tissues and organs, usually high in leaves, meristematic tissues, flowers, or young fruits and low in non-photosynthetic organs such as stems and roots

·[Mark W Davey, Marc Van Montagu, Dirk Inzé, Maita Sanmartin, Angelo's Kanellis, Nicholas Smirnoff, Iris JJ Benzie, John J Strain, Derek Favell, John Fletcher ,2002]. In some organ or tissue, vitamin C content is influenced by the plant developmental stage and environmental changes [**R.N. Tharanathan ,**H.M. Yashoda & T.N. Prabha Pages 95-123 | Published online: 06 Feb 2007]Vitamin C is involved in the synthesis of the plant hormone ethylene, acting as a cofactor of 1-aminocyclopropane-1- carboxylic acid oxidase, the enzyme that catalyzes the biosynthetic.[Mario C.De Tullio Volume 178, October 2020, 104173]. Contributes to the ring opening of 1-aminocyclopropane-1- carboxylic acid by supplying the electron to the active site of the enzyme [Solomon, Thomas , Mindy I. Davis , Jyllian N. Kemsley ,Sang-Kyu Lee ,Nicolai Lehnert , Frank Neese ,Andrew J. Skulan ,Yi-Shan Yang ,and Jing Zhou , December 18, 1999]

Being a cofactor of dioxygenases, vitamin C could be involved in the synthesis of abscisic acid and gibberellins, as well as in catabolism of auxins. In plants, vitamin C can controls division, elongation, and differentiation of cells, as well as programmed cell death. [Gabriela M. Pastori, Guy Kiddle, John Antoniw, Stephanie Bernard, Sonja Veljovic-Jovanovic, Paul J. Verrier, Graham Noctor, Christine H. Foyer ,Issue 4, April 2003]Vitamin C plays an important role in controlling of cell division. This metabolite in the meristematic cells of root, meristems can shorten the G1 phase and stimulate the entry of S phase [NICHOLAS SMIRNOFF , 30 July 1996] Vitamin C, regulating the processes at molecular and cellular levels, therefore it is involved in different phases of plant growth and development, such as seed maturation and germination, flowering, fruit ripening, and senescence [*Int. J. Mol. Sci.* **2021**, *22*(9)]

Materials and Chemical required

Beakers, glass rods, spatulas, volumetric flask, measuring cylinder, conical flasks, burette, and burette stand, weighing scale. Oxalic acid, Sulphur acid, distilled water, DCIP dye (taken 26mg of DCIP dye + 21mg of sodium bicarbonate and was dissolved in 100ml of distilled water and after dissolving the solution has filter) , Sodium Bicarbonate, and Ascorbic acid.

Methodology

Fresh vegetables Pisum sativum (Green peas), *Spinacia oleracea L*.(Spinach), *Daucus carota* (Carrot), *Solanum tuberosum* (Potato), *Brassica oleracea* (Broccoli), *Phaseolus vulgaris L*.(Beans),*Brassica oleracea* (Cabbage),*Solanum Lycopersicum* (Tomato), *Capsicum Annuum* (capsicum), *Brassica Juncea* (Mustard) were collected from local market and thoroughly washed with distilled water followed by surface sterilization using sodium hypo chloride

The filtered DCIP+ Sodium Bicarbonate solution was taken in the burette which is act as self-indicator, 5ml of the stock solution was taken in a conical flask and was titrated against DCIP+ Sodium bicarbonate solution taken in burette till the pale pink color is seen. The titration is stopped and the titration value was noted and again the process was repeated one more time to get concurrent value (Blank titration)., then 5 ml of each sample were taken in conical flasks and titrated against DCIP+ Sodium Bicarbonate solution taken in burette till pale pink color is seen, the titration was repeated until concurrent values were obtained and were noted. From these values determined, the vitamin C content by using following formula.

$$\textbf{Vitamin C} = \frac{0.5mg}{V2} \times \frac{V1}{5ml} \times \frac{100ml \times 10}{wt.\ of\ the\ sample} \times 100$$

Where
V1 = blank titration value

V2 =sample titration value

Wt. of the sample= 100gm

Result:

In this experiment we estimated Vitamin C (Ascorbic acid) content , in the following vegetables they are- *Pisum sativum* (Green peas) -83.3mg/100g, *Spinacia oleracea L.* (Spinach) -33.3mg/100g *Daucus carota* (Carrot) - 111mg/100g, *Solanum tuberosum* (Potato) - 125mg/100g,*Brassica oleracea* (Broccoli) - 111mg/100g, *Phaseolus vulgaris L.*(Beans) - 125 mg/100g,*Brassica oleracea* (Cabbage) - 166mg/100g , *Solanum Lycopersicum* (Tomato) -59mg/100g, *Capsicum Annuum* (capsicum)-48mg/100g, *Brassica Juncea* (Mustard) – 47mg/100mg . By using titrimetric method.

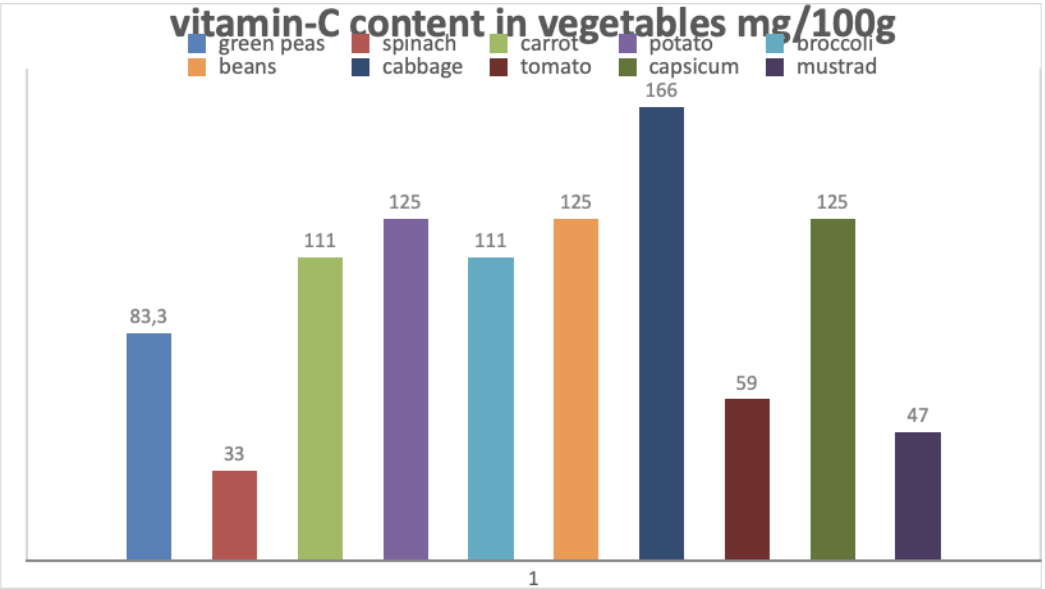

Figure: vitamin-C content in vegetables mg/100g

Discussion

In the above experiment, the Vitamin C content of various fresh vegetable results indicated that the given vegetables studied contained Vitamin C, but in different concentration.

The Vitamin C concentration is greater in Cabbage i.e. 166mg/100g, and less in Spinach I.e. 33.3mg/100g.

Some vegetables had the same amount of Vitamin C concentration such as Potato and Beans(125mg/100g/) and Broccoli and Carrot (111mg/100g)

Thus, we can see that there may be some factors that can affect the Vitamin C level invegetables, such as analytical method, production process, and degree of ripeness, temperature, climate, maturity state, and soil nutrients.

This experiment, therefore, may serve as a good guide on selection of a particular vegetable for the daily requirement of Vitamin. C.

This method is simple, convenient and less time consuming.

Conclusion

The obtained result is also good agreement with given data regarding the contents of vitamin C (Ascorbic acid) in vegetables. The result obtained in the study shows that the titrimetric method can be used as a part of quality management in food industry successfully, for accessing the Vitamin C content in vegetables. Vitamin C found in vegetables are useful for the body for healing wounds and form scar tissue, repair and maintain cartilage ,bones and teeth, helping to protect cells and keep them healthy. It is a powerful antioxidant and plays an important role in immune system, neurotransmitter production, in collagen synthesis. It improves the absorption of non- heme iron. Vitamin C is also a powerful antioxidant. It helps in removing unwanted substances, it also help to reduce inflammation and lower the risk of developing various conditions , which includes cancer. Low level of vitamin C can lead to swollen joints, bleeding gums, and loose teeth , anemia and tiredness.

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UGC CARE Group 1

THE EFFECT OF EMPLOYEE ENGAGEMENT ON EMPLOYEE PERFORMANCE: A CASE STUDY

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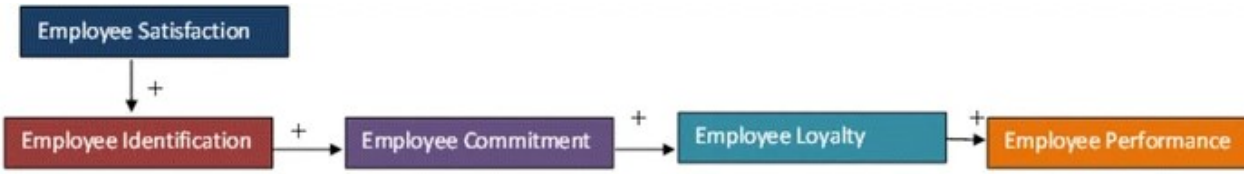
Abstract

The amount of dedication and interest displayed by workers in their organisation and the ideals it promotes is referred to as "employee engagement and the word comes from the phrase "engaged employees." In addition to investigating the link between employee engagement and performance, the outcomes of this research will analyse the variables that influence employee engagement. In addition to compensation and benefits, evaluations look at health and safety conditions as well as the company's overall performance, as well as internal communication, leadership development, and other aspects of the job. As part of this study's design, it included both the causal and descriptive parts of research.In order to obtain the data, a standardised questionnaire was used, and the number of workers that comprised the sample was predetermined to be 100. When doing an analysis of the data, descriptive statistics are an important tool to have at your disposal.

Keywords: Employee, engagement, factors, performance

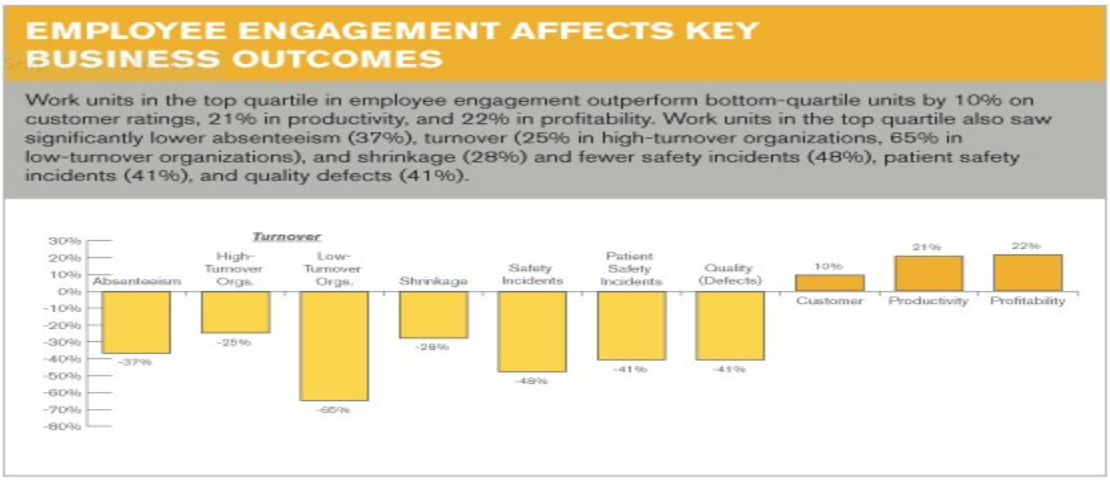
Introduction

An organization's production and profitability are directly correlated to its employees' levels of engagement. This book deals with a variety of topics, including commitment, fulfilment, and organisational behaviour. "Employee engagement" refers to a worker's commitment to his or her employer and its values. When employees are engaged, they are aware of the company's issues and work together with their coworkers to improve their job. Emotional connection to the work of colleagues is associated with higher productivity. People exhibit their involvement in a variety of ways, including via their actions and personality traits. "The purpose of this study is to identify the factors that help employees feel like they belong at work". It is therefore possible to enhance employee performance by discovering the main elements that characterize engagement. A person's performance at work may be defined as their accomplishments and successes there. The importance of a company's workforce's sense of purpose is well-documented. According to research, greater levels of employee commitment to the company are associated with better quality work product.



The influence of employee engagement on productivity is studied using a variety of factors. Engaged workers are thought to boost organisational performance, reduce attrition, and have a more favourable attitude about their jobs. One of the most important concerns is the broad interest in employee engagement. Identifying and conveying expectations of one another may lead to clarity in expressing a clear vision, and this can be achieved via the use of an intranet.

It fosters a sense of trust and cooperation among team members and inside the company. Even under the most difficult of circumstances, the employee works hard to keep the company's reputation intact. To put it another way, these factors help to clarify the relationship between employee involvement and job success inside a business.



Review of Literature

As stated by T.Suhasini and K. Kalpana (2018b), According to the report, employee engagement is a complex issue that cannot be solved with successful training programmes. Through opportunity thinking, decision making, and employee involvement, organisations may boost engagement. Reducing the likelihood of employee attrition and fostering more creative thinking at work are both aided by high levels of employee engagement.

A research by Sivasubramanian and Rupa, 2017, found that stronger incentive programmes and techniques that indicate an interest in employee career development are a sign of employee satisfaction. In order to enhance employee engagement, organisations need to find means of acknowledging the contributions of their workers.

In the work of Dr Naveen Prasadula. MSC (I.T), MBA, PHD (2021) Department of Business Management Osmania University, as a retention strategy and a growth driver, high levels of employee engagement have been

shown to have a positive impact on organisations. Employee involvement at private banks was found to be lacking in this research.

Researcher: NP Myilswami, Dr: R. Gayati to put it another way, the amount of employee engagement has a direct impact on productivity and staff retention. Certainly. Increased loyalty, retention, safety, productivity, and profitability are all bolstered by a more engaged workforce. People who are really invested in their jobs are more likely to be loyal to the company as a whole. It's all about how deeply and for how long a person becomes involved with the hub and how long they stay there as a consequence.

Employee engagement, according to Robinson et al. (2004), is defined as a favourable attitude among workers toward the company and its principles. Employees that are actively involved in their work adapt to changes in the business environment and collaborate with their coworkers to better their own performance and that of the company as a whole. Employers and employees need to work together to foster an environment of mutual respect and cooperation if they are to achieve meaningful employee engagement.

Goals of Research

1. Insights about the relationship between employee motivation and output.
2. Employee engagement may be studied by looking at the elements that impact it.
3. Employee involvement as a setting for investigating job satisfaction.

Research Methodology

In order to collect data, a structured questionnaire is employed to collect methodological information. Employees of manufacturing enterprises are the focus of this research. Non-probability sampling method called "convenient sampling" is utilised to get answers from workers, and 100 employees are the chosen sample size for the study. The effect of employee engagement may be measured using a variety of methods.

Analysis and Interpretation

Table : Descriptive Statistic

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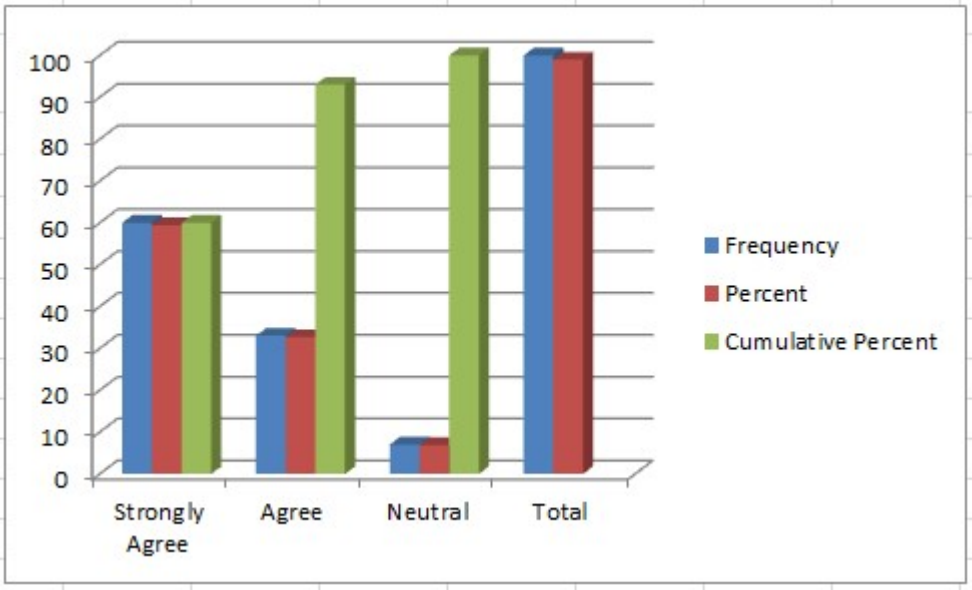
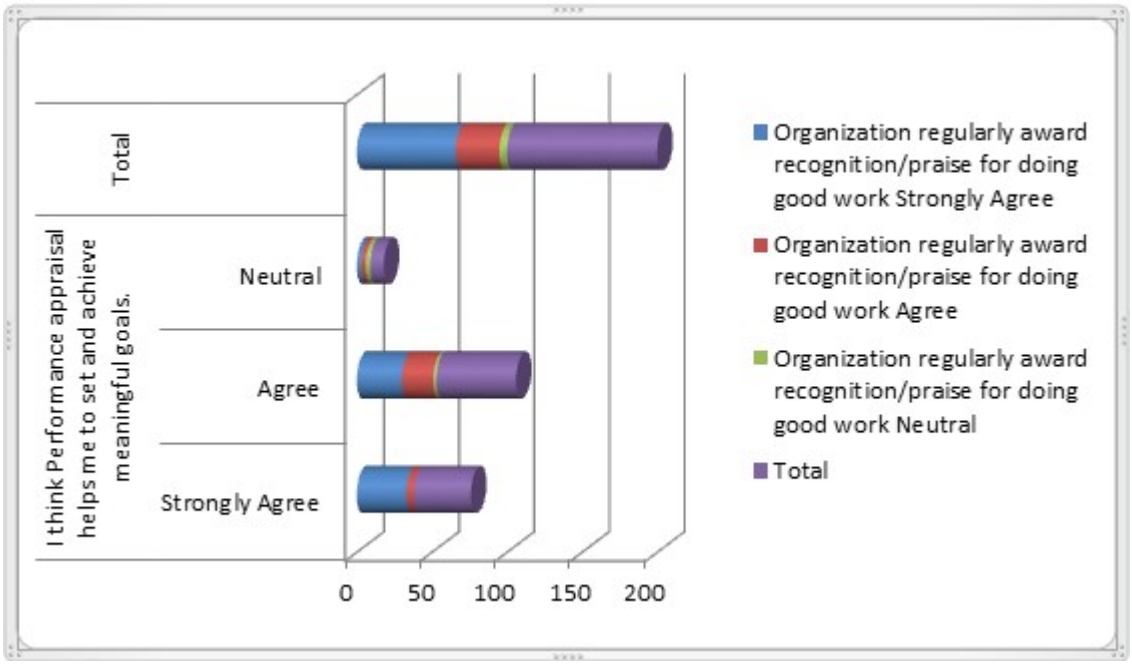


Only 7 percent of workers disagreed and were indifferent; no one was displeased. This data is based on the question answered by employees: "How satisfied are you with the total salary in your organisation?" No one objected to the assertion about overall compensation satisfaction. From the replies, it can be determined that the overall remuneration received by workers is satisfactory. "

Table: Commendation and praise for excellent work * performance evaluation aids in goal setting and attainment.



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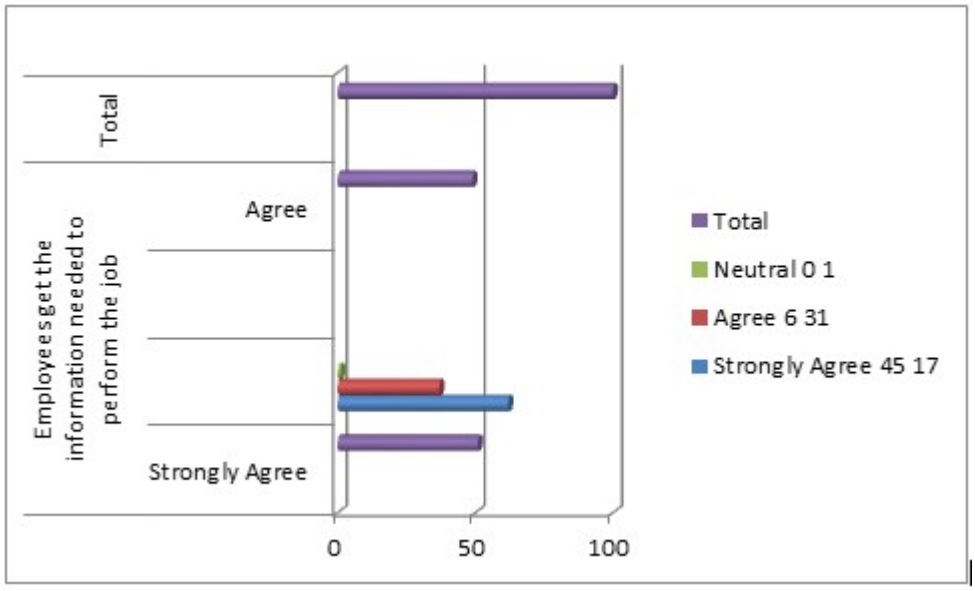
Workforce members are happy with the company's reward and recognition systems, as seen in the graph above, and the performance evaluation component aids them in setting and achieving their desired goals. When asked whether their company provided frequent appreciation for exceptional performance, 33 workers said they did, and they also said it helped them create and accomplish significant objectives. The impact of one on the other, however, is well-known for both.

Get all the information you need for the work with the help of this table.



Observation: It was discovered that 45 workers in one business had given their express approval to receive all of the information required to carry out their jobs, according to the interpretation provided by the researcher. Both views were supported by 31 workers. Employees can acquire the knowledge they need to execute their jobs when there is open communication throughout the company's ranks. Again, none of the workers disagreed with any of the comments made so far.

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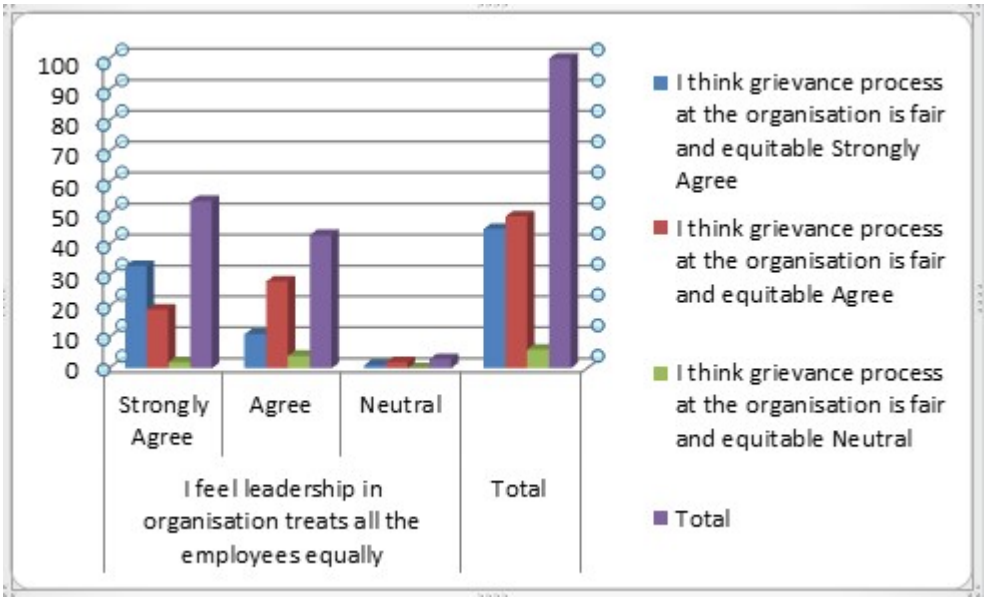

It can be deduced from the data in the table above that the company's workers are dedicated to ensuring that all employees are treated equally and fairly. More than two-thirds of workers have agreed to both standards, but just 28 have agreed to both considerations. This indicates that employees are fair and just, but they also believe that these conditions dominate management since all employees are treated equally.

Findings

The remuneration package offered by the corporation was generally well-liked by employees. It's common for employees to be rewarded and acknowledged for their efforts. Occupational health and safety are just as important as output and quality in the workplace. After a performance review, employees are given a raise in pay. As a whole, employees are pleased with how well the company is doing its job. Employees are made aware of company policies, objectives, and plans. Employees like their time spent at the company. The workers have all the tools and supplies they need to get the task done. Employees at the company are given the proper training by their managers. Employees have confidence in their abilities and are motivated to succeed. Employees are well-versed in the ins and outs of their professional paths. Managers provide their employees with helpful criticism. The improvement in total pay satisfaction as a result of the wage increases. The research found that employees are more likely to create and accomplish meaningful objectives if they have a positive attitude about workplace incentives and recognition and the performance evaluation process. Additionally, managers' input is addressed by the firm in its training programmes for staff.

Conclusion

According to the study's findings, workers are happy with the company's overall performance. Employees are provided with the knowledge they need to perform their duties effectively. Engagement has a favourable effect on employee output in the company in all of its facets. The total remuneration of the company is well-received

by all workers. To ensure that all workers are aware of the organization's objectives and initiatives, training and communication are available. I discovered a substantial correlation between the variables using the crosstab test. People agree that rules are conveyed and that they are provided with the knowledge they need to execute their jobs, and they believe that performance appraisals assist the company achieve an important objective. The business has leadership because all workers are treated equally.

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