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PHYTOCHEMICAL COMPOSITION OF *Fimbristylis ferruginea* (L.) Vahl. AND EVALUATION OF ITS ANTIOXIDANT POTENTIAL

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ABSTRACT

Medicinal plants are rich in bioactive compounds, crucial for disease prevention and treatment. This study explored the phytochemical composition, antioxidant, antimicrobial, and antifungal potential of *Fimbristylis ferruginea* (L.) Vahl. Phytochemical screening revealed phenolics, flavonoids, terpenoids, alkaloids, glycosides, and anthraquinones, particularly in ethyl acetate, ethanol, acetone, and chloroform extracts. The ethyl acetate extract had highest phenolic content (1.62 mg GAE g^{-1}), while the chloroform extract exhibited highest flavonoid content (59 mg QE g^{-1}). Antioxidant potential was evaluated using DPPH and FRAP assays, while antimicrobial and antifungal potential was evaluated against *Staphylococcus aureus, Escherichia coli, Shigella flexneri, Enterococcus faecalis, Candida albicans,* and *Fusarium oxysporium* using agar well diffusion and MIC/MBC assays, with ethyl acetate and chloroform extracts exhibiting the highest efficacy. The study employed gas chromatography-mass spectrometry (GC-MS), ultraviolet-visible (UV-Vis) spectroscopy, Fourier transform infrared (FT-IR) spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy for the identification and structural elucidation of bioactive compounds. GC-MS, UV-Vis, FT-IR, and NMR confirmed the presence of key compounds, including 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester. These findings suggest the potential of *F. ferruginea* in pharmaceutical and nutraceutical applications.

Keywords: Antimicrobial activities, antioxidant activity, bioactive compounds, *Fimbristylis ferruginea*, phytochemical screening

INTRODUCTION

Medicinal plants are fundamental part of traditional healing practices, offering bioactive compounds with therapeutic potential. India, the home of approximately 45,000 medicinal plant species, has deeply rooted medicinal traditions in Ayurveda, Siddha, and Unani systems (Singh *et al.,* 2023; Kothiyal *et al.,* 2023). These plants contain secondary metabolites such as flavonoids, alkaloids, sterols, tannins, and phenols, which exhibit antioxidant, antimicrobial, anti-inflammatory, and anticancer properties (Bhat and Sharma, 2022). The family Cyperaceae comprises of around 5,687 species, and is well known for its ecological, economic, and pharmacological significance (Hamid *et al.,* 2023). Several species within this family have demonstrated antimicrobial, antioxidant, and anti-inflammatory activities due to the presence of phenolic derivatives, stilbenoids, and flavonoids (Taheri *et al.,* 2021). *Fimbristylis ferruginea* (L.) Vahl, a member of this family, is traditionally used for wound healing, treating skin infections, and reducing inflammation (Nair and Viji, 2023). Its phytochemical components include flavonoids, terpenoids, phenolics, tannins, alkaloids, etc., similar to other *Fimbristylis* species known for their antioxidant and antimicrobial activities (Ramli *et al.,* 2022; Abdullah *et al.,* 2022).

The antioxidant potential of *Fimbristylis* species is well-documented, with phenolics and flavonoids playing a key role in mitigating the oxidative stress-related diseases such as cancer and cardiovascular disorders (Ramli *et al.,* 2022). *F, ovata* has demonstrated neuroprotective effects by reducing oxidative stress and inflammation (Sirirattanakul and Santiyanont, 2021). The species like *F. miliacea* and *F. dichotoma* also exhibit antimicrobial properties, attributed to their alkaloid and flavonoid content, making them effective against multidrug-resistant pathogens (Ramli *et al.,* 2022).

Phytochemical characterization of medicinal plants enhances the understanding of their bioactive compounds, facilitating the development of pharmaceutical and nutraceutical applications. Techniques such as gas chromatography-mass spectrometry (GC-MS), ultraviolet-visible (UV-Vis) spectroscopy, Fourier transform infrared (FT-IR) spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy are widely used for compound identification (Prakash, 2023; Chen, 2024). These analytical methods provide insights into the structure and function of key metabolites, supporting their therapeutic applications (Sahoo and Umashankar, 2022). The present study was aimed to evaluate the phytochemical composition, antioxidant, and antimicrobial properties of *F. ferruginea*, with a focus on its potential use in the pharmaceutical and nutraceutical industries. By analysing its phytochemical profile the study aimed to identified key secondary metabolites responsible for its biological activities.

MATERIALS AND METHODS

Sample collection and solvent Extraction

F. ferruginea (L.) Vahl, stem samples were collected from Mulki, Dakshina Kannada, Karnataka (India), and the identity of plant was subsequently authenticated by the Botanical Survey of India under voucher specimen No. BSI/SRC/5/23/2022/Tech./298. The plant stem samples were thoroughly rinsed with tap water, air-dried in shade and finely ground using a blender before storage in airtight containers. For extraction, 10 g shade-dried stem powder was subjected to solvent extraction using 100 mL solvents like water, ethanol, methanol, hexane, chloroform, acetone and ethyl acetate, with the help of a Soxhlet apparatus. The extracts were concentrated by evaporation under reduced pressure using a rotary vacuum evaporator. The dried residues were stored in desiccators till use.

Phytochemical analysis

Phytochemical screening of *F. ferruginea* extracts was performed as per the method of Harborne (1973) and Trease and Evans (1987). Saponins were identified using foam test, while alkaloids were detected through Wagner's, Dragendorff's, Mayer's, and Hager's tests. Tannins and phenolics were confirmed via ferric chloride and gelatin tests. Sterols and terpenoids were identified using Libermann-Burchard test, and flavonoids by using lead acetate and alkaline reagent tests. Glycosides were screened with Keller-Killani, Liebermann's, Borntrager's, and Baljet tests. Proteins and amino acids were confirmed by using biuret and ninhydrin tests, while carbohydrates were detected through Benedict's and Fehling's tests. The presence of fixed oils was determined by saponification test.

Antioxidant activity

Total phenol content: The total phenolic content of *F. ferruginea* was determined as per Gao *et al.,* (2000). Extract (20 µL) was mixed with 1 mL of 10 % Folin-Ciocalteu reagent, incubated for 5 min at 37°C, followed by the addition of 1 mL of 6% Na_2CO_3, and incubation for 30 min. Absorbance was measured at 750 nm using a UV-Vis spectrophotometer (Labtronics; LT-2205), with a reagent blank as a reference. Results were expressed as gallic acid equivalents.

Total flavonoids content: The total flavonoid content in *F. ferruginea* was determined as per the standard protocol (Kim *et al.,* 2003). Plant extract (20 µL) was mixed with 4 mL distilled water, 0.3 mL $NaNO_2$ (5%) and 0.3 mL $AlCl_3$ (10%). After 5 min, 2 mL of 1 mM NaOH was added. The absorbance was measured at 510 nm spectrophotometerically, and expressed as quercetin equivalents.

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay: To assess the DPPH radical scavenging activity of *F. ferruginea*, 20 µL plant extract was dissolved in 1.98 mL methanol and then combined with 2 mL of 0.16 mM DPPH solution. The mixture was incubated in dark at 37°C for 30 min. The absorbance was recorded at 517 nm using a UV-Vis spectrophotometer (Labtronics; LT-2205), with a reagent blank serving as a reference. A control sample containing only the sample buffer was included in the reaction, and a sample blank was prepared by substituting DPPH with methanol (Duan *et al.*, 2006). The scavenging activity was calculated using the formula:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Absorbance in control} - \text{Absorbance in sample}}{\text{Absorbance in control}} \; x\; 100$$

Ferric reducing antioxidant power (FRAP) assay: The FRAP assay for *F. ferruginea* was performed by adding 20 µL extract to 3 mL FRAP reagent, followed by incubation in a water bath at 37°C for 30 min. The absorbance was measured spectrophotometrically (Labtronics; LT-2205) at 593 nm. The difference in absorbance between the sample and blank was used to determine the FRAP value. The results were expressed as EDTA equivalents (Dudonne *et al.*, 2009).

Antimicrobial activity

Antibacterial activity assessment: The antibacterial activity of *F. ferruginea* extract was evaluated against four bacterial strains *viz*., *Staphylococcus aureus* MTCC96, *Escherichia coli* MTCC118, *Shigella flexneri* MTCC731, *Enterococcus faecalis* MTCC5153 obtained from the Microbial Type Culture Collection (MTCC), India. These strains were cultured in brain heart infusion (BHI) medium and incubated at 37°C for 24 h with agitation (150 rpm). Minimum inhibitory concentration (MIC) was determined by using broth dilution method. Bacterial cultures (10^8 CFU mL^{-1}) were inoculated into 96-well microtiter plates containing serially diluted extract (25-0.19 mg mL^{-1}). The lowest concentration preventing the visible turbidity after 24 h at 37°C was recorded as MIC. Positive (bacterial suspension without extract) and negative (broth only) controls were included (Andrews, 2001). For minimum bactericidal concentration (MBC), 100 µL from MIC wells showing no visible bacterial growth or turbidity were plated onto agar and incubated at 37°C for 24-48 h. The MBC at lowest concentration that completely inhibited bacterial growth indicated the bactericidal activity. IC_{50}, the concentration required to inhibit 50% of bacterial growth, was assessed by using agar well diffusion method. BHI agar pre-inoculated with bacterial pathogens was poured into petri-dishes and solidified. Wells (4 mm dia.) were created and filled with 80 µL extract (100 mg mL^{-1}). After 30 min. at 4°C for diffusion the plates were incubated at 37°C for 24 h. Antibacterial activity was measured as inhibition zone diameters, with chloramphenicol (1 mg mL^{-1}) as a positive control.

Antifungal activity: The antifungal activity of *F. ferruginea* extract was tested against *F. oxysporum* MTCC 1755 (obtained from the MTCC, India) and *Candida albicans* ATCC 90028 (obtained from the American Type Culture Collection (ATCC), USA). Both fungal strains were cultured in potato dextrose broth (PDB) at 30°C for 3 days under continuous agitation (150 rpm). Potato dextrose agar (PDA) pre-inoculated with 1% fungal suspension was poured into sterile petri dishes and allowed to solidify. Wells (4 mm dia.) were made using a sterile cork borer and filled with 100 µL extract (100 mg mL^{-1}). Plates were kept at 4°C for 30 min. to ensure proper diffusion, followed by incubation at 30°C for 2-3 days. Fungal inhibition was assessed by measuring the zones of inhibition (mm), with nystatin used at a concentration of 1 mg mL^{-1} used as a positive control (Brantner, 1994).

Column chromatography

Column chromatography was performed on extracts obtained from all tested solvents to separate and purify bioactive compounds from the crude extracts of *F. ferruginea*. This technique helped in fractionating the extract based on polarity, allowing the isolation of individual compounds with potential biological activities. The collected fractions were subsequently evaluated for antioxidant activity using DPPH assay and total flavonoid content**.** Fractions exhibiting the highest antioxidant activity were selected for further analysis to identify the most potent bioactive constituents (Mahabaleshwara *et al.*, 2016).

GC-MS analysis

GC-MS analysis was performed using a Perkin Elmer Clarus 680 GC coupled with a Clarus SQ 8C mass spectrometer, equipped with an HP-5MS fused silica column with 30.0 m × 0.25 mm ID × 0.25 µm df (Agilent Technologies, 7890B GC). Helium was used as the carrier gas @ 2 mL min^{-1}, with an injector temperature of 250°C and a 1.0 µL sample injected at a split ratio of 1:10. The temperature program started at 100°C (2 min), increased by 10°C min^{-1} to 200°C (1 min hold), then by 25°C min^{-1} to 300°C (8 min hold), totalling 25 min runtime. The mass spectrometer operated in electron impact mode (70 eV) with an inlet line temperature 250°C, source temperature 230°C, and a scan range of 40–600 Da at 0.2 sec and 0.1 sec interval. The spectral data were matched with the NIST 2017 library for compound identification.

UV-Vis spectral analysis

The UV-vis analysis of *F. ferruginea* extracts was conducted by using a PerkinElmer UV WinLab Data Processor, along with PerkinElmer Spectrum IR version 10.7.2 and Viewer version 10.6.2. Known quantity of extracts were dissolved in suitable solvents, like methanol or ethanol, to prepare stock solutions, which were then diluted for analysis. The spectrophotometer was warmed up for 30 min. and calibrated using a solvent blank (200-700 nm) to set the baseline. Absorption spectra were recorded for each sample using a quartz cuvette across this wavelength range.

FT-IR spectroscopy

FT-IR analysis of *F. ferruginea* extract was conducted using a PerkinElmer Spectrum II FT-IR Spectrometer**.** Dried powdered plant samples were mixed with potassium bromide (KBr) and pressed into pellets. These pellets were placed in spectrometer, and spectra were recorded within 4000-400 cm^{-1} range**.** The PerkinElmer software analysed the spectra to identify the characteristic absorption bands, indicating the presence of various functional groups and phytochemicals in the extracts.

NMR analysis

NMR spectroscopy of *F. ferruginea* was done by using a Bruker Ascend 400 NMR spectrometer at a frequency of 400 MHz. Extracts were dissolved in deuterated chloroform ($CDCl_3$) and transferred to 5 mm NMR tubes. The instrument parameters were optimized to acquire high-resolution ^1H NMR spectra at approximately 25°C. Data processing was done using Bruker TopSpin software, with chemical shifts referenced to residual solvent peaks, providing detailed insights into the structural characteristics of the phytochemicals in the samples (Zhao, *et al.*, 2022).

Statistical analysis

Statistical analysis was conducted to ensure the reliability of data. Experiments followed a completely randomized design (CRD) with three biological and three technical replicates for accuracy. Data were expressed as mean ± standard deviation (SD), and significance was determined using ANOVA.

RESULTS AND DISCUSSION

The extraction yields of *F. ferruginea* varied depending on the solvent used. Water extraction gave highest yield of 16.51 ± 0.20% (w/w), followed by methanol (10.97 ± 0.10% w/w) and ethanol (8.85 ± 0.11% w/w). Other solvents showed lower extraction efficiencies with acetone (2.74 ± 0.09% w/w), ethyl acetate (1.97 ± 0.12% w/w), chloroform (1.67 ± 0.25% w/w), and petroleum ether (1.31± 0.10% w/w). These variations revealed that water and methanol are the most effective solvents for extracting phytochemicals from *F. ferruginea*, presumably due to their polarity and ability to dissolve a broad range of bioactive compounds.

The phytochemical analysis of *F. ferruginea* extracts revealed variations in bioactive compounds across the solvents. Carbohydrates were detected in all extracts, except water and methanol, while

proteins, amino acids, and saponins were absent in all. Tannins and phenolic compounds were present in all extracts, except methanol, and flavonoids were found in all, except water. Terpenoids were identified in ethyl acetate, ethanol, acetone, chloroform, and petroleum ether extracts, whereas alkaloids were present in all extracts, though inconsistently detected in water. Glycosides, cardiac glycosides, steroids and anthraquinones were found in ethyl acetate, ethanol, acetone, and chloroform extracts. Oils and fats were present in all extracts (Table 1).

Table 1: Phytochemical analysis of *F. ferruginea*

Parameters	Tests	Water	Ethyl acetate	Ethanol	Acetone	Chloroform	Petroleum ether	Methanol
Carbohydrate	Benedict's test	-	+	+	+	+	+	-
	Fehling' test	+	+	+	+	+	+	+
Proteins & amino acids	Biuret test	-	-	-	-	-	-	-
	Ninhydrin test	-	-	-	-	-	-	-
Tannins & phenol	$FeCl_3$ test	+	+	+	+	+	+	-
	Gelatin test	-	+	-	+	+	+	-
Saponin	Foam test	-	-	-	-	-	-	-
Flavonoid	Lead acetate test	-	+	+	+	+	+	-
	Alkaline test	+	+	+	+	+	+	+
Terpenoids	Salkowski's test	-	+	+	+	+	+	+
	Libermann-Buchard test	-	+	+	+	+	+	+
Alkaloids	Dragondroff's test	-	+	+	+	+	+	+
	Wagner's test	+	+	+	+	+	+	+
	Mayer's test	-	+	-	-	+	-	-
	Hager's test	-	+	-	-	+	-	-
Glycosides	Liebermann's test	-	+	-	-	+	+	-
	Borntrager's test	-	+	+	+	+	-	-
	Baljet test	+	+	+	+	+	+	+
Cardiac glycosides	Keller kiliani's test	-	+	-	+	+	+	-
Steroids	Liebermann Burchard's test	-	+	-	+	+	+	-
Anthraquinone	Borntrager's test	-	+	+	+	+	-	-
Oils and fats	Saponification test	+	+	+	+	+	+	+

+ : Present; -: Absent.

The absence of saponins in *F. ferruginea* contrasts with their reported presence in *F. miliacea* and *F. dichotoma* (Nurul *et al.*, 2022). However, the presence of phenolics and flavonoids reveals its potential antioxidant activity. *F. miliacea* reportedly exhibits allelopathic effects due to the presence of compounds like gallic acid, chlorogenic acid, rutin, luteolin, apigenin, acacetin, and alkaloids (Silva *et al.,* 2020), suggesting that *F. ferruginea* may also possess similar properties due to its alkaloid content. The neuroprotective effects observed in *F. ovata* (Sirirattanakul and Santiyanont, 2021) and antidiarrheal properties in *F. miliacea* (Mukta, *et al.,* 2020) indicate potential therapeutic relevance of *F. ferruginea*, especially due to the presence of terpenoids and phenolics. The presence of alkaloids, flavonoids, tannins, terpenoids, glycosides, and steroids across various *Fimbristylis* species (Mandal *et al.,* 2015) highlights their broad-spectrum antimicrobial and medicinal properties. The high presence of these compounds in ethyl acetate, ethanol, acetone, and chloroform extracts deteriorate the medicinal potential of *F. ferruginea*. The absence of proteins, amino acids, and saponins further distinguishes its phytochemical profile, potentially contributing to its unique biological properties.

Antioxidant activity of F. ferruginea extracts

The antioxidant activity of *F. ferruginea* extracts was evaluated through total phenolic content across the solvents. The methanol extract exhibited highest phenolic content of 1.66 ± 0.027 mg GAE g^{-1}, followed closely by ethyl acetate at 1.62 ± 0.059 mg GAE g^{-1}, both were significantly higher than other solvents. Acetone and ethanol also showed considerable phenolic content, though lower than

methanol and ethyl acetate. Chloroform extract had moderate phenolic content, while water and petroleum ether were the least effective in phenolic extraction (Table 2). These findings align with previous reports that methanol and ethyl acetate are among the most effective solvents for extracting phenolics (Muntean and Vulpie, 2023; Gnanasri *et al.,* 2023). Phenolic compounds play a crucial role in antioxidant defence mechanisms, providing protection against oxidative stress-related diseases, including cancer, cardiovascular and neuro-degenerative disorders (Fernandes *et al.,* 2023; Sharma and Rajpal, 2023). The high phenolic content in *F. ferruginea* is consistent with the reports from other *Fimbristylis* species, like *F. miliacea* and *F. dichotoma*, wherein strong antioxidant activity was linked to phenolic content (Mukta *et al.,* 2020; Ramli *et al.,* 2022). The presence of phenolic compounds in *F. ovata* supports the genus's potential for antioxidant properties (Mukta *et al.,* 2020). These findings show *F. ferruginea* as a promising source of natural antioxidants and endorse the role of methanol and ethyl acetate as preferred solvents for phenolic extraction.

Table 2: Total phenol and total flavonoid contents in *F. ferruginea* extracts

Extracts	Total phenols (mg GAE g^{-1})	Total flavonoids (mg QE g^{-1})
Water	0.64 ±0.007 [b]	4.00 ±2.683 [a]
Ethyl acetate	1.62 ±0.059 [e]	14.00 ±2.366 [c]
Ethanol	1.30 ±0.009 [d]	24.00 ±4.472 [d]
Acetone	1.37 ±0.038 [d]	29.00 ±0.000 [d]
Chloroform	1.04 ±0.041 [c]	59.00 ±1.789 [e]
Petroleum ether	0.45 ±0.001 [a]	9.00 ±2.683 [b]
Methanol	1.66 ±0.027 [e]	4.50 ±2.236 [a]

The values are mean ± SD (n=2); GAE = gallic acid equivalent; Values in columns with different superscripts differ significantly ($p<0.05$)

The total flavonoid content in *F. ferruginea* extracts varied significantly within the solvent used. The chloroform extract gave highest flavonoid content of 59.00 ± 1.789 mg QE g^{-1}, indicating its effectiveness in flavonoid extraction, which aligns with the studies showing non-polar solvents extract flavonoids efficiently (Mathesius, 2018). Acetone (29.00 ± 0.00 mg QE g^{-1}) and ethanol (24.00 ± 4.47 mg QE g^{-1}) also demonstrated notable flavonoid content, supporting their effectiveness as extraction solvents. Ethyl acetate showed moderate flavonoid content (14.00 ± 2.366 mg QE g^{-1}), while petroleum ether (9.00 ± 2.68 mg QE g^{-1}) was more effective than water and methanol, which had low flavonoid contents of 4.00 ± 2.683 and 4.50 ± 2.236 mg QE g^{-1}, respectively (Table 2).

Flavonoids are known for their antioxidant, anti-inflammatory, and anticancer properties, contributing to plant defense mechanisms and therapeutic potential (Nagar *et al.,* 2022). The high flavonoid content in the chloroform extract aligns with studies where non-polar solvents effectively extracted flavonoids (Oladeji, 2016). Both polar and non-polar solvents have been reported to be effective depending on flavonoid solubility. Similar findings in *Fimbristylis* species, such as *F. miliacea* and *F. dichotoma*, indicate flavonoids contribute significantly to their antioxidant activity (Mukta *et al.,* 2020; Ramli *et al.,* 2022). The high flavonoid content in *F. ferruginea* supports its potential as a valuable source of natural antioxidants. The effectiveness of chloroform, acetone, and ethanol in flavonoid extraction aligns with previous studies emphasizing the importance of solvent selection in optimizing extraction efficiency (Sulaiman & Balachandran, 2012). This underscores the plant's potential for developing novel therapeutic agents (Amaliaa *et al.,* 2023).

DPPH scavenging activity

The DPPH scavenging activity of *F. ferruginea* extracts was assessed to evaluate their antioxidant potential. The DPPH assay measures the ability of plant extracts to neutralize free radicals, which play a role in diseases such as cancer, cardiovascular diseases, neurodegenerative disorders, and inflammation (Johari and Khan, 2022). Despite some limitations, such as pigment interference and the the need for standardization, this assay remains a widely used method for evaluating antioxidant activity (Gulcin and Alwasel, 2023). In present study, ethanol extract of *F. ferruginea* exhibited highest DPPH scavenging activity at 25.90 ± 0.539%, with EC_{50} value of 2.60 ± 0.107 mg mL^{-1} (Fig. 1) indicating its strong antioxidant potential. The high scavenging activity and favourable EC_{50} value suggest ethanol effectively extracts antioxidant compounds, aligning with its higher total phenolic and

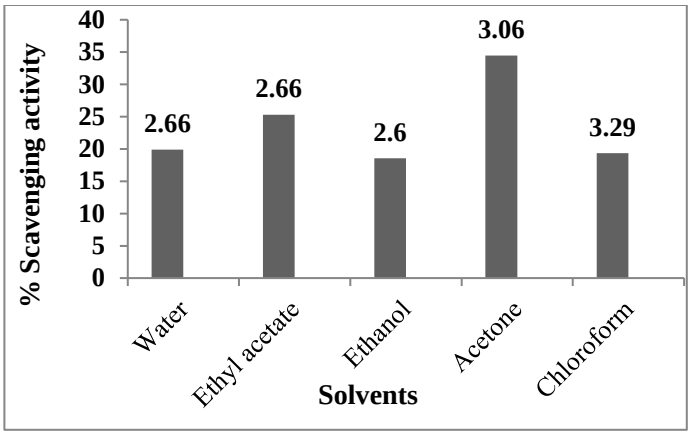

Fig. 1: **Antioxidant activity of *F. ferruginea* extracts evaluated using DPPH scavenging assays. Each bar represents the EC_{50} value (mg mL^{-1}) of the extract, which denotes the concentration needed to reduce DPPH radical activity by 50%. Lower EC_{50} values indicate higher antioxidant effectiveness. The bars are labeled with their respective EC_{50} values to illustrate the relative antioxidant potency of each extract**

flavonoid content. Ethyl acetate and water extracts showed notable scavenging activities of 24.10 and 22.89%, respectively, with similar EC_{50} values of 2.66 mg mL^{-1}, making them slightly less effective than ethanol. The moderate activity of these extracts suggests the presence of antioxidant compounds, though in lower concentrations. In contrast, acetone and chloroform extracts exhibited lower antioxidant activity, with scavenging values of 19.28% and EC_{50} values of 3.06 ± 0.054 mg mL^{-1} and 3.29 ± 0.027 mg mL^{-1}, respectively. The lower scavenging activity and higher EC_{50} values indicate that these extracts are less effective in neutralizing free radicals. The results highlight ethanol as the most effective solvent for extracting antioxidant compounds from *F. ferruginea*, supporting previous findings on the role of solvent selection in optimizing antioxidant extraction in medicinal plants.

FRAP assay

The FRAP assay evaluates the antioxidant capacity of medicinal plant extracts by measuring their ability to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions. In present study, *F. ferruginea* extracts exhibited varying antioxidant efficacy across the solvents as revealed by their EC_{50} values and percent reduction activities (Fig. 2). Acetone extract had highest scavenging activity of 34.47%, with EC_{50} value of 1.99 ± 0.098 mg mL^{-1}, revealing its strong ferric-reducing power. The ethyl acetate extract followed a scavenging activity of 25.29% and EC_{50} of 3.57± 0.098 mg mL^{-1}. Interestingly, water extract, despite having a lower scavenging activity (19.88%), recorded lowest EC_{50} value 1.84 ± 0.085 mg mL^{-1}) suggesting its effectiveness at lower concentrations. In contrast, chloroform extract displayed lower

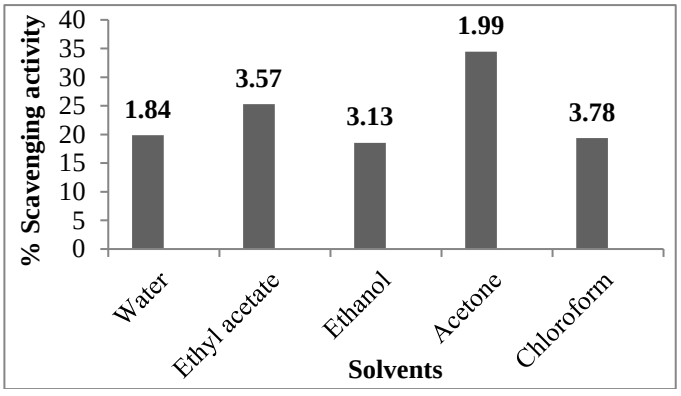

Fig. 2: Antioxidant activity of *F. ferruginea* extracts assessed by FRAP assay. Each bar displays EC_{50} value (mg mL^{-1}), which represents the concentration needed to achieve 50% reduction in DPPH radical activity. The EC_{50} values, marked on bars, reflect the antioxidant potency of extracts, with lower values indicating higher antioxidant activity

lower activity (19.35%) with higher EC_{50} of 3.78 ±0.089 mg mL^{-1}, showing less antioxidant potential. The ethanol extract had lowest scavenging activity (18.53%) with EC_{50} value of 3.13 ± 0.103 mg mL^{-1}. Our findings revealed highest ferric reducing power in acetone extract, while water extract was most efficient in terms of its lower EC_{50} value. This study is the first report about the effectiveness of FRAP assay in assessing the antioxidant capacity of *Fimbristylis* genus. The results highlight the potential of these extracts, particularly those obtained with acetone and water, as valuable sources of natural antioxidants (Amaliaa *et al.*, 2023).

Antimicrobial activities of F. ferruginea extracts

The antibacterial activity of *F. ferruginea* varied across the pathogens and solvents. Against *S. aureus*, all extracts exhibited similar MIC and MBC values, but IC_{50} variations indicated that ethyl acetate, chloroform, and acetone extracts were more effective than water (Fig. 3A-D; Tables 3, 4). This aligns with findings of Ranasinghe *et al*. (2023) on *C. rotundus* who reported strong antibacterial effects against *S. aureus*. The water extract showed highest potency against *E. coli*, with lowest IC_{50} value, consistent with the studies on *C. rotundus* and *C. articulatus* (Sartini *et al.*, 2023). Acetone extract was least effective, emphasizing the role of solvent choice in antibacterial activity. For *S. flexneri*, MIC, MBC, and IC_{50} values remained consistent across the extracts, indicating that active compounds were effectively extracted by various solvents. Our findings are in agreement with Hamid and Eltayeb (2019) and Kiteme *et al.* (2023). In *E. faecalis*, ethyl acetate and chloroform extracts

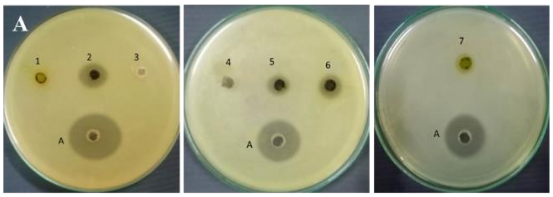

Fig. 3A: Antibacterial activity of *F. ferruginea* against *E. coli*; A-Antibiotic Chloramphenicol; (1) Ethanol extract; (2) Ethyl acetate extract; (3) Water extract; (4) Petroleum ether extract; (5) Chloroform extract; (6) Acetone extract; (7) Methanol extract

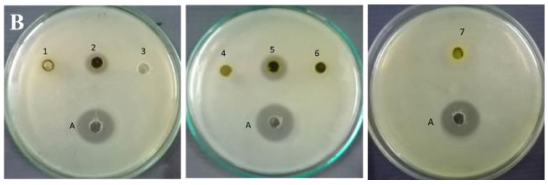

Fig. 3B: Antibacterial activity of *F. ferruginea* against *E. faecalis*; A-Antibiotic Chloramphenicol; (1) Ethanol extract; (2) Ethyl acetate extract; (3) Water extract; (4) Petroleum ether extract; (5) Chloroform extract; (6) Acetone extract; (7) Methanol extract

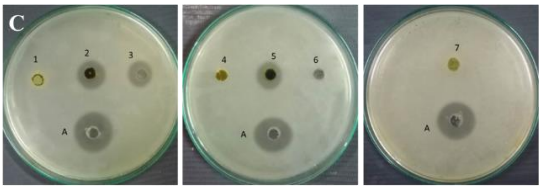

Fig. 3C: Antibacterial activity of *F. ferruginea* against *S. aureus*; A-Antibiotic Chloramphenicol; (1) Ethanol extract; (2) Ethyl acetate extract; (3) Water extract; (4) Petroleum ether extract; (5) Chloroform extract; (6) Acetone extract; (7) Methanol extract

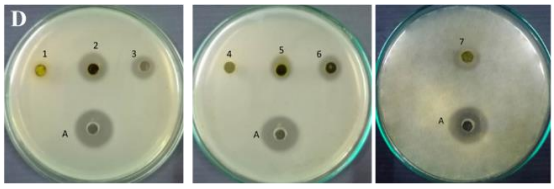

Fig. 3D: Antibacterial activity of *F. ferruginea* against *S. flexneri*; A-Antibiotic Chloramphenicol; (1) Ethanol extract; (2) Ethyl acetate extract; (3) Water extract; (4) Petroleum ether extract; (5) Chloroform extract; (6) Acetone extract; (7) Methanol extract

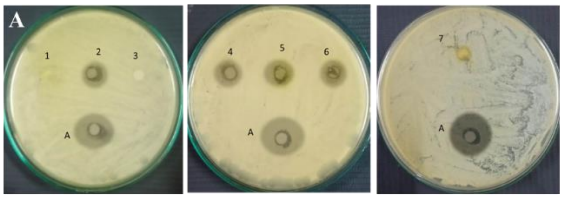

Fig. 4A: Antifungal activity of *F. ferruginea* against *C. albicans*; A-Antibiotic Chloramphenicol; (1) Ethanol extract; (2) Ethyl acetate extract; (3) Water extract; (4) Petroleum ether extract; (5) Chloroform extract; (6) Acetone extract; (7) Methanol extract. Antibiotic-Nystatin (1mg/ml)

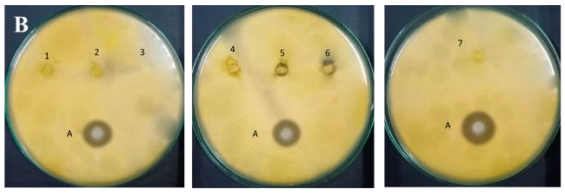

Fig. 4B: Antifungal activity of *F. ferruginea* against *F. oxysporium*; A-Antibiotic Chloramphenicol; (1) Ethanol extract; (2) Ethyl acetate extract; (3) Water extract; (4) Petroleum ether extract; (5) Chloroform extract; (6) Acetone extract; (7) Methanol extract. Antibiotic-Nystatin (1mg/ml)

Table 3: MIC, MBC and IC_{50} of *F. ferruginea* extracts

Pathogens	Solvent used for extraction	*F. ferruginea*		
		MIC (mg mL^{-1})	MBC (mg mL^{-1})	IC_{50} (mg mL^{-1})
S. aureus	Water	50	50	3.12
	Ethyl acetate	50	50	1.56
	Chloroform	50	50	1.56
	Acetone	50	50	1.56
E. coli	Water	25	50	0.39
	Ethyl acetate	25	50	0.58
	Chloroform	25	50	1.17
	Acetone	>50	>50	25
S. flexneri	Water	25	50	1.17
	Ethyl acetate	25	50	1.17
	Chloroform	25	50	1.17
	Acetone	25	50	1.17
E. faecalis	Water	nd	nd	Nd
	Ethyl acetate	25	50	0.97
	Chloroform	25	50	0.97
	Acetone	nd	nd	Nd

exhibited strongest activity, with lowest IC_{50} values, similar to studies on *C. rotundus* and *C. nardus* (Marques *et al.*, 2020).

The antifungal activity of *F. ferruginea* extracts against *C. albicans* and *F. oxysporum* varied depending on the solvent used. Chloroform, acetone, ethyl acetate, and petroleum ether extracts exhibited significant inhibition against *C. albicans*, while ethanol, water, and methanol showed no activity (Fig. 4A,B). These findings align with Al-Hazmi *et al.* (2018) who observed significant impact of solvent selection on antifungal efficacy in case of *C. conglomeratus* and *C. rotundus*. For *F. oxysporum*, petroleum ether, chloroform, and acetone extracts were effective, whereas ethanol, ethyl acetate, water, and methanol showed no activity. Similar observations have been reported in other plant-based antifungal studies, signifying the role of solvent choice in bioactive compound extraction (Haruna *et al.*, 2024). The efficacy of petroleum ether, chloroform, and acetone extracts aligns with previous works showcasing their ability to extract antifungal compounds from plants (Salim *et al.*, 2023). Conversely, the lack of activity in ethanol, ethyl acetate, water, and methanol extracts reflects their inability to extract relevant antifungal compounds (Padmalatha and Prabha, 2023).

Table 4: Zone of inhibition depicted by *F. ferruginea* extracts against various bacteria

Pathogens	Zone of inhibition (mm in dia.)						
	Ethanol	Ethyl acetate	Water	Petroleum ether	chloroform	Acetone	Methanol
S. aureus	0.0 ± 0.0	11.5 ± 0.58	0.0 ± 0.00	0.0 ± 0.00	8.5 ± 0.58	8.5 ± 0.58	6.0 ± 0.00
E. faecalis	0.0 ± 0.0	10.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	11.5 ± 0.58	0.0 ± 0.00	0.0 ± 0.00
E. coli	0.0 ± 0.0	14.0 ± 0.00	13.5 ± 0.58	0.0 ± 0.00	11.5 ± 0.58	0.0 ± 0.00	0.0 ± 0.00
S. flexneri	0.0 ± 0.0	13.5 ± 0.58	11.5 ± 0.58	0.0 ± 0.00	9.5 ± 0.58	10.5 ± 0.58	10.0± 0.00

The values are zone of inhibition (mm in dia). Values are the average of two independent experiment. Antibiotic chloramphenicol showed an inhibition zone of 20 mm diameter

Column chromatography

The ethyl acetate fraction (fraction 28.25) of *F. ferruginea* exhibiting the highest total phenol content (0.272 mg mL^{-1}) and DPPH antioxidant activity (7.41%) was selected for further analysis, while the fractions from chloroform, hexane, and methanol were excluded. GC-MS analysis identified a major peak at a retention time of 19.14 min, corresponding to 1,2-enzenedicarboxylic acid, bis(2-ethylhexyl) ester, a compound known for its biological activities, including anticancer potential (Kumar *et al.*, 2021) (Fig. 5). UV-vis spectroscopy revealed the absorption peaks at 203.0 and 281.2 nm, suggesting conjugated and aromatic structures, aligning with GC-MS findings (Fig. 6). FT-IR spectroscopy

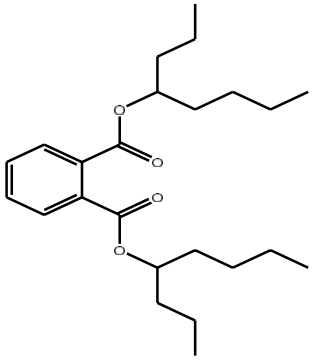

Fig. 5: Chemical structure of 1,2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester in *F. ferruginea* identified by GC-MS analysis

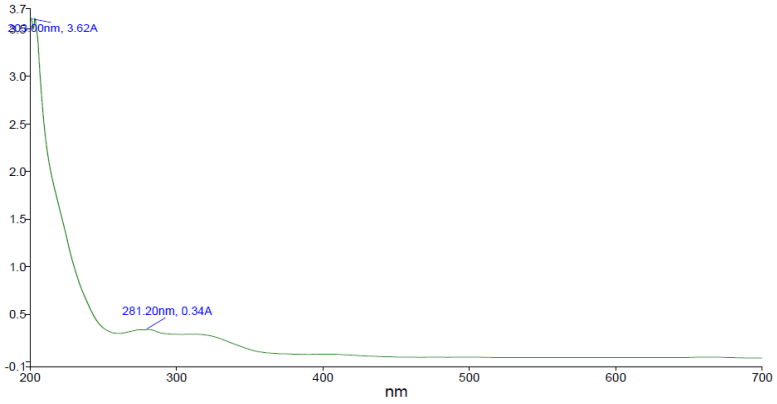

Fig. 6: UV-visible absorption spectrum of ethyl acetate fraction of *F. ferruginea*

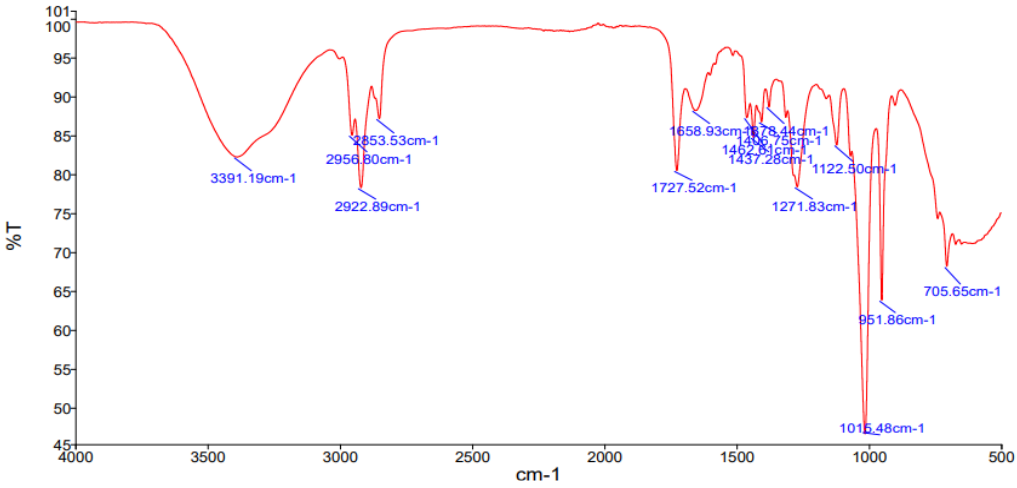

Fig. 7: FT-IR spectrum of 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester with peak assignments in *F. ferruginea*

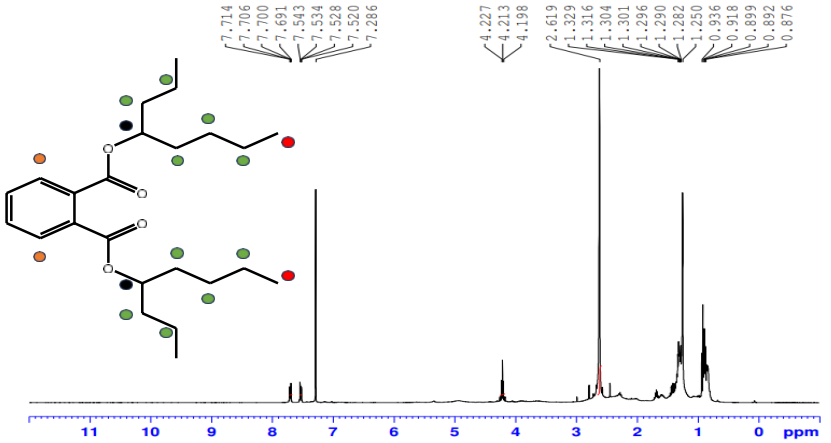

Fig. 8: ^1H NMR spectrum of 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester with key peak annotations in CDCl3 in *F. ferruginea*

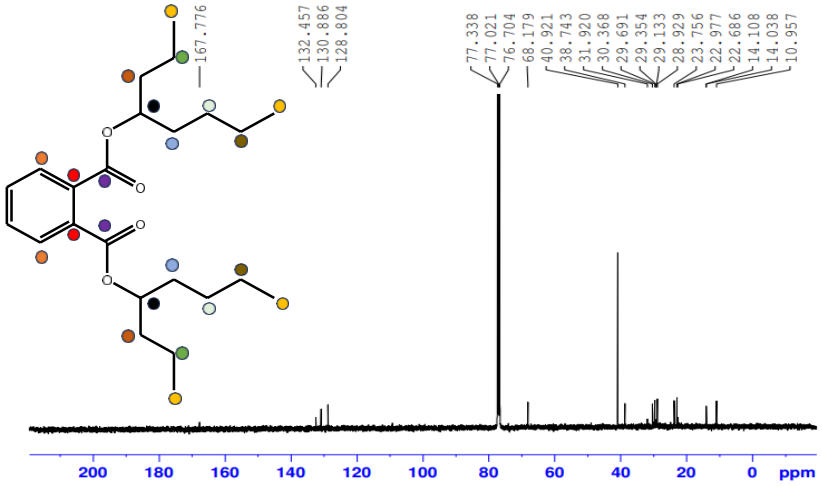

Fig. 9: Carbon-13 NMR spectrum of 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester with peak assignments in CDCl3 in *F. ferruginea*

confirmed the presence of esters, aromatic rings, and alkyl chains, with key peaks at 1727 cm^{-1} (C=O stretching), 1271 cm^{-1} (C-O-C bending), and a broad band at 3391 cm^{-1} (-OH group), indicative of alcohols, phenols, and carboxylic acids (Fig. 7). ^{1}H and ^{13}C NMR analyses further validated the molecular profile, showing aromatic rings, esters, and alkyl chains, with a characteristic ^{13}C peak at 40.92 ppm suggesting primary alkyl carbons (Fig. 8 and 9). These spectroscopic analyses provide detailed phytochemical profile of *F. ferruginea*, revealing its therapeutic potential, including antioxidant, anti-inflammatory, anti-cancer, and antimicrobial properties (Dar and Shahnawaz, 2023; Salem *et al.*, 2023). The combined results from GC-MS, UV-Vis, FT-IR, and NMR underscore the extract's bioactive potential and its significance for further pharmaceutical exploration.

Conclusion: The comprehensive analysis of *F. ferruginea* extracts revealed a rich phytochemical profile, including phenolics, flavonoids, terpenoids, alkaloids, glycosides, and anthraquinones, which contribute to the plant's diverse biological activities. The ethyl acetate and chloroform extracts, in particular, demonstrated strong antioxidant, anti-microbial, and antifungal activities, making them promising candidates for pharmaceutical applications. The identification of 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester as a major compound in the ethyl acetate extract, supported by GC-MS, UV-Vis, FT-IR, and NMR spectroscopy, underscores the extract's potential for therapeutic development. These findings suggest that *F. ferruginea* holds significant potential as a natural source of bioactive compounds, with applications in developing antioxidant-rich and antimicrobial agents. Further studies, including *in vivo* assessments and mechanistic investigations, are warranted to fully explore its therapeutic benefits and pharmacological applications.

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Conflict of interest: The authors declare that there are no conflicts of interest related to this study.

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