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COMPARATIVE BIOEFFICACY OF *IPOMOEA PES-CAPRAE* ROOT AND STEM EXTRACTS: PHYTOCHEMICALS, ANTIOXIDANTS, AND ANTIMICROBIALS

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ABSTRACT

Introduction: Active natural compounds from *Ipomoea pes-caprae* plant were strongly acknowledged in various literatures for their biological and pharmacological significance.

Aim: The study aims to identify bioactive fractions from stem and root parts of *Ipomoea pes-caprae.*

Method: Solvents like ethanol, ethyl acetate and diethyl ether used for stem extraction, whereas chloroform-methanol and acetone were used for root extraction which is followed by phytochemical analysis using standard procedure and partial purification by column chromatography. The bioactive fractions collected from stem extracts i.e., ethanol *Ipomoea* extract fraction (EIEF) ethyl acetate *Ipomoea* extract fraction (EAIEF), Diethyl ether *Ipomoea*

extract fraction (DEIEF) & from root extracts i.e., chloroform-methanol *Ipomoea* extract fraction (CMIEF) and acetone *Ipomoea* extract fraction (AIEF) were subjected to antimicrobial and antioxidant screening along with spectral studies like UV-Visible, Fourier transform infrared spectroscopy (FTIR) and Gas Chromatography Mass Spectrophotometry (GCMS) to identify and characterize the biochemicals. However, with the limited experimental studies, solvents based on polarity were used for stem and root extraction followed by phytochemical analysis and partial purification.

Results: The phytochemical analysis of extracts revealed the presence of polyphenols, flavonoids, saponins, fatty acids, and proteins which were further authenticated by UV-Visible, FTIR and GCMS. The highest zone of inhibition was identified against *P. aeruginosa* and *B. subtilis* among bacterial pathogens tested in case of CMIEF. CMIEF and EIEF showed the half-maximal inhibitory concentration (IC_{50}) values of 35.87 ± 0.28 and 39.89 ± 0.28 µg/ml respectively for 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) assay.

Conclusion: The present study confirmed that CMIEF has excellent antibacterial and antioxidant properties against infectious organisms.

Keywords: ***Ipomoea pes-caprae,*** **phytochemical analysis, GC-MS, antibacterial, antioxidant**

INTRODUCTION

Coastal sand dunes (CSDs) are unique and dynamic ecosystems that exist at the intersection of the marine and terrestrial worlds and making them as vital sites for environmental protection **[1]**. CSD legumes offers a variety of useful quality and tend to have a wide range of applications, including medical, pharmacological, industrial, nutritional, and ecosystem restoration. Many studies have shown that the presence of secondary metabolites, particularly antioxidants and polyphenolic compounds like flavonoids, tocopherol, carotene, ascorbic acid, anthocyanins, alkaloids, tannins, triterpenes, coumarins, carbohydrates, steroids and other phenolic components, have a major role in their therapeutic potential **[2]**. The diverse spectrum of biological activity seen in *I. pes-caprae* plant and its extracts could possibly associate with the existence of such bioactive components. Among the secondary metabolites, the most important one's are phenolics and flavonoids, which are produced during stress and are found to have strong antioxidant, anticancer, antimicrobial, antihistaminic, insulinogenic, hypoglycaemic, immunostimulant, anti-inflammatory, and antispasmodic properties **[3,4]**.

Ipomoea pes-caprae is a member of the *Convolvulaceae* family that grows wild along India's ocean coasts and is found all over the world. At significant

drought and saline stress, it acts as a sand binder, growing down and most well-drained soils will support this full-sun plant **[5]**. *I. pes-caprae R.Br.,* also known as Goat's foot or Beach Morning Glory is a prevalent tropical encroaching vine that is part of the 600-species of *Convolvulaceae* family **[6]**. Along coastal beaches, it grows just over the high tide line, creating enormous mats that helps to stabilize the sand. This perennial plant is evergreen and has a big, deep root that can grow up to 10 feet long and 2 inches in diameter. All of the plant is rather mushy and glabrous, flowers are upright as the stem roots at the nodes and runs parallel to the ground **[7,8]**.

Ipomoea pes-caprae **(Figure 1)** belongs to Kingdom: Plantae; Sub kingdom: Tracheobionta; Division: Spermatophyta; Subdivision: Magnoliophyta; Class: Magnoliopsida – Dicotyledons; Subclass: Asteridae; Order: solanales; Family: Convolvulaceae; Genus: *Ipomoea*; Species: *pes-caprae*. Alkaloids, flavonoids, norisoprenoids, phenolics, saponins, terpenoids, steroids, glycosides, leucoanthocyanidins, and carbohydrates are the main constituents of the phytochemical examination of the *I. pes-caprae* acetone extract **[9]**. According to Sheeba *et al*. (2019), *I. pes-caprae's* aqueous flower extract has the potential to be a medicinal agent that targets particular bacteria and cancer cells.

Figure 1: *Ipomoea* plant (Sand dune - Beach Morning Glory)

I. pes-caprae can be a substantial supplier of antioxidant phytochemicals and has the ability to scavenge free radicals **[10]** betulinic acid, glochidone, alpha and beta-amyrin acetate, isoquercitrin in the formalin and writhing test in mice, and for managing dolorous procedures, among other substances, have strong antinociceptive properties **[11]**. It has been suggested that the leaf extract has laxative, diuretic, and

astringent qualities and are employed traditionally as a tonic, stomachic, and rheumatism remedy **[12]**. Additionally, it is utilized to treat piles, vomiting, diarrhoea, and for platelet aggregation inhibition **[13]**. The majority of the antioxidant chemicals found in a normal diet usually comes from plant sources, and higher plants polyphenolic components frequently function as antioxidants or through other means to prevent cancer **[14]**. The biological activity of the plant varies depending on its sections, accordingly pharmacognostic investigations of medicinal plants are therefore crucial and significant **[15]**. For example, Pothula and Kanikaram assessed the antimalarial activity of *I. pes-caprae* root extracts in vitro and found that the methanolic extract has an excellent antimalarial activity towards *Plasmodium falciparum* strain 3D7 as indicated by half-maximal inhibitory concentration (IC_{50}) value of 15 µg/mL **[16]**.

I. pes-caprae was also found to contain a few physiologically active substances, including eugenol, 4-vinyl guaiacol, actinidol, (E) phytol, betulinic acid, iso quercitin, stoloniferin, and pescaprosides A and B **[17, 18]**. Even though our study proposed was based on the previous findings, however, literature reviews revealed that less scientific studies had been done on *Ipomoea pes-caprae*. Henceforth, the objective our work was to extract and identify distinct biomedical compounds from the diverse solvent extracts of *Ipomoea pes-caprae* stem and root and to analyse further its pharmacological properties i.e., antimicrobial and antioxidant activities to explore the *I. pes-caprae* untapped potential.

2. MATERIALS AND METHODS

2.1 Materials

Chloroform, methanol, acetone and were procured from Virat Lab, Hyderabad, India. 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and l-ascorbic acid were acquired from Sigma-Aldrich, India. Folin–Ciocalteu phenol reagent, ninhydrin reagent, Liebermann–Burchard reagent, anisaldehyde spray, vanillin, hydrochloric acid, n-hexane, sulfuric acid, methanol, chloroform, acetonitrile, and benzene were acquired from SD Fine Chemicals Limited, Mumbai. Nutrient broth, Mueller Hinton agar, silica gel G and ethanol were purchased from Siri scientific Limited, (Rajahmundry, India). Other chemicals employed were of analytical grades.

2.2. Collection & Authentication of Plant material

I. pes-caprae (with its leaves, stems, flowers and roots) plant were amassed from the coastal shorelines of Vishakhapatnam, Andhra Pradesh and later verified by comparison with the authenticated herbarium specimens available at the

Department of Botany, Andhra University, Vishakhapatnam.

2.3 Preparation of solvent extract

The plant's freshly constellated roots were shade-dried **(Figure 2a)** pulverized, and sieved using a sieve with a mesh size of 20. The powdered sample was consecutively extracted using different solvents by Soxhlet extraction method. A dried sample powder of approximately 25 g was weighed, and the extraction procedure ensued for 48 hours using 100 ml of Chloroform-Methanol solvent mixture. After being concentrated to the consistency of gel for eight hours at 40–50 °C in a hot air oven, the extract was dried, which was then kept at room temperature **(Figure 2b)**. For preparing stem extract, ethanol, ethyl acetate and diethyl ether were utilized and for root extract, chloroform:methanol and acetone solvents were employed. The obtained solid extract residues were subjected to partial purification by column chromatography **[4,19]**.

Figure 2a: Dried root parts of *Ipomoea sp.*

Figure 2b: (a) Solvent (Chloroform-Methanol & Acetone) root extracts of *Ipomoea sp.* and (b) *Ipomoea* root extracted residue

2.4 Characterization of solvent extracts

2.4.1 Detection of Phytoconstituents

A tiny bit of the dried extract was employed for phytochemical analysis to identify the prevalence of tannins, phenols, carotenoids, alkaloids, flavonoids, steroids, proteins, saponins, carbohydrates, reducing sugars, quinones, in consonance with the regular standard approaches with minor modifications and standard protocol given in Brian and Turner 1975 and Evans 1996 **[20, 21]**. All the phytochemical tests were carried out separately for each extract like EIE (Ethanol *Ipomoea* stem extract), EAIE (Ethyl acetate *Ipomoea* stem extract), DEIE (Diethyl ether *Ipomoea* stem extract), CMIE (Chloroform-Methanol *Ipomoea* root extract) and AIE (Acetone *Ipomoea* root extract).

2.4.2 Partial purification of bioactive compounds

For separating active compounds, the crude stem extracts (EIE, EAIE, DEIE) and root extracts (CMIE and AIE) were partially purified via Silica open-column chromatography and separated by step-gradient elution with successive ratios of solvent mixtures like hexane: ethyl acetate, chloroform: ethanol, diethyl ether: ethyl acetate for stem extracts and chloroform: methanol and acetone for root extracts and the collected fractions were amassed **[22] (Figure 3)**.

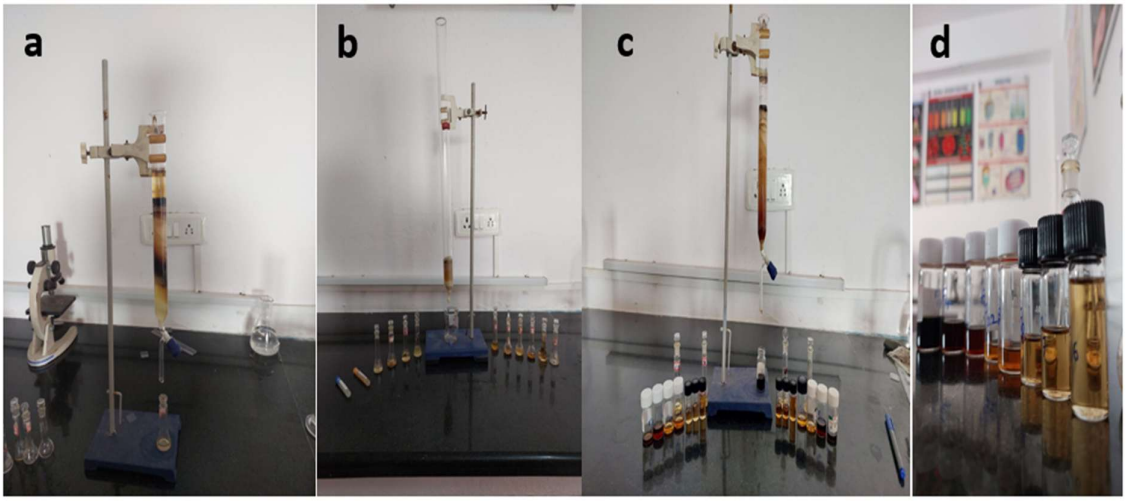

Figure 3: Partial purification of *Ipomoea* extracts; (a) Loading the sample; (b) Collection of fractions from Chloroform-Methanol extract; (c) Acetone extract fractions and (d) Collected fractions

2.4.3 Determination of total phenolic content (TPC)

TPC (Folin-Ciocalteu test) were estimated for stem (EIE, EAIE, and DEIE) and root (CMIE and AIE) extracted fractions. To 20 µl extract + 100 µl of Folin-Ciocalteau's reagent; 5 minutes at 37 °C; add 75 µl of 60 gL^{-1} sodium bicarbonate solution; stir the mixture; incubated for 120 min at 37°C (dark place) & absorbance measured at 765

nm. Quantification was done based on the linearity plot of GA concentration from 50 to 500 mg/mL (r^2 = 0.9829). The TPC was estimated from the calibration curve, stated as mg of GA/g of DW **[23]**. The experiment was done in triplicates.

2.4.4 Determination of total flavonoid content (TFC)

The TFC of stem (EIE, EAIE, and DEIE) and root (CMIE and AIE) extracted fractions was ascertained by $AlCl_3$ calorimetric technique **[24]**. Extract (0.5 mL) + 1.5 mL methanol + 0.1 mL $AlCl_3$ (10 %), 0.1 mL of 1 M CH_3CO_2K + 2.8 mL DW; mixture kept in dark (30 min); read at 415 nm (absorbance). TFC was estimated from the calibration curve [Quercetin concentration extending from 50 to 500 mg/ml (r^2 = 0.9583)] and explicated as mg of QE/g of dry weight. Every measurement was done in triplicates.

2.5 Spectroscopy methods

2.5.1 UV-Visible spectrophotometry

The fractions obtained from stem extracts, namely EIE, EAIE, and DEIE, as well as the fractions obtained from root extracts, CMIE and AIE, were subjected to UV-Visible spectroscopy using a Lab India 3200 instrument. The wavelength range used for scanning was from 200 to 800 nm. The UV-Vis spectra exhibited apparent peaks, which were subsequently identified and their corresponding values were documented. The wavelength bands corresponding to distinct secondary metabolites are presented (**Table 1) [25-28]**.

Table 1: The ranges of wavelength corresponding to particular secondary metabolites

S. No	Absorption maxima (wavelength range nm)	Phytochemical compounds (Bioactive metabolites)
1	234 - 676	Phenolic compounds, Flavonoids, and Alkaloids
2	230 - 285 (band I)	Flavonoids and Flavonoid derivatives
3	230 - 290 (band I)	Flavonoids
4	300 - 350 (band II)	Flavonoids and Flavonoid derivatives
5	250 - 500	Tannins
6	400 - 450	Carotenoids
7	400 - 550	Terpenoids
8	600 - 700	Chlorophyll

2.5.2 FTIR spectra

The bioactive fractions such as EIEF-S and CMIEF-R were dried, blended with KBr to attain a pellet and subjected to standard procedure for obtaining FT-IR spectra (Shimadzu IR spectrophotometer, model 840, Japan) **[29]** in a wavelength between 4000 and 400 cm^{-1} at a resolution of 4 cm^{-1}. From the absorption bands, the vibrational assignments and wave number (cm^{-1}), the intensities of predominant and weak peaks were documented.

2.5.3 Gas Chromatography – Mass Spectrometry

The bioactive fractions, namely EIEF-S and CMIEF-R, were subjected to GC-MS analysis utilizing the Shimadzu/ QP2020 GC instrument, which was linked with the

MS-5975 inert MSD and triple axis mass selective ion detector. The experiment utilized a DB-5MS capillary column with dimensions of 30m x 0.2mm. The initial temperature was fixed at 150 °C and the maximum temperature was set to 300 °C. A sample volume of 1 µl was injected using the split mode at a ratio of 10:1. Helium functioned as the carrier gas with a flow rate of 0.8 ml/min. The overall run duration for the experiment was 25 minutes. Phytochemical substances were discovered utilizing the NIST-MS library **[29]**.

2.6 Screening for Antimicrobial efficacy

2.6.1 Agar well diffusion (AWD) assay

Antimicrobial action of extracted fractions was checked by Agar well Diffusion method using a standard protocol. Gram – ve bacteria (*P. aeruginosa* and *E. coli*), Gram +ve bacteria (*S. aureus* and *B. subtilis*) and fungi (*C. albicans* and *A. niger*) test microorganisms were acquired from the GSL Institute of Medical Science (Department of Microbiology), Rajahmundry, India. To culture bacterial and fungal clinical pathogens, Mueller Hinton (MH) broth were exploited accordingly. The test microorganisms were inoculated in to the media using cotton swab (30 min to dry); wells formed (6 mm in diameter); concentrations of 10, 20 and 30 µL of EIEF-S and CMIEF-R (1 mg mL^{-1}) filled in to the respective wells; incubated at 37 °C (24 h) & inhibition zones measured **(Figure 4)**. Streptomycin (1 mg mL^{-1}) was added as standard separately & all the experiments were executed in triplicates **[30]**. Antimycotic activity screening was carried out exercising a similar method as for bacteria. The test organisms *C. albicans* and *A. niger* was set to turbidity of 0.5 McFarland standard were utilized as an inoculum. In place of nutrient agar, Sabouraud dextrose agar was employed; inoculated & incubated at 25°C for 2 days. Nystatin (100 µg/ml) (+ve control, standard) and DMSO (–ve control) for antifungal assays.

Figure 4: Antimicrobial assay of *Ipomoea* root extracts

2.6.2 Antioxidant activity

a) DPPH assay

The following methodology was adopted: Control types used in the assay includes negative control i.e., mixture of 50 µL DPPH solution + 200 µL ethanol, whereas the positive control consists of Ascorbic Acid (AsA) in concentrations range from 10 – 100 µg mL-1 corresponding to that of extracts. Sample extracts + 250 µL ethanol mixture were used as a blank **[31]**. The experiment involved adding 50 µL of freshly made ethanol-DPPH solution (0.3 mM) to a mixture containing 200 µL of CMIEF and AIEF. The resulting solution was then incubated at a temperature of 37°C in a dark environment for one hour. Following the incubation period, the 517 nm wavelength was used to test the solution's absorbance.

$$\% \text{ DPPH scavenging activity} = \frac{(\text{Control absorbance} - \text{Sample absorbance}) \times 100}{\text{Control absorbance}}$$

The DPPH% values generated from the aforementioned equation were graphed against the concentration of AsA standard. The RSA of the samples, reported as mg (AsAE)/100 g sample, was calculated using linear regression ($R^2 = 0.989$). The measure of antioxidant efficacy was expressed as the IC_{50}, which represents the level of antioxidant required that mitigates the starting concentration of DPPH by 50%.

b) Hydrogen peroxide assay (HPA)

HPA was estimated using 1 ml each of EIEF, EAIEF, and DEIEF with varying concentrations from 10 – 100 µg/ml. To which phosphate buffer (2.5 ml, pH 7.4, 100 mM) + H_2O_2 (400 µl, 5 mM) were added; incubated at 37°C (20 min) & read at 610 nm (absorbance). The blank (mixture without sample) and control (AsA) were considered for every experiment **[32]**.

c) FRAP

Concentrations that range from 10–100 µg/ml of EIEF, EAIEF and DEIEF (1 ml) was taken; to which phosphate buffer (0.2 M, pH 6.6) + 2.5 ml of 1% Potassium ferricyanide was added; the reaction was stopped with 1 ml trichloro acetic acid (10%); incubated at 50°C (20 min); centrifuged (3000 rpm, 15 min); Ferrous chloride dissipated into the supernatant & the solution absorbance was read at 700 nm using AsA as standard and FRAP was determined **[33]**.

2.7 Statistical Analysis

The data were evaluated using GraphPad Prism (Windows version 7.04) for linear regression. SPSS version 16 software (SPSS Inc., Chicago, IL) and one-way ANOVA were used to calculate the statistical analysis's findings. The results of each experiment were shown as mean ± SD (triplicates). If $p < 0.05$, the findings were deemed statistically significant.

3. RESULTS and DISCUSSION

The current study, stem extracts (EIE, EAIE, DEIE) and root extracts (CMIE and AIE) were prepared from *Ipomoea pes-caprae* and subjected to the evaluation of antibacterial and antioxidant activity.

3.1 Percentage yield of solvent extracts

The dry weight of *Ipomoea* biomass was determined to be 25 g. The obtained results for percentage yield of stem extract - ethanol (12.3 g), ethyl acetate (8.1 g), and diethyl ether (4.6 g) was found to be 49.20%, 41.28%, and 18.40% w/w respectively, the substantial yield was found in stem extract of ethanol and for the root extract, chloroform-methanol (16.7 g) and acetone (8.3 g), corresponding to be 66.8% and 33.2% w/w respectively. Plant-derived substances and extracts from plants are widely utilized in the management of various diseases, including neoplastic ailments, since they offer several advantages, such as reduced adverse effects, affordability, and widespread accessibility **[34]**. The highest yield was found in chloroform-methanol solvent mixture.

3.2 Phytochemical screening

Phytochemical screening for stem extracts (EIE, EAIE, DEIE) and root extracts (CMIE and AIE) was performed and the obtained results were shown (**Table 2**). According to Ramesh (2018), within the field of stem research, phenols were found to be the most abundant phytoconstituents, with other constituents present in moderate quantities. However, anthocyanins, coumarin, and quinones were not detected.

Table 2: Phytochemical analysis of *Ipomoea* Solvent extracts

S. No	Phytoconstituents	Tests	EIE	EAIE	DEIE	CMIE	AIE
1	Tannins	Gelatin test	+++	+++	+++	+++	+++
2	Phenols	Ferric chloride test	+++	+++	+++	+++	+++
3	Carotenoids	Chloroform and sulphuric acid test	-	-	-	-	-
4	Alkaloids	Mayer's test, Wagner's test	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++
5	Flavonoids	Alkaline reagent test Ammonium test	++++	++++	++++	++++	++++
6	Proteins	Biuret test	+	+	+	+++	++
7	Saponins	Foam test	+++++	+++++	++++	+++++	+++++
8	Terpenoids	Liebermann-Burchard reaction	+	+	+	+	+
9	Carbohydrates	Molisch's test Benedict's test	+++	+++	++	+++	+++
10	Reducing sugars	Fehling's test	++	++	++	++	++
11	Quinones	Borntrager´s test	-	-	-	-	-
12	Glycosides	Shinoda test	+++	+++	+++	+++	+++

+++ = a substantial quantity (positive within 5 minutes); ++ = a moderate quantity (positive after 5 minutes but within 10 minutes); + = Minimal quantity (positive after 10 minutes but within 15 minutes); - = Negligible.

The examination of the extracts of *I. pes-caprae* also indicated the existence of secondary metabolites, including flavonoids, saponins, alkaloids, tannins,

triterpenes, coumarins, phenols, steroids, phlobatannins, and carbohydrates **[16]**. Previous studies have established a correlation between plant phytochemicals and their bioactive properties, specifically highlighting the antibacterial and free radical scavenging capabilities exhibited by these phytochemical elements **[35]**.

3.3 Partial purification

The bioactive fractions were collected, concentrated by evaporation under reduced pressure at 4°C. After OSCC, a total of 12 fractions (F1 – F12) were collected for ethanol, ethyl acetate and diethyl ether solvent for stem extracts. Based on the UV-Vis spectral absorbance values of stem extract, bioactive metabolites were detected for ethanol *Ipomoea* extracted fraction EIE (F6), ethyl acetate *Ipomoea* extracted fraction EAIE (F7) and diethyl ether *Ipomoea* extracted fraction DEIE (F5) which were further evaporated to get a thick concentrated residue. After OSCC, a total of 15 fractions (F1 – F15) were generated for chloroform:methanol and acetone for root extracts. Based on the UV-Vis spectral absorbance values of root extract, bioactive metabolites were detected for Chloroform *Ipomoea* extracted fraction CMIEF (F7) and Acetone *Ipomoea* extracted fraction AIEF (F9) which were further evaporated to get a thick concentrated residue.

3.4 Spectroscopical techniques

3.4.1 UV-Visible Spectral analysis

The UV-Vis profile of the stem extracts (EIEF, EAIEF, DEIEF) and root extracts (CMIEF and AIEF) of *Ipmoea sp.* was assigned from 200 nm to 750 nm to reflect the prominence of distinctive peaks and proper baseline. From the UV-Visible profile, EIEFs F6 fraction revealed the absorbance at 261 nm, EAIEs, F7 fraction revealed the absorbance at 325 nm and DEIEFs, F5 fraction revealed the absorbance at 285 nm.

From the UV-Vis profile, CMIEFs F7 fraction revealed the absorbance at 256 nm. From the earlier research findings, the unique absorption bands observed in the wavelength range of 234–676 nm is an indication for the predominance of alkaloids, flavonoids, and phenolic chemicals and it becomes apparent that all peaks manifest within this specified range **[25, 36]**. Whereas, UV-Visible profile of the AIEFs, F9 fraction revealed the absorbance at 312 nm.

Previous research has indicated that the prevalence of alkaloids, flavonoids, and phenolic compounds which can be identified by absorption bands within the wavelength range of 234–676 nm **[25,36]**. In the current study, all observed peaks fall within this specified range. The peaks at 256 and 312 were recognized as flavonoids and their derivatives based on their absorption spectra, which exhibited characteristic bands in the wavelength extending from 230

to 285 nm (band I) and 300 to 350 nm (band II)[28,44,45]. An additional study also emphasized that the existence of peaks within the wavelength range of 280 to 330 nm was an indicative of phenolic derivatives suggesting that the extract used in the present investigation contains phenolic chemicals **[27]**. The stem extracts (EIEF, EAIEF, DEIEF) and root extracts (CMIEF and AIEF) of *Ipmoea sp.* were subjected to determination of TPC, TFC, antimicrobial and antioxidant screening.

3.4.2 Determination of total phenolic content

The total phenolic content value was computed using a gallic acid standard curve equation of y = 0.05x + 0.0912, R^2 = 0.99 varies from 78.41 to 117.65 mg GAE / g for EIEF, EAIEF, and DEIEF **(Figure 5)**. For stem extract, TPC of EIEF, EAIEF and DEIEF was found to be 117.65, 93.31, and 78.41 mg GAE / g respectively. For root extract, TPC of 215.53 & 103.25 mg GAE / g was obtained for CMIEF & AIEF respectively.

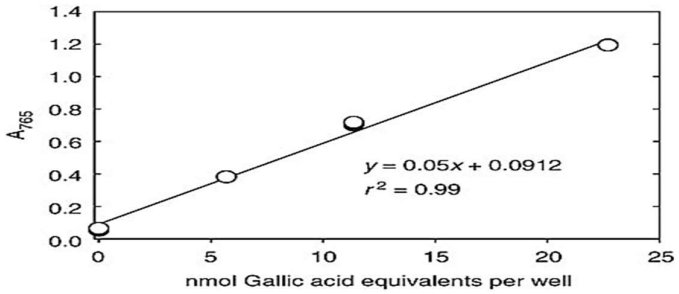

Figure 5: Calibration curve of TPC (mg/g GAE)

3.4.3 Determination of total flavonoid content

The total flavonoid content value computed tend to vary from 161.28 to 197.37 mg QE / g for EIEF, EAIEF, and DEIEF **(Figure 6)**. The TFC value obtained for each solvent used for preparing the stem extract was 197.37 for EIEF, 173.31 for EAIEF and 161.28 mg QE / g for DEIEF. Similarly, the TFC value obtained for each solvent used for preparing the root extract was 238.11 for CMIEF and 181.23 QE / g for AIEF.

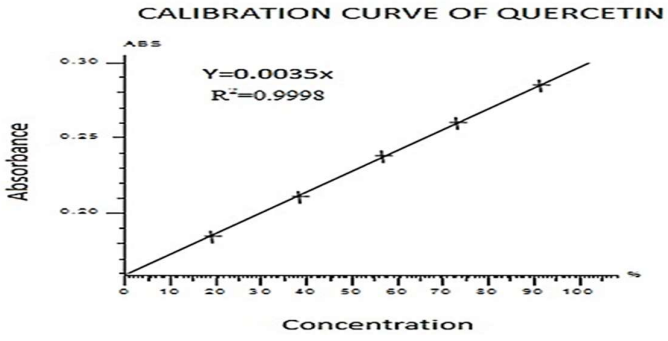

Figure 6: Calibration curve of TFC (mg QE/100g of dry weight)

The findings were acceptable with the former statements on the studied sand dunes reported by several workers **[39,40,41]**.

3.4.4 Fourier Transform Infrared study

The FTIR spectrum of partially purified EIEF-S (F6) and CMIEF-R (F7) were presented **(Figure 7a & 7b)**. The prototype in the fingerprint sector was used for spotting the presence of bioactive compounds.

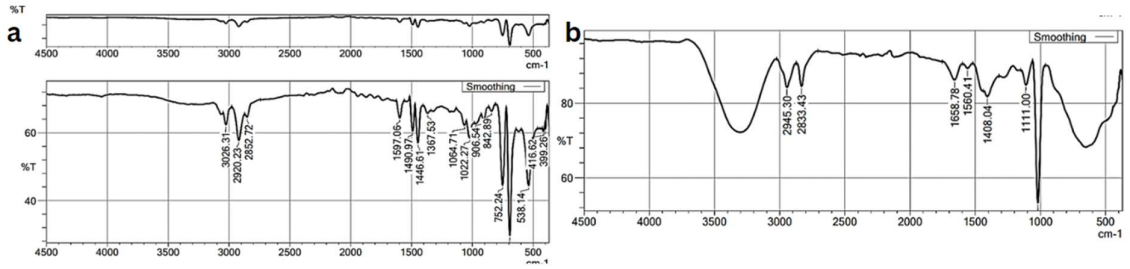

Figure 7a: FTIR spectra of EIEF *Ipomoea* stem extract; Figure 7b. FTIR spectra of CMIEF *Ipomoea* root extract

Table 3: FTIR peak spectral values with corresponding functional groups

Group frequency, wavenumber (cm^{-1})	Origin	Functional group
399, 416 (broad band)	C–Cl or C–I stretching	Halo (chloro/iodo) compound
538, 692, 752 (sharp peak)	C–H bending	Alkyl
842 (weak band)	C–H bending	Alkene
906 (weak band)	C–H bending	Alkane
1022 (sharp band)	C–O stretching	Flavonoid, phenol
1064 (intense band)	C–O stretching	primary alcohol
1367 (weak band)	C–H bending	Isopropyl alkane
1446 (sharp band) 1490 (sharp band)	CH_2 and CH_3 bending C–H bending	Lipids & proteins Alkane
1597 (sharp band)	C=O and C–O–H stretching	Carboxylic acid
2852 (weak band)	C–H stretching	Aldehydes
2920 (sharp peak)	C–H stretching	Aliphatic alkane/alkyl group
3026 (sharp band)	O–H stretching	Alcohol/polyphenols

Table 4: FTIR peak spectral values with corresponding functional groups

Group frequency, wave number (cm^{-1})	Origin	Functional group
1020 (sharp band)	C–O stretching	Flavonoid, Phenol
1111 (sharp peak)	C–O stretching S=O stretching	Aryl alkyl ether Sulphonic acid
1408 (broad band)	C=O stretching C=C stretching	Phenol, Flavanols
1564 (weak band)	C=O stretching C–O–H stretching	Carboxylic acid
1658 (sharp band)	C=O stretching	Fatty acid esters
2833 (sharp band)	C–H stretching	Aldehydes
2945 (intense band)	C–H stretching O–H stretching	Alkane Sulphonic acid
3387 (broad band)	O–H stretching C–H stretching	Polyphenol, Alcohol, Naphthalene

Based on the inference presented in **(Table 3 and Table 4)**, the prevalence of flavonoids, carboxyl groups of fatty acids, ether, ester, flavanols, carboxylic acid, aldehydes, alkanes, carboxyl and hydroxyl groups of alcohols & polyphenols in EIEF-S and CMIEF-R extracts are authenticated and the findings also conforms the results from previous studies **[42]**.

3.4.5 GCMS Spectra analysis

The results of GC-MS analysis of EIEF (F6) fraction reflected 16 major compounds and nearly 200 trace elements. The biological activities and peak values of predominant compounds were depicted **(Table 5a; Figure 8a)**. The major compounds identified such as 1,1 diethoxy ethane (56.24%), Hexadecanoic acid (1.04%), Octadecanoic acid (1.06%), Dodecanoic acid (5.26%), Behenic alcohol (1.95%), Phenol (1.09%), Hexanoic acid (1.08%), Decanoic acid (1.04%), Benzene dicarboxylic acid (2.64%), 1 Eicosanol (1.27%), Octadienol (1.28%), n-Pentadecanol (1.89%), n-Nonadecanol (2.89%), Glycerin (1.32%). The biological activities were documented in Dr. Duke's Phytochemical and Ethnobotanical Databases.

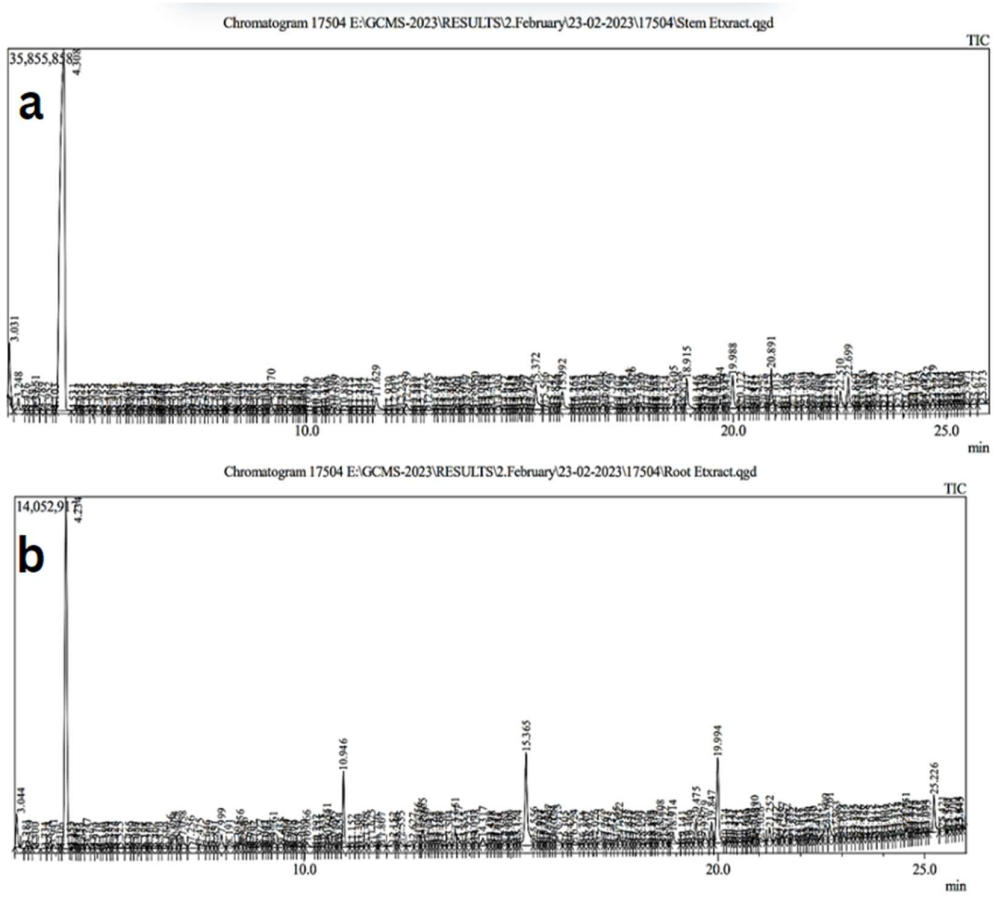

Figure 8a: GCMS analysis of EIEF *Ipomoea* stem extract; Figure 8b. GCMS analysis of CMIEF-R *Ipomoea* root extract

Table 5a: GC-MS analysis of EIEF-S stem extract and its biological activities

S. No	Compound name	Biological activity
1	Hexadecanoic acid (fatty acids)	Antioxidant, pesticide, Antifibrinolytic, Anti-hypercholesterolemic [43]
2	Octadecanoic acid (fatty acids)	Cancer preventive, anti-inflammatory, Anti-hypercholesterolemic [44]
3	Dodecanoic acid (fatty acids)	Cancer preventive, Fatty Acid Oxidation ailment, liver fatty acid metabolism intermediate, Anti-hypercholesterolemic [45]
4	Behenic alcohol/1- Docosanol (saturated fatty alcohol)	Antiviral agent, emollient, emulsifier, thickener in cosmetics [46]
5	Phenol/Polyphenol	Antioxidant, Anti-inflammatory, Cause cell cycle arrest, antibacterial, act as an insect pheromone, flavoring agent [47]
6	Hexanoic acid, Decanoic acid (saturated fatty acids)	Antimicrobial, Antioxidant [48]
7	Benzene dicarboxylic acid	Cancer preventive [48] [39]
8	1 Eicosanol, Octadienol, Pentadecanol, n-Nonadecanol (long chain aliphatic fatty alcohols)	Antimicrobial, Cosmetics, anti-hair fall agent [48]
9	Glycerin/Diglycerol	Hand sanitizers, skin moisturizers, deodorants, chewing gum, combination medications used to treat respiratory and urinary tract issues, insect repellent lotions and sprays, food additive and flavoring agent [49]

Table 5b: GC-MS analysis of CMIEF-R root extract and its biological activities

S. No	Compound name	Biological activity
1	Benzene sulphonic acid	Surfactants [48]
2	Hexadecanoic acid (fatty acids)	Antioxidant, pesticide, Antifibrinolytic [43]
3	Octadecanoic acid (fatty acids)	Cancer preventive, anti-inflammatory [44]
4	Undecanoic acid (fatty acids)	Antifungal [48]
5	Dodecanoic acid (fatty acids)	Cancer preventive, Fatty Acid Oxidation ailment, liver fatty acid metabolism intermediate [47]
6	Phenol	Antioxidant, Anti-inflammatory, Cause cell cycle arrest, antibacterial, act as an insect pheromone, flavoring agent [45]
7	Hexanoic acid, Decanoic acid (saturated fatty acids)	Antimicrobial, Antioxidant [48]
8	Carboxylic acid	Antimicrobial [48]
9	1 Eicosanol, Octadienol, Pentadecanol (long chain aliphatic fatty alcohols)	Antimicrobial, Cosmetics, anti-hair fall agent [48]
10	Dibutyl phthalate	Antimicrobial [49]

The results of GC-MS analysis of CMIEF fraction reflected 16 major compounds and nearly 200 trace elements. The biological activities and peak values of predominant compounds were depicted **(Table 5b; Figure 8b)**. The major compounds identified such as 1,1 diethoxy ethane (27.48%), Benzene sulphonic acid (1.31%), Hexadecanoic acid (1.73%), Octadecanoic acid (1.07%), Undecanoic acid (1.04%), Dodecanoic acid (8.38%), Phenol (5.05%), Ethanol (5.29%), Hexanoic acid (1.48%), Decanoic acid (2.24%), Carboxylic acid (1.2%), 1 Eicosanol (1.37%), Octadienol (1.03%), Pentadecanol (1.15%), Dibutyl phthalate (1.2%), Naphthalene (6.41%). The biological activities were documented according to Dr. Duke's Phytochemical and Ethnobotanical Databases.

Likewise, previous findings identified the presence of 79 compounds, 55 of which are known for their medicinal properties. The

predominant chemical identified was Dodecanoic acid, 3-hydroxy- (10.41%), utilized in cancer therapies. The compound with the lowest percentage was 3,3,7,11-Tetramethyltricyclo and undecan-1-ol (0.06%) in the GC-MS analysis of the leaf ethanolic extract of *Ipomoea* staphylina **[50,51]**. Vitamin E, a phytochemical, has been isolated in the entire plant of *I. pes-caprae*, and has notable antioxidant properties. The presence of the chemical stigmasterol was detected in both the stem and leaf of *I. pes-caprae*, and it exhibited potential anticervical cancer properties. According to Ramesh *et al*., squalene exhibits several biological activities, including antioxidant, antibacterial, anticancer, immunostimulant, and lipoxygenase inhibitor properties **[51]**.

3.5 Antimicrobial efficacy

Six clinical pathogenic organisms were tested to check the antimicrobial property of EIEF-S. The results depicted antimicrobial activity which is of broad spectrum that tend to vary in inhibition against tested clinical pathogens. The inhibition diameter zone was monitored around each well stacked with EIEF. Thus, inhibition zone diameter increases by increasing the concentration of the fractionated EIEF from 15 to 45 µg/ml, which exemplify dose dependent activity for all clinical pathogens. The maximum inhibition zone was established against *P. aeruginosa* (17.41 mm for EIEF) and *B. subtilis* (16.19 mm for EIEF). The minimum diameter zone was observed for *E. coli* (15.33 mm for EIEF) and *S. aureus* (11.23 mm for EIEF) among bacterial pathogens. For fungal pathogens the highest zone of inhibition was noticed with *A. niger* (16.15 mm for EIEF) and minimum for *C. albicans* (12.32 mm for EIEF) **(Table 6a; Figure 9a)**.

Table 6a: Antimicrobial assay – EIEF-S

Microorganism	STD (15µg/mL)	Extract concentration		
		15 µg/mL	30 µg/mL	45 µg/mL
E. coli	15.31 ± 0.45	11.21 ± 0.09	13.41 ± 0.23	15.33 ± 0.06
P. aeruginosa	13.19 ± 0.12	13.34 ± 0.12	15.53 ± 0.29	17.41 ± 0.15
S. aureus	16.23 ± 0.17	7.13 ± 0.02	9.15 ± 0.06	11.23 ± 0.13
B. subtilis	15.20 ± 0.06	12.09 ± 0.04	14.07 ± 0.04	16.19 ± 0.08
C. albicans	16.23 ± 0.16	8.18 ± 0.12	10.26 ± 0.10	12.32 ± 0.20
A. niger	15.12 ± 0.11	11.41 ± 0.19	13.33 ± 0.14	16.15 ± 0.16

All the values are expressed as mean ± SEM, n=3.

Table 6b: Antimicrobial assay – CMIEF-R

Microorganism	STD (15µg/mL)	Extract concentration		
		15 µg/mL	30 µg/mL	45 µg/mL
E. coli	15.31 ± 0.45	10.21 ± 0.09	12.41 ± 0.23	14.33 ± 0.06
P. aeruginosa	13.19 ± 0.12	14.34 ± 0.12	16.33 ± 0.29	18.11 ± 0.15
S. aureus	16.23 ± 0.17	8.13 ± 0.02	10.15 ± 0.06	12.23 ± 0.13
B. subtilis	15.20 ± 0.14	13.09 ± 0.09	15.07 ± 0.21	16.97 ± 0.18
C. albicans	16.23 ± 0.15	9.15 ± 0.23	11.22 ± 0.12	13.18 ± 0.16
A. niger	15.12 ± 0.11	11.14 ± 0.19	14.32 ± 0.24	16.21 ± 0.14

All the values are expressed as mean ± SEM, n=3.

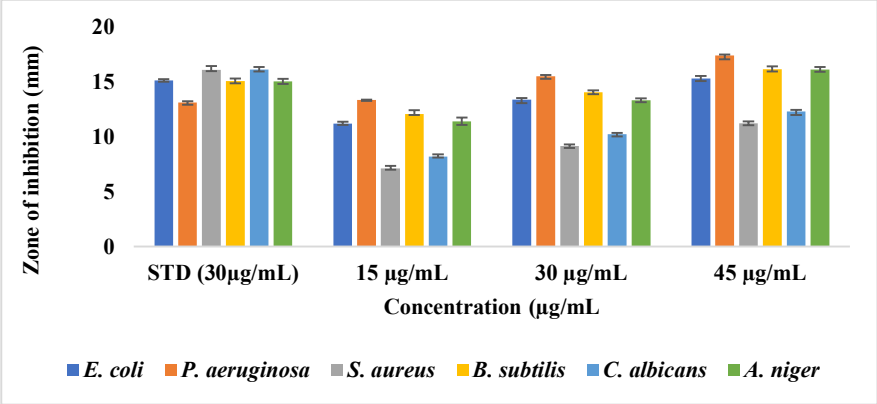

Figure 9: Antimicrobial efficacy of EIEF-S fraction

The results depicted broad spectrum antimicrobial competency exhibiting variations in the level of inhibition against certain clinical pathogens. The inhibition diameter zone was monitored around each well stacked with CMIEF-R **(Figure 9c)**. Thus, inhibition zone diameter increases by increasing the concentration of the fractionated CIEF from 15 to 45 µg/ml, which exemplify dose dependent activity for all clinical pathogens. The maximum zone of inhibition was established against *P. aeruginosa* (18.11 mm for CMIEF) and *B. subtilis* (16.97 mm for CMIEF). The minimum diameter zone was observed for *E. coli* (14.33 mm for CMIEF) and *S. aureus* (12.23 mm for CMIEF) among bacterial pathogens. For fungal pathogens, the maximum zone of inhibition zone was attained against *A. niger* (16.21 mm for CMIEF) and minimum for *C. albicans* (13.18 mm for EIEF) **(Table 6b; Figure 9d)**.

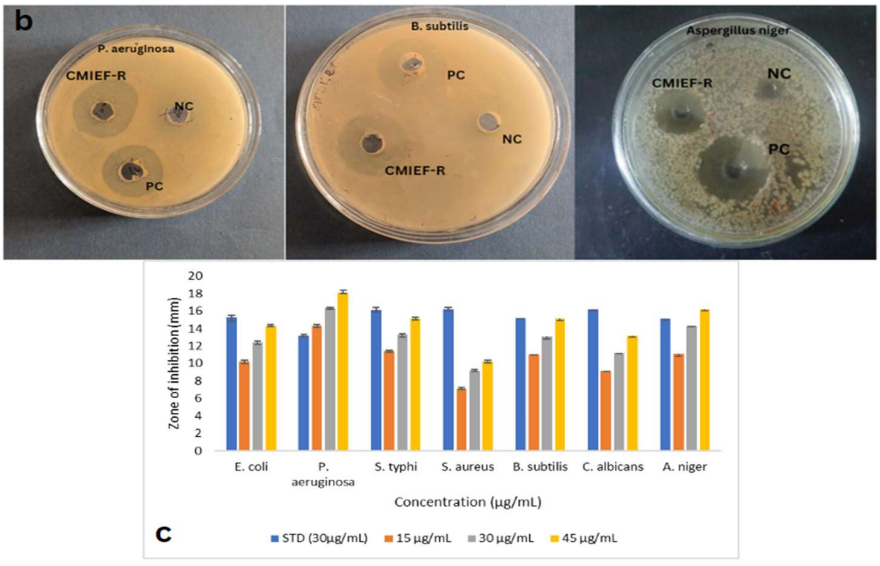

Figure 9b: Inhibition zone diameter of CMIEF-R fraction against *P aeruginosa, B subtilis* and *A. niger*; Figure 9c: Antimicrobial assay of CMIEF-R fraction

Results showed that EIEF-S and CMIEF-R was found to be more effective for G-ve bacteria than G+ve bacteria and efficacious against fungi too. Previous studies showed, a concentration of 400 g/disc was used, both the extract and standard exhibited the highest average zone of inhibition. The range of inhibition zones for the extract was between 13 and 19 mm, while for the standard it was between 37 and 42 mm. Additionally, the largest zone of inhibition towards the gram-positive bacteria *Bacillus cereus* was observed for *I. mauritiana*, measuring 19.25 mm **[52]**. Past studies disclosed that phytochemicals like flavonoids, fatty acids, saponins and phenols that has been found in *Ipomoea* species were accountable for its antibacterial efficacy **[6,53]**.

3.6 Antioxidant activities

The quantitative antioxidant DPPH, HPA and FRAP activities were conducted for stem extracts (EIEF, EAIEF, DEIEF) and root extracts (CMIEF and AIEF) of *Ipmoea sp.* fractions, which illustrated effectual antioxidant potential in a dose-dependent mode.

3.6.1 DPPH assay

The results of the quantitative DPPH assay demonstrated the concentration-dependent antioxidant activity of *Ipomoea*. In terms of scavenging activity, the concentration of 100 µg/ml demonstrated the superior activity. The IC_{50} values of stem extracts (EIEF, EAIEF, DEIEF) obtained from *Ipomoea* were found to be 39.89 ± 0.28, 44.67 ± 0.26, and 52.70 ± 0.31 µg/ml, respectively **(Figure 10a)**. The root extracts (CMIEF, AIEF) of *Ipomoea* have demonstrated IC_{50} values of 35.87 ± 0.28 and 54.88 ± 0.31 µg/ml, correspondingly. The determined IC_{50} value for standard Ascorbic acid (AsA) was found to be 5.59±0.32 µg/ml **(Figure 10b)**.

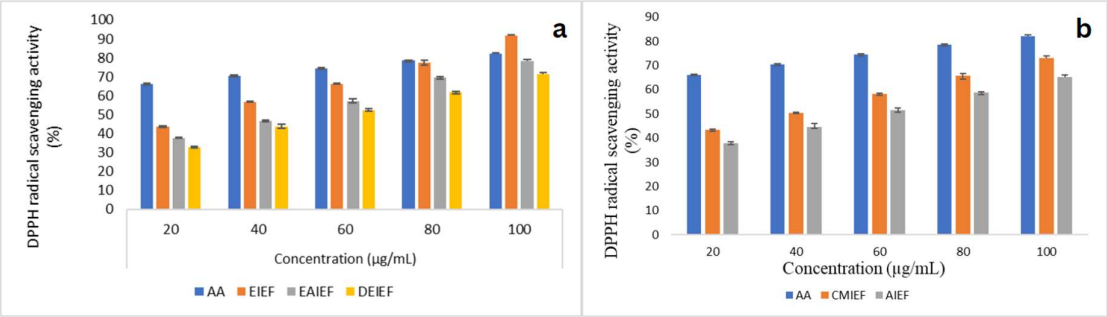

Figure 10a: DPPH of stem extracts (EIEF, EAIEF, and DEIEF); Figure 10b: DPPH of root extracts (CMIEF and AIEF)

3.6.2 HPA

This assay showed the potentiality of *Ipomoea* solvent extracts as antioxidants. The concentration of 100 µg/ml exhibited a greater degree of inhibition for all of the extracts. IC_{50} values of EIEF, EAIEF,

DEIEF, and standard (AsA) were found to be 57.33±0.36, 59.17±0.25, 62.03±0.21, and 8.14±0.19 µg/ml, respectively **(Figure 11a)**. IC_{50} values of CMIEF, AIEF and standard (AsA) were found to be 38.32±0.12, 43.62±0.21 and 5.59±0.32 µg/ml, respectively **(Figure 11b)**.

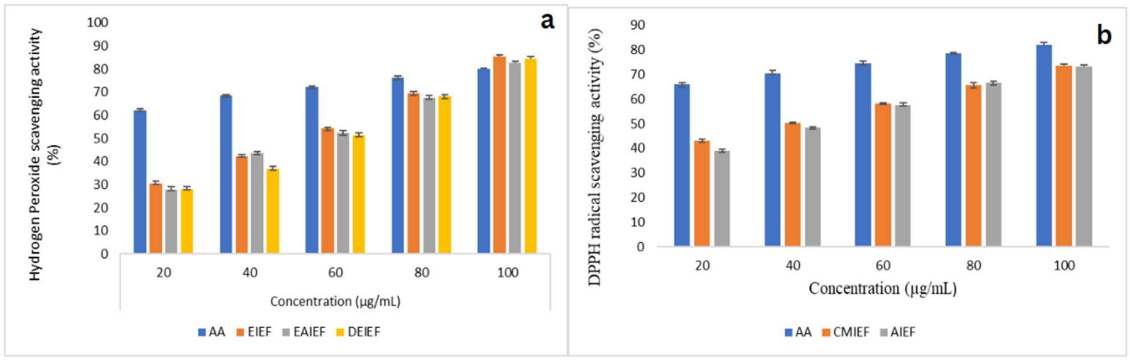

Figure 11a: HPA of stem extracts (EIEF, EAIEF, and DEIEF); Figure 11b: HPA of root extracts (CMIEF and AIEF)

3.6.3 FRAP assay

The reductive ability was measured by investigating the transformation of Fe3+ ions into Fe2+ ions with the extracts. IC_{50} values of EIEF, EAIEF, DEIEF, and standard (AsA) were found to be 61.89±0.23, 65.52±0.15, 66.01±0.22, and 8.09±0.13 µg/ml, respectively **(Figure 12a)**. IC_{50} values of CMIEF, AIEF and standard (AsA) were found to be 37.63±0.22, 42.76±0.17 and 5.59±0.32 µg/ml, respectively **(Figure 12b)**.

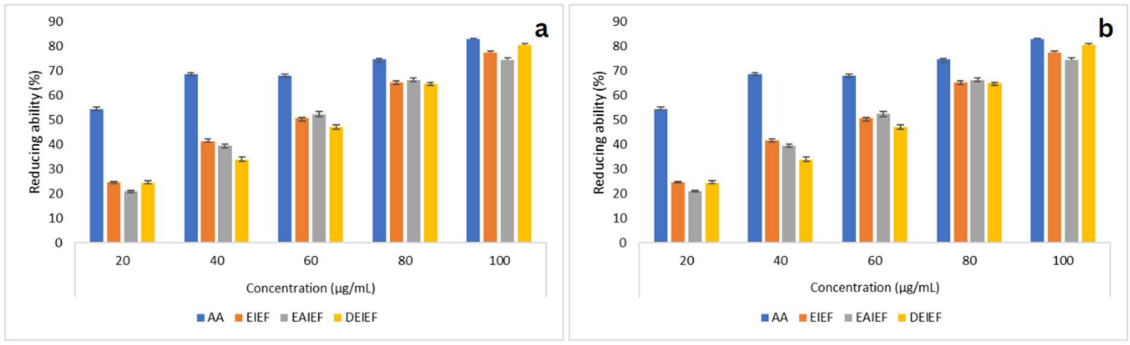

Figure 12a: FRAP of stem extracts (EIEF, EAIEF, and DEIEF); Figure 12b: FRAP of root extracts (CMIEF and AIEF)

An antioxidant possesses the ability to diminish and avert the process of oxidation in other molecules or moieties. Within a biological context, antioxidants have the capacity to safeguard cells against harm induced by reactive chemicals that lack stability. According to Forni *et al*. (2019), it was well acknowledged that they possess the capacity to contribute to the mitigation of chronic ailments, including but not limited

to cancer, cardiovascular disorders, stroke, alzheimer's disease, and rheumatoid arthritis **[54]**. The results obtained from the quantitative DPPH experiment demonstrated that the antioxidant activity of *Ipomoea* exhibited a dependence on the concentration. The DPPH experiment demonstrated a lower IC_{50} value for the ethanolic extract in comparison to the other extracts. The HPA demonstrated the competency of the extracts from *Ipomoea* to effectively suppress the hydroxyl radical inside the hydrogen peroxide reaction mixture. The observation of a transition from yellow to varying hues of blue and green in the test solution, contingent upon the concentration of the extract, serves as an indicator of antioxidant activity. The absorbance of *Ipomoea* extracts and ascorbic acid demonstrates an upward trend as their concentration increases, suggesting an elevation in their reducing potential.

Ethanol extract of *Ipomoea* shows maximum scavenging capacity for DPPH, HPA and FRAP among all the extracts. The antioxidant score: AsA>EIEF >EAIEF >DEIEF. Chloroform-Methanol extract of *Ipomoea* shows effective scavenging capacity for DPPH than acetone extracts. The antioxidant score: AsA > CMIEF > AIEF. The prominent concentration of biochemicals, notably flavonoids, flavanols, fatty acids, polyphenols, ester, ether, and aldehydes in stem and root extracts likely explains their increased scavenging ability. Agoramoorthy *et al*., 2008 found that most *I. pes-caprae* active chemicals are antioxidants **[10]**. The overall antioxidant, and elemental composition analysis found that plant encompasses loads of active chemicals that synergistically scavenge reactive oxygen species **[55]**. Ultimately, *Ipomoea staphylina Roem. & Schult.'s* ethanolic leaf extract found to contain nutrient-rich compounds with antibacterial, antioxidant, and anticancer properties and many antimicrobial-active plants have also yielded numerous natural medicines **[50]**. Methanol and acetone leaf extracts of *I. pes-caprae* inhibited *Escherichia coli* and *Salmonella paratyphi*, *Mucor sp.* and *Candida albicans* respectively **[56]**. Methanol had strong antibacterial effect on human infections, while Kumar *et al*., 2014 found no activity in hexane, dichloromethane, or ethyl acetate **[57]**.

4 CONCLUSION

The results of this experiment validate *Ipomoea pes-caprae* potential for antibacterial and free radical scavenging action. The findings imply that the root extract attained highest antioxidant and antimicrobial activities. Based on the inhibition zone diameter and IC_{50} values EIEF-S and CMIEF-R were found to have valuable antimicrobial and antioxidant efficacy, which may be due to the dominance of phytochemicals like

flavonoids, fatty acids, flavanols, polyphenols, ester, ether and aldehydes as evidenced by FTIR & GCMS. GC-MS analysis showed increased percentages of dodecanoic acid and saturated fatty acids in all the extracts. Thus, the research findings revealed that *Ipomoea pes-caprae* could be used to produce lead molecules to prevent and treat cancer disease because of its antioxidant potentialities.

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