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Original Research Article

Phytochemical analysis and evaluation of antimicrobial activity of leaf and stem extracts of *Ecbolium viride* Forssk.

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Abstract

The rise and dissemination of drug-resistant microorganisms pose a serious threat to public health. There is an urgent need for an alternative therapeutic option, particularly those derived from traditionally used medicinal plants. The aim of this study was to evaluate the antimicrobial efficacy of leaf and stem extracts of *Ecbolium viride* Forssk., against human pathogenic bacterial and fungal strains. Additionally, this study sought to investigate the phytochemical composition of these extracts using spectral analysis techniques. Soxhlet extraction was conducted using solvents of varying polarities, including ethanol, methanol, acetone, and hexane. The methanolic extract underwent phytochemical analysis *via* FTIR and GC-MS. Antimicrobial activity was assessed by determining the MIC and MBC/MFC using the broth dilution method. The methanolic extracts of the leaf and stem of *E. viride* exhibited significant inhibition against all tested microorganisms. The highest inhibition was observed against *Bacillus subtilis* (22.3 ± 0.15 mm), followed by *Salmonella typhi* (19.9 ± 0.11 mm), and *Aspergillus niger* (21.8 ± 0.21 mm) at a concentration of 500 µg/mL. The methanolic leaf extract demonstrated strong antimicrobial activity with MIC values ranging from 15.625 to 125 µg/mL. FTIR and GC-MS analyses confirmed the presence of bioactive compounds with broad therapeutic potential. This study highlights *E. viride* as a promising source for the bioactive compounds responsible for its broad-spectrum antimicrobial activity, as evidenced by the diverse phytoconstituents identified through phytochemical analysis. This also underscores its potential applications in combating resistant microbial pathogens.

Keywords: Antimicrobial, *Ecbolium viride*, FTIR, GC-MS, Phytochemical.

Introduction

The World Health Organization (WHO) reports that traditional medicine continues to meet the primary healthcare needs of approximately 80% of the population in developing countries (Dai *et al*., 2022). The discovery and utilization of plants with bioactive compounds date back to antiquity, when they were used for medicinal, dietary and nutraceutical purposes (Hussain *et al*., 2008). Over time, plant-based products have evolved from being used as folk remedies to becoming an integral part of both traditional and modern allopathic practices (Dubey *et al*., 2012). In many developing nations, especially across Asia and Africa, medicinal plants continue to play a vital role in daily healthcare, with India being a notable example (Agidew, 2022). In India, medicinal plants are extensively employed by various sectors like pharmaceuticals, traditional medicine systems (such as Ayurveda, Unani, and Siddha), cosmetics, and the food and nutraceutical industries, either directly or indirectly through traditional remedies (Hussain *et al*., 2014).

Medicinal plants have been considered a major source of phytochemicals, which are crucial for identifying potential lead compounds in drug discovery and contribute to diverse biological activities, including antioxidant, antifungal, antibacterial, and anticancerous properties (Johnson and Ayoola, 2015). The utilization of natural products for drug discovery and development highlights their value as alternatives or complementary options to synthetic drugs, further promoting the advancement of phytomedicine research and production (Pougoue *et al*., 2020).

Phytochemicals from traditional medicinal plants have demonstrated the ability to inhibit the growth of microbes that are resistant to multiple antibiotic classes (Álvarez-Martínez *et al*., 2021) and the bioactive compounds in plants, are obtained from various plant parts, including seed coats, flowers, roots, leaves, bark, seeds, and pulp (Altemimi *et al*., 2017). The rise of antibiotic-resistant bacteria, combined with the adverse side effects of synthetic antibiotics, emphasizes the need to develop

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plant-based drugs as an effective antimicrobial agent (Ameya *et al*., 2022). Several key factors contribute to the widespread use of plant-derived therapeutics, including their affordability, accessibility, and reduced risk of side effects compared to synthetic drugs (Maduka *et al*., 2020). The secondary metabolites derived from plants exhibit a wide range of biological activities, making them crucial not only in the food and healthcare industries but also in pharmaceutical development, particularly for managing inflammatory conditions and chronic diseases (Abdelmoumen *et al*., 2023).

The present study was aimed at qualitative phytochemical analysis to assess the antimicrobial activity of solvent extracts derived from the leaves and stems of *Ecbolium viride*, to identify functional groups present in the methanolic leaf extract using FTIR and to analyze the bioactive compounds in the methanolic leaf extract through GC-MS.

Materials And Methods

Collection of plant materials and preparation of extracts

Leaves and stems of *E. viride* Forssk., were selected for this study and mature plants were collected from Annamalai Nagar (11^{o} 24′ N latitude and 79^{o} 44′ E longitude), Cuddalore district of Tamil Nadu, India. The leaves and stems were washed thoroughly, shade-dried, and ground into a fine powder and underwent soxhlet extraction at 50 to 80°C for 24 hours, utilizing hexane, acetone, ethanol and methanol (Umar *et al*., 2013).

Phytochemical analysis

The presence of various phytochemical components, including alkaloids, phenols, saponins, tannins, glycosides, flavonoids, quinones, steroids and terpenoids, was screened in the crude extracts of *E. viride* plant leaf and stem using a standard procedure outlined by Harborne (1998).

Cultivation and maintenance of test microorganisms

The microorganisms used for the antimicrobial tests were clinical isolates categorized into two groups: (1) Bacterial pathogens, including *Staphylococcus aureus* ATCC 23235, *Bacillus subtilis* ATCC 6051, *Klebsiella pneumoniae* ATCC 13883, *Salmonella typhimurium* ATCC 19430, *Streptococcus pyogenes* ATCC 19615 and *Escherichia coli* ATCC 25922; and (2) fungal pathogens including *Aspergillus flavus* ATCC 9643, *A. niger* ATCC 16888 and *Candida albicans* ATCC 10231. These isolates were obtained from Raja Muthiah Medical College and Hospital, Annamalai University, India. The bacterial and fungal clinical isolates were identified through morphological and biochemical characterization after immediate sub-culturing on freshly prepared nutrient agar broth at 37 ± 0.5°C and on Sabouraud dextrose agar, respectively (Cuevas-Cianca *et al*., 2022).

Preparation of test solutions and antimicrobial assay

Each crude extract from the leaves and stem was dissolved in dimethyl sulfoxide (DMSO) to achieve a concentration of 500 µg/mL, which was then tested for antimicrobial activity. The antimicrobial activity against the selected human pathogens was evaluated using aliquots from various leaf and stem extracts at a concentration of 500 µg/mL, following the agar well diffusion method described by Valgas *et al*. (2007) with slight modifications. Inocula were prepared from overnight cultures, suspended in nutrient broth, and adjusted to 0.5 McFarland standard. Mueller-Hinton agar served as the growth medium for bacteria, while Sabouraud dextrose agar was used for fungi. The test organisms were evenly swabbed over the media, and 7 mm diameter wells were made. From each extract concentration, 50 µL was added to the wells, followed by incubation at 37°C for 24 hours for bacterial species and *Candida albicans*. For fungi, the plates were incubated at 37°C for 60 to 72 hours. The inhibitory activity of each extract was assessed by measuring the zones of inhibition around the wells. Ciprofloxacin and Fluconazole are used as positive controls for bacteria and fungi, respectively (Manilal *et al*., 2020).

Minimum inhibitory concentration and minimum bactericidal/fungicidal concentration

To assess the MIC of the plant extracts, the broth dilution method was used, following the procedure described by Kowalska-Krochmal and Wicher (2021). Each extract, initially at a concentration of 5 mg/mL, was serially diluted in eight test tubes to obtain concentrations of 1000, 500, 250, 125, 62.50, 31.25, 15.60, and 7.80 µg/mL. A 1-mL sample of microbial suspension was added to each tube and incubated at 37°C for 24 hours. Control tubes containing the test strain without the plant extract and the solvents used to dissolve the extracts were also included. After incubation, turbidity was visually compared to the negative control. The turbidity of the microbial suspension in the inoculum was measured using spectrophotometry, adjusted to 0.5 McFarland standards (1×10^8 CFU/mL), and recorded. To determine the minimum bactericidal/fungicidal concentration (MBC/MFC), a 5 µL sample was taken from each tube that showed no growth and incubated at 37°C for 24 hours. Ciprofloxacin and fluconazole were used as positive controls for bacteria and fungi, respectively (Manilal *et al*., 2020).

FTIR spectroscopic analysis

For FTIR analysis of the methanolic leaf extract, 2 mg of the sample was encapsulated in a 100 mg KBr pellet (FTIR grade). The spectra were then recorded using a Jasco

FTIR-6300 (Shimadzu, Japan) in transmittance mode, with a scanning range of 450 to 4000 cm^{-1} and a resolution of 4 cm^{-1} (Nandiyanto *et al*., 2019).

GC–MS analysis

The phytochemical composition of the methanolic leaf extract was analyzed using GC–MS at the Central Instrumentation Lab, Central University of Punjab, India. The analysis was performed on an Agilent Techn-7820A GC system, with a mass spectrometer (5975C V2-MSD with a Triple-Axis Detector) and an Agilent DB-WAX column (30 m × 320 × 0.25 μm), equipped with an autosampler and injector (G4513A-CA, United States). Helium (99.99%) was used as the carrier gas at a constant flow rate of 1-mL/min. The injection volume was 1-mL, with a split ratio of 50:1. The injector temperature was set to 60°C, and the ion source temperature to 250°C. Mass spectra were recorded at 70 eV. Spectral and chromatographic data were analysed using the GC–MS Mass Hunter software, and the relative percentage of each component was determined by comparing the average peak areas to the total area. The NIST GC–MS library and WILEY-08 database were used for data interpretation (Mulyaningsih *et al*., 2010).

Statistical analysis

Data are presented as the mean ± SD (n = 3). To evaluate the significance of differences between groups, one-way ANOVA was conducted, followed by a post hoc test. The *p-value* of ≤ 0.05 was considered statistically significant.

Results

The therapeutic potential of plants is closely linked to the presence and concentration of key metabolites. In the present study, we investigated the crude extracts from *E. viride* leaves and stems by using various solvents. Our analysis included qualitative phytochemical screening, antimicrobial activity and spectral analysis.

Phytochemical analysis

The qualitative phytochemical analysis of *E. viride* leaves and stems is presented in Table 1. Methanol emerged as the most effective solvent, extracting all phytochemicals from both the leaves and stems. Whereas, hexane was the least effective, yielding the lowest quantity of secondary metabolites. The analysis confirmed the presence of various phytochemicals, including flavonoids, glycosides, alkaloids, terpenoids, saponins, quinones, and steroids, in both leaf and stem extracts. Notably, the methanolic extracts demonstrated a broader range of phytochemicals than the ethanol, acetone, and hexane extracts. These findings are aligned with previous research (Wani *et al*., 2012, 2013; Kumar and Dhillon, 2015), further validating the reported phytochemical composition. Numerous plant extracts are known to contain phytochemicals with therapeutic, psychological, and medicinal benefits (Shukla *et al*., 2021). Given the significant biological effects of these phytochemicals, further processes for isolation, purification, and characterization may be warranted to support the development of new medications (Lone *et al*., 2023).

Antimicrobial activity

The antimicrobial efficacy of *E. viride* leaf and stem extracts was assessed against six pathogenic bacterial strains and three fungal strains, as detailed in Tables 2 and 3. The results demonstrated that the methanolic extracts from both leaves and stems exhibited the strongest antimicrobial activity. The methanolic leaf extract showed significant inhibition against all tested strains, with the largest inhibition zone observed in *Bacillus subtilis* (22.3 ± 0.15 mm), followed by *Salmonella typhi* (19.9 ± 0.11 mm) and the fungal strain *Aspergillus niger* (21.8 ± 0.21 mm) at a concentration of 500 μg/mL. Ethanol extracts displayed moderate antimicrobial activity, with the highest of 18.8 ± 0.22 mm against *Bacillus subtilis* and the lowest 14.3 ± 0.48 mm against *A. flavus*. The acetone and hexane extract

Tables 1: Preliminary phytochemical analysis of various solvent extracts of leaf and stem of *E. viride*

Plant secondary metabolites	*Method used*	*Leaf*				*Stem*			
		Ethanol	*Methanol*	*Acetone*	*Hexane*	*Ethanol*	*Methanol*	*Acetone*	*Hexane*
Alkaloids	Mayers's test	+++	+++	++	+	+++	++	+	+
Phenol	Ferric chloride test	+++	+++	+	+	+++	+++	+	+
Saponins	Foam test	++	++	-	-	+	+	-	-
Tannins	Braymer's test	++	+	-	-	++	+	+	-
Glycosides	Liebermann's test	++	++	-	-	+	+	+	-
Flavonoids	Shinoda test	++	++	+	+	++	++	+	+
Quinones	Phillipson's test	++	++	-	-	-	+	-	-
Steroids	Chloroform test	++	++	+	-	+	+	-	-
Terpenoids	Salkowski's test	+++	++	+	+	+++	++	+	-

(+++) Strongly present, (++) Moderately present, (+) Present, (−) Absent.

Table 2: Antimicrobial activity of *E. viride* leaf extracts at 500 μg/mL

Test Organisms		*Zone of Inhibition (mm)**				
		Ethanol	*Methanol*	*Acetone*	*Hexane*	*Ciproflaxin*
Bacteria	*S. aureus*	16.5 ± 0.30	19.4 ± 0.35	17.6 ± 0.44	14.7 ± 0.28	25.6 ±0.24
	B. subtilis	18.8 ± 0.22	22.3 ± 0.15	14.15±0.11	12.3 ± 0.34	25.8 ±0.58
	K. pneumoniae	16.7 ± 0.41	19.8 ± 0.13	16.4 ± 0.5	14.3 ± 0.48	25.2 ±0.17
	S. typhi	18.4 ± 0.17	19.9 ± 0.11	14.6 ± 0.28	13.6 ± 0.12	26.6 ±0.14
	S. pyogenes	16.3 ± 0.96	18.8 ± 0.10	13.8±0.16	13.7 ± 0.53	25.7 ± 0.16
	E. coli	18.5 ± 0.20	19.2 ± 0.25	16.7 ± 0.31	14.9 ± 0.29	26.5 ±0.12
Fungi		*Ethanol*	*Methanol*	*Acetone*	*Hexane*	*Flucanozole*
	A. flavus	14.3 ± 0.48	17.3 ± 0.24	13.6 ± 0.12	12.2 ± 0.11	27.5 ±0.19
	A. niger	18.3 ± 0.65	21.8 ± 0.21	14.5 ± 0.24	12.8 ± 0.19	27.10 ±0.26
	C. albicans	15.2 ± 0.27	17.8 ± 0.45	13.4 ± 0.21	12.8 ± 0.19	27.4 ±0.36

* Including the diameter of the well.

Table 3: Antimicrobial activity of *E. viride* stem extracts at 500 μg/ml.

Test Organisms		*Zone of Inhibition (mm)**				
		Ethanol	*Methanol*	*Acetone*	*Hexane*	*Ciproflaxin*
Bacteria	*S. aureus*	17.6 ± 0.44	18.7 ± 0.5	13.7 ± 0.31	12.2 ± 0.5	27.3 ±0.93
	B. subtilis	14.5 ± 0.26	14.9 ± 0.33	12.15±0.11	10.9 ± 0.26	27.2 ±0.87
	K. pneumoniae	16.4 ± 0.5	18.9 ± 0.13	13.9 ± 0.23	12.2 ± 0.3	25.9 ±0.76
	S. typhi	16.7 ± 0.41	17.4 ± 0.37	13.6 ± 0.28	14.2 ± 0.6	27.4 ±1.12
	S. pyogenes	15.2 ± 0.27	16.1 ± 0.26	12.8 ± 0.19	11.5 ± 0.19	26.2 ± 0.16
	E. coli	18.3 ± 0.65	20.2 ± 0.29	15.1 ± 0.21	13.2 ± 0.23	27.6 ±1.06
Fungi		*Ethanol*	*Methanol*	*Acetone*	*Hexane*	*Flucanozole*
	A. flavus	12.0 ± 0.34	12.7 ± 0.18	10.1 ± 0.24	9.1 ± 0.22	26.9 ±0.19
	A. niger	14.6 ± 0.35	14.9 ± 0.21	13.5 ± 0.24	12.1 ± 0.19	26.2 ±0.20
	C. albicans	13.4 ± 0.21	13.9 ± 0.23	12.8 ± 0.11	11.3 ± 0.21	27.5 ±0.67

* Including the diameter of the well.

exhibited the least inhibition across all tested strains at the concentrations used. In the stem extracts, methanol demonstrated the highest inhibition zone against *Escherichia coli* (20.2 ± 0.29 mm), followed by *Klebsiella pneumoniae* (18.9 ± 0.13 mm) and *A. niger* (14.9 ± 0.21 mm). The antimicrobial activity of an agent is primarily based on two mechanisms: chemically interfering with the synthesis of essential bacterial components or bypassing conventional antibacterial resistance mechanisms. However, the bacteria can develop resistance to multiple antimicrobials either through inherent selective pressures or by acquiring resistance mechanisms from neighboring microbes (Magiorakos *et al*., 2012). Certain antibiotics, such as polymyxins, can bind to the lipid component of lipopolysaccharides, leading to structural changes *via* phospholipid exchange, ultimately causing osmotic imbalance and rapid bacterial death (Poirel *et al*., 2017).

MIC, MBC and MFC

The leaf extracts exhibited significantly higher antimicrobial activity with lower MIC values against the tested organisms, compared to the stem extracts, as illustrated in Tables 4 and 5. Notably, the methanolic leaf extract demonstrated the most promising results, with MIC and MBC values of 15.625 and 31.25 μg/mL, respectively, against *B. subtilis*. For the fungal strain *A. niger*, the lowest MIC and MFC values recorded were 31.25 and 62.50 μg/mL. The MBC and MFC values corresponded directly to their respective MIC values. Similar findings have been reported in *E. coli* (Nadeem *et al*., 2021), *Bacillus* sps. (Mahdavi *et al*., 2020) and *Aspergillus* sps. (Beyene, 2021). Our data indicated that the most active compounds contributing to the antimicrobial effects were predominantly found in the methanolic leaf extract. As the extract concentration increased, there was a rise in the microbial inhibition zones, aligning with the results of Nigussie *et al*. (2021), who studied the antibacterial activity of methanolic extracts from the leaves of three medicinal plants against bacteria isolated from lymphoedema wounds. Ethanol and acetone extract also displayed significant activity against tested organisms, consistent with the antimicrobial activity reported for

Table 4: MIC and MBC/MFC of different leaf extracts of *E. viride* against bacterial and fungal strains

Test Organisms		*Leaf extracts (µg/mL)*									
		Ethanol		*Methanol*		*Acetone*		*Hexane*		*Ciproflaxin*	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Bacteria	*S. aureus*	62.50	125	62.50	125	125	250	250	500	31.25	62.50
	B. subtilis	31.25	125	15.62	31.25	125	250	250	500	7.81	15.62
	K. pneumoniae	31.25	62.25	31.25	125	125	250	250	500	15.62	31.25
	S. typhi	62.50	125	31.25	125	62.25	125	250	250	15.62	31.25
	S. pyogenes	62.50	125	31.25	125	62.25	125	250	500	62.25	125
	E. coli	31.25	125	31.25	125	62.25	125	250	500	62.25	125
Fungi		*Ethanol*		*Methanol*		*Acetone*		*Hexane*		*Flucanozole*	
		MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
	A. flavus	125	250	62.25	125	125	250	250	500	31.25	62.50
	A. niger	62.50	125	31.25	62.25	62.50	250	250	1000	62.50	125
	C. albicans	62.50	125	62.50	125	125	250	500	1000	31.25	62.50

MIC -Minimum Inhibitory Concentration, MBC – Minimum Bactericidal Concentration, MFC – Minimum Fungicidal Concentration.

Table 5: MIC and MBC/MFC of different stem extracts of *Ecbolium viride* against bacterial and fungal strains

Test organisms		*Stem extracts (µg/mL)*									
		Ethanol		Methanol		Acetone		Hexane		Ciproflaxin	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Bacteria	*S. aureus*	125	250	125	250	125	250	500	1000	62.50	125
	B. subtilis	125	500	31.25	125	125	250	250	500	15.62	31.25
	K. pneumoniae	250	500	125	500	250	500	125	500	62.50	125
	S. typhi	125	250	62.50	125	125	250	500	1000	31.25	62.50
	S. pyogenes	250	250	62.50	125	125	250	250	500	125	500
	E. coli	125	250	125	250	125	250	500	1000	62.50	125
Fungi		*Ethanol*		*Methanol*		*Acetone*		*Hexane*		*Flucanozole*	
		MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
	A. flavus	250	500	125	250	250	500	500	1000	62.25	125
	A. niger	125	250	62.50	125	125	250	250	1000	125	250
	C. albicans	250	500	125	250	500	500	250	500	62.50	125

MIC -Minimum Inhibitory Concentration, MBC – Minimum Bactericidal Concentration, MFC – Minimum Fungicidal Concentration.

Humiria balsamifera (Dias *et al*., 2021). In contrast, the hexane extract exhibited the lowest antimicrobial activity across all tested strains, findings previously reported by Mogana *et al*. (2020) in their study on the antimicrobial properties of *Canarium patentinervium*.

FTIR analysis

In this study, FTIR analysis of the methanolic leaf extract of *E. viride* displayed a broad and strong peak at 3339.7 and 2922.2 cm^{-1}, also within the 3400 to 2900 cm^{-1} range, indicating O–H stretching and the presence of alcohol groups. A peak at 2102.2 cm^{-1}, corresponding to C≡C stretching, indicated the presence of alkynes, while a peak at 1707.1 cm^{-1} with C=O stretching confirmed conjugated aldehydes. A peak at 1513.3 cm^{-1}, linked to N–O bending, revealed nitro compounds, while a medium and broad peak at 1438.8 cm^{-1}, attributed to S=O stretching, identified sulfonates. Additionally, a peak at 1349.3 cm^{-1} with C–N stretching signified the presence of amines, and peaks at 1207.7 and 1021.3 cm^{-1}, corresponding to C–F stretching, indicated fluoro compounds (Figure 1, Table 6).

GC–MS results

The methanolic leaf extract of *E. viride* exhibited the strongest antibacterial activity, so GC–MS analysis was conducted to identify the compounds potentially responsible for the observed biocidal effects. Only compounds whose mass spectra showed at least 95% similarity to the NIST and WILEY-O8 libraries

Table 6: Interpretation of the FTIR spectra of the methanolic leaf extract of *E. viride*

S. No.	*Frequency (Cm^{-1})*	*Absorption intensity*	*Band assignment*	*Type of compounds*
1	3339.7	Strong	O-H stretching	Alcohol
2	2922.2	Strong	O-H stretching	Carboxylic acid
3	2102.2	Weak	C≡C stretching	Alkyne
4	1707.1	Strong	C=O stretching	Conjugated aldehyde
5	1647.5	Strong	C=C stretching	Alkene
6	1513.3	Strong	N-O bending	Nitro compound
7	1438.8	Medium	S=O stretching	Sulfonate
8	1349.3	Medium	C-N stretching	Amine
9	1207.7	Strong	C-F stretching	Fluoro compound
10	1021.3	Strong	C-F stretching	Fluoro compound
11	834.9	Strong	C=C stretching	Alkene
12	730.6	Strong	C-Cl stretching	Alkene

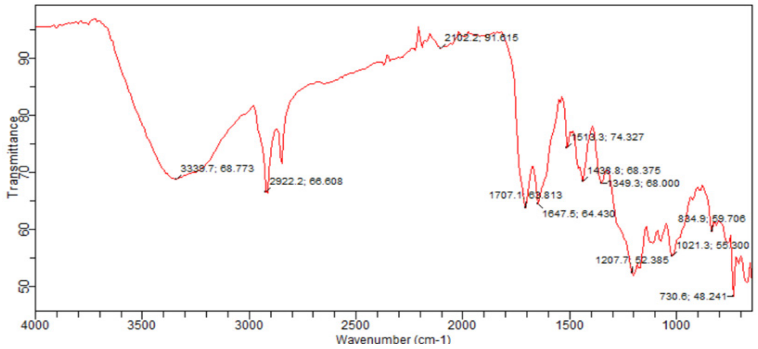

Figure 1: FTIR spectrum of the methanolic leaf extract of *E. viride*

were reported, ensuring precise characterization of the identified compounds. The analysis revealed the presence of 34 distinct compounds (Figure 2). Among these, the most prominent, based on the peak area, were: 13-Docosenamide, (Z)- (16.89%); Beta-sitosterol (12.98%); (3aR,4S,11E,12aR)-11-Methyl-3-met (6.69%); Decanedioic acid, bis(2-ethylhexyl) ester (11.94%); Phytol (5.50%); Cyclononasiloxane, octadecamethyl- (4.0%); Tris(2,4-di-tert-butylphenyl) phosphate (3.74%); 1-Octadecanesulphonyl chloride (2.84%); Cyclohexane, hexadecyl- (2.75%); Cetene (2.72%); Heptadecane, 2,3-dimethyl- (2.47%); and Supraene (2.03%). The presence of a broad spectrum of bioactive compounds with potent therapeutic activities confirms that the methanolic leaf extract of *E. viride* has significant antimicrobial activity. A previous study by Nadeem *et al*. (2024) on methanolic extract of *Thymus linearis* revealed the presence of 25 phytocompounds, which are less in number as compared to our study, thus confirming the significance of this study.

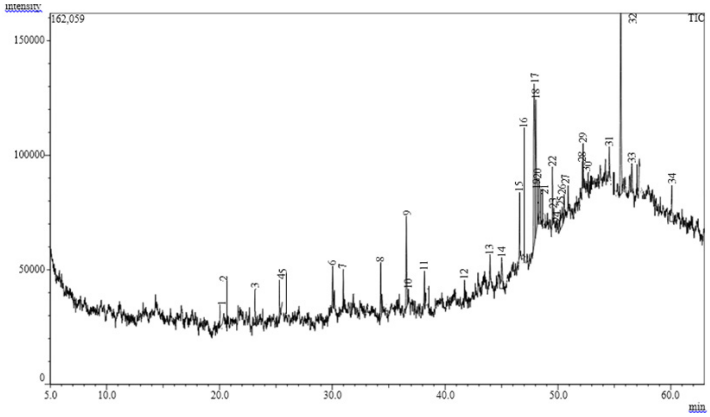

Figure 2: GC–MS chromatogram of the methanolic leaf extract of *E. viride*

Conclusion

The present study reveals that the methanolic leaf extract of *E. viride* possesses notable antibacterial and antifungal properties. Phytochemical analysis using FTIR and GC-MS revealed the presence of bioactive compounds with broad-spectrum pharmacological and therapeutic properties, supporting the plant's long-standing use against antimicrobial activity. The leaf and stem extracts of *E. viride* demonstrated significant antimicrobial activity, with the methanolic leaf extract exhibiting superior efficacy against both bacterial and fungal pathogens. These findings provide valuable insights, paving the way for future research into the therapeutic potential of this plant. Furthermore, the development of novel broad-spectrum chemotherapeutic agents will necessitate ongoing efforts in the isolation, identification, and purification of the bioactive compounds present in these extracts. These processes are currently underway, highlighting the promising role of *E. viride* in advancing antimicrobial drug discovery and development.

Conflict of Interest

The authors have no conflict of interest.

Author's Contribution

Rudhra conducted fieldwork, plant collection, methodology, data collection, and manuscript writing. Killivalavan was responsible for data analysis and interpretation, statistical analysis, and manuscript writing; Venkatesan was responsible for the research concept, literature review, edited the manuscript, and overall supervision.

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