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PHYTOCHEMICAL PROFILING, GC-MS CHARACTERIZATION, ANTIBACTERIAL ACTIVITY OF *PLECTRANTHUS VETTIVEROIDES* AND *KIGELIA PINNATA*

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ABSTRACT
*Plectranthus vettiveroides***,** belonging to the Lamiaceae family is a valuable herb, rich in bioactive compounds and *Kigelia pinnata*, sausage tree belonging to Bignoniaceae family has medicinal value and is rich in naphthoquinones, iridoids, and flavonoids. The current study was conducted to examine the phytochemical profiling, GC-MS analysis and antibacterial activity of different parts of *P. vettiveroides*- Root (PVR), Stem (PVS), Leaf (PVL) and *K. pinnata* – Root (KPR), Bark (KPB), Leaf (KPL). In qualitative analysis, high concentration of alkaloids was present in all extracts of *P. vettiveroides* and *K. pinnata.* Further, in quantitative analysis PVL had the highest alkaloid content (13.44 mg/mL) and PVS had the highest flavonoid content (4.03 mg/mL) in *P. vettiveroides,* whereas in *K. pinnata* bark and root had the highest terpenoid content 5.7 and 13.2 mg/mL, respectively. The phytochemical analysis of both selected plants, indicate the potential for biological activities. GC-MS analysis revealed different phytocompounds in both *P. vettiveroides* and *K. pinnata* which are of great use in pharmacological activities. Well-diffusion assays carried out the root and leaf extracts of *P. vettiveroides* and the bark and leaf extracts of *K. pinnata* produced the most consistent zones of inhibition against the selected bacterial strains, signifying antibacterial potential that requires further investigation.

KEYWORDS: *Plectranthus vettiveroides, Kigelia pinnata,* GC-MS, methanolic extract, bioactive compound, antibacterial activity.

1. INTRODUCTION

Medicinal plants have been employed since ancient times as a rich source of Phytoconstituents such as flavonoids, alkaloids, terpenoids, and saponins, which impart significant anti-bacterial properties (Atanasov *et al*., 2021; Alasbahi and Melzig, 2018; Ahamed *et al*., 2023). *Plectranthus vettiveroides* (K. C. Jacob) N.P. Singh and B.D. Sharma (Lamiaceae), is a succulent aromatic herb endemic to South India, whose roots are traditionally used for treating inflammation, nausea and vomiting, and are valued in the perfumery industry for their pleasant fragrance (Nisheeda *et al*., 2016). The presence of diterpenoids, alkaloids, tannins and essential oils imparts notable pharmacological and anti-bacterial activity to this plant (Ahamed *et al*., 2023; Mussay *et al*., 2022). *Kigelia pinnata* (Jacq.) DC. (Bignoniaceae) commonly known as the sausage tree and is distributed across tropical India for its medicinal properties, used traditionally in the treatment of wound infections, inflammatory disorders and skin ailments (Burkill, 1985; Grace *et al*., 2002; Agyare *et al*., 2009; Gorman and Adikwu, 2010).

Although the individual medicinal properties of *P. vettiveroides* and *K. pinnata* have been reported earlier, comparative studies integrating phytochemical screening, GC–MS characterisation and antibacterial evaluation of these two plants remain scarce. In view of growing problem of antibacterial resistance, such comprehensive profiling of bioactive compounds is essential to provide scientific validation for their traditional uses and to aid in the discovery of novel plant-derived antibacterial compounds. The present study was therefore undertaken to carry out phytochemical evaluation, GC–MS characterisation, and antibacterial studies of the methanolic extracts of root, stem & leaf of *P. vettiveroides* and leaf, bark & root of *K. pinnata*, respectively.

2. MATERIALS AND METHODS

Plectranthus vettiveroides (*P. vettiveroides*) (authentication number RRCBI-mus660) was collected from Chidambaram, Tamil Nadu and *Kigelia pinnata* (*K. pinnata*) (authentication number RRCBI-mus539) was collected from FRLHT, Bengaluru. Both plant samples were identified and authenticated from Central Ayurveda Research Institute, Government of India, Bengaluru by a plant taxonomist. The plant materials were washed thoroughly with tap water followed by 2 – 3 times distilled water wash, so as to cleanse all the dust, contaminants and impurities from the materials. The root, stem, bark and leaf were segregated, dried in shade at 25-30 ˚C for 10-15 days and then pulverised into a fine powder using an electric grinder. Further, the plant powders were stored separately in airtight containers for further use.

2.1. Preparation of the plant extract

The plant extracts were prepared by the Soxhlet extraction method. 10 g of each powdered plant material (root, stem, bark, and leaf) was extracted separately with 200 mL of methanol for 6 hrs or until the solvent in the siphon tube became clear (Ozturk *et al*., 2022). Later the extraction was recovered and the resultant extracts were concentrated by evaporating the excess methanol. The dried extracts were stored in sterile vials for further studies.

2.2. Phytochemical screening

Standard procedures were followed to investigate the preliminary analysis present in the methanolic extracts, such as terpenoids, flavonoids, alkaloids, phenolics, steroids, and Saponins (Harborne, 1998; Trease and Evans, 2009).

2.3. Quantitative analysis

Estimation of phenols: The total phenolic content of plant extract was estimated by using Folin- ciocalteu (FC) method (Singleton *et al*., 1999). Estimation of total flavonoids: The total flavonoids were determined by the aluminium chloride method (Rajendra *et al*., 2014)**.** Estimation of Terpenoids: The total terpenoids in the plant extract was determined by (Ghorai *et al*., 2012)**.** Estimation of Alkaloids: The total alkaloid content in the sample was estimated by 1,10- phenanthroline method (Singh *et al*., 2017).

2.4. Gas chromatography mass spectroscopy (GC MS analysis)

GC–MS analysis was carried out to categorize the volatile and semi-volatile compounds present in the plant extracts. The analysis was performed for the leaf, stem, bark, and root extracts of *P. vettiveroides* and *K. pinnata*. The substances were identified based on their mass spectra and comparison with the NIST library 2023, The GC–MS analysis was performed using a Shimadzu QP2010S GC–MS system. The identified compounds were subsequently recorded based on the molecular characteristics, retention time, and spectral matching.

2.5. Anti-bacterial Activity

The anti-bacterial studies were conducted by agar well-diffusion technique for selected strains of Gram +ve bacteria i.e., *Bacillus cereus* (NCIM 2155), *Staphylococcus aureus* (NCIM 5718) and Gram-negative bacteria i.e., *Escherichia coli* (NCIM 2345), *Pseudomonas aeruginosa* (NCIM 5029) (Hassan and Ullah, 2019) were selected as test organisms. The sterile nutrient agar was poured on to the petri-plates and allowed for solidification. Different concentrations of plant samples i.e. 25, 50, 75 & 100 µL were left for the determination of clear zones with selected bacterial strains of test organisms and compared with positive control Antibiotic Ciprofloxacin (Sarkar *et al*., 2007; Mounyr *et al*., 2016) and distilled water as negative control. Experiments were performed in triplicates.

3. Statistical analysis

The trials were conducted in three replications and are expressed in mean ± SD. The results were calculated using one-way ANOVA followed by Duncan's multiple range tests using SPSS software, and $p \leq 0.05$ were considered statistically significant.

4. RESULTS AND DISCUSSION

4.1. Phytochemical analysis

Qualitative phytochemical screening of methanolic extracts of *P. vettiveroides* (PVL, PVS, PVR) and *K. pinnata* (KPL, KPB, KPR) revealed the presence of alkaloids in all samples, while least concentration of tannins and saponins were detected in PVL. Whereas, the lowest concentration of flavonoids, phenols, terpenoids, and steroids was noticed in PVR (Table 3). Studies by Wakchoure *et al*. (2022) and Ashalatha *et al*. (2019), observed the presence of major phytoconstituents such as steroids, carbohydrates, proteins, tannins, flavonoids and alkaloids in methanolic leaf and root extracts of *Kigelia pinnata* and *Hemidesmus indicus*.

All the extracts were further subjected to quantitative estimation of alkaloids, flavonoids, phenols and terpenoids (Harborne, 1998). PVL exhibited the highest alkaloid content (13.44 mg/mL), followed by PVS and KPL, signifying considerable potential for biological activity, whereas the maximum flavonoid content was recorded in PVS (4.03 mg/mL), compared to PVL and KPL, suggesting considerable antioxidant capacity. While KPB and KPR were found to be rich in phenolic content (1.75 and 1.27 mg/mL) and terpenoid content (15.7 and 13.2 mg/mL) as related to other samples, indicative of possible anticancer, antibacterial, and anti-inflammatory potential (Fig. 1a & b). Our results highlight the diverse phytochemical strengths inherent in these plant extracts.

Table 1: Qualitative analysis of root, stem, bark and leaf of *P. vettiveroides* and *K. pinnata*

Phytochemicals→ Sample↓	*Alkaloids*	*Flavonoids*	*Phenols*	*Tannins*	*Terpenoids*	*Saponins*	*Steroids*
PVL	+++	++	++	+	++	+	++
PVS	+++	+++	++	++	++	++	++
PVR	+++	+	+	+++	+	++	+
KPL	+++	++	+++	++	++	+	++
KPB	+++	+++	+++	+++	+++	++	+++
KPR	+++	+++	+++	+++	+++	++	+++

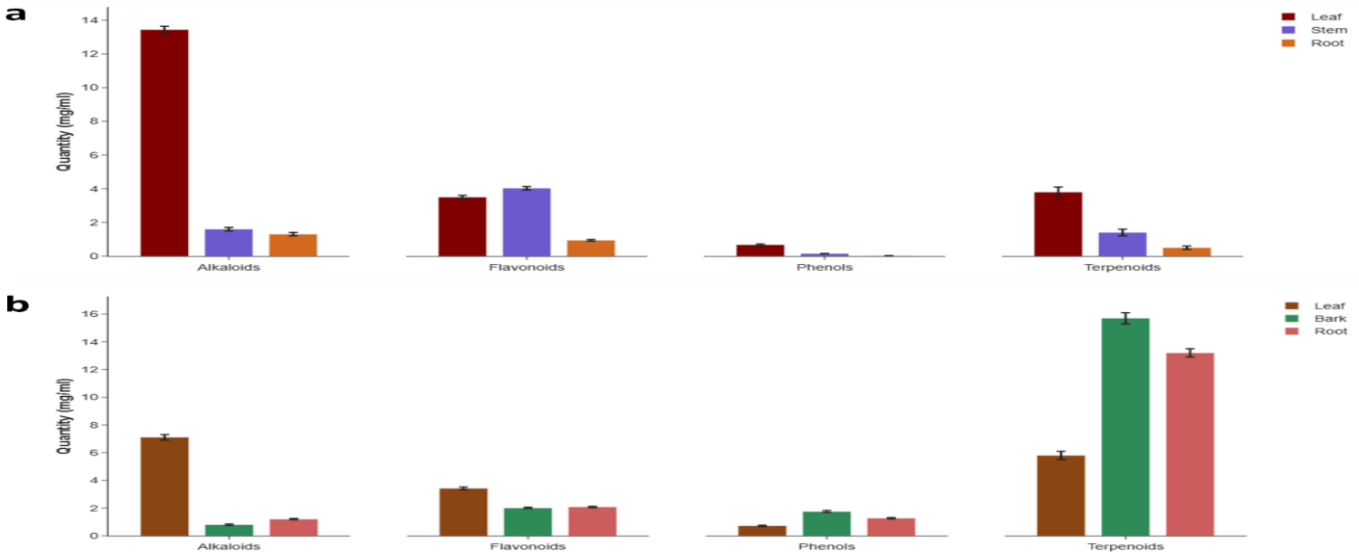


Fig.1: Quantitative analysis of root, stem, bark and leaf of a) *P. vettiveroides* and b) *K. pinnata*

4.2. GC-MS analysis

GC-MS analysis of the methanolic extracts revealed distinct and tissue-specific phytochemical profiles. In *P. vettiveroides*, the root and stem extracts were found to be rich in triterpenoid and phytosterol constituents, predominantly the cycloartane triterpene 9,19-cyclolanost-25-ene-3,24-diol (21.8%), lupeol acetate (8.3%), stigmasterol (5.8%) and 1-glyceryl ricinoleate (7.6%). The leaf extract (PVL), in contrast, exhibited a comparatively weaker phytochemical signal, with 3,7,11,15-tetramethyl-2-hexadecen-1-ol (10.0%), phenol (4.9%) and a TMS-derivatised roburic acid (8.4%) as the major identifiable constituents (Table 2, 3, & 4; Fig. 2 A, B & C). In *K. pinnata*, the leaf extract (KPL) displayed a lipid profile characteristic of chlorophyll turnover, dominated by phytyl linoleate (76.9%) and a stigmastane-type phytosterol oleate (11.9%), whereas the bark and root extracts (KPB and KPR) were markedly rich in hydroxy-fatty-acid and oxylipin constituents, principally ricinoleic acid (39.8%) and its lactone (Z)-18-octadec-9-enolide (19.9%), together with isomers of linoleic acid (~24% combined), the close resemblance between The two chromatograms indicating a shared lipid pool in these tissues (Table 5, 6 & 7; Fig. 3 A, B & C). Hassin *et al*. (2016), also reported various compounds in ethanol leaf extract of *K. africana*. with a similarity index (SI) of ≥85, including ricinoleic acid, phytyl linoleate, caryophyllene, and lupeol acetate.

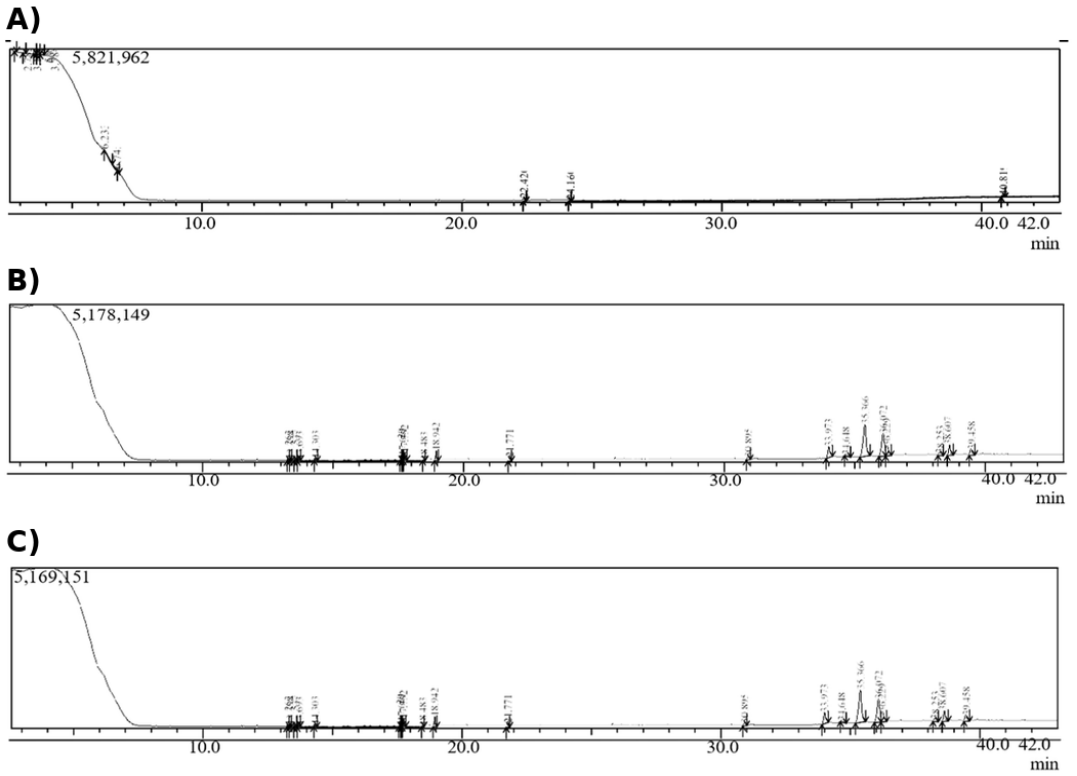


Fig. 2: Chromatograph of methanolic extract of P. vettiveroides by GC- MS A) leaf B) Stem and C) Root

Table 2: Phytoconstituents identified from methanolic leaf extract of *P. vettiveroides* by GC-MS analysis

Peak#	R. Time	Area	Area%	Similarity	Base m/z	CAS#	Name
1	3.536	485506	20.12	95	73.05	68-12-2	Formamide, N,N-dimethyl-
2	6.233	823624	34.13	90	40.00	463-49-0	Allene
3	22.420	240187	9.95	94	95.05	102608-53-	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
4	40.819	203038	8.41	78	218.15	0-00-0	Roburic acid, TMS

Table 3: Phytoconstituents identified from methanolic stem extract of *P. vettiveroides* by GC-MS analysis

Peak#	R. Time	Area	Area%	Similarity	Base m/z	CAS#	Name
1	13.533	253945	1.04	95	93.05	87-44-5	Caryophyllene
2	17.732	823040	3.33	86	91.05	137695-18-	(E)-2-((8R,8aS)-8,8a-Dimethyl-3,4,6,7,8,8a-hexahydro-1,3,4,6,7,8,8a-hexahydro-1,
3	18.942	984246	3.99	85	131.10	61847-19-4	4a,5-Dimethyl-3-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydro-1,2,3,4,4a,5,6,7-octahydronaphthyl
4	21.771	429281	1.74	67	98.05	19407-28-4	Phenanthrene, 1,2,3,4,4a,9,10,10a-octahydro-1,1,4a-trimethyl-
5	30.895	356545	1.44	86	55.05	213738-77-	Glycidyl palmitoleate
6	33.973	186978	7.57	83	55.05	141-08-2	1-Glyceryl ricinoleate
7	34.648	287432	1.16	66	143.10	0-00-0	Dihydromonolin dicasitate
8	35.366	8095816	32.79	84	109.10	0-00-0	6 beta.Bicyclo[4.3.0]nonane, 5 beta.-iodomethyl-1.beta.-isopr
9	36.072	5375020	21.77	83	81.10	0-00-0	9,19-Cyclolanost-25-ene-3,24-diol
10	36.229	2047363	8.29	85	109.10	1617-68-1	Lup-20(29)-en-3-ol, acetate, (3 beta.)-
11	38.607	1424787	5.77	82	83.10	83-48-7	Stigmasterol

Table 4: Phytoconstituents identified from methanolic root extract of *P. vettiveroides* by GC-MS analysis

Peak#	R. Time	Area	Area%	Similarity	Base m/z	CAS#	Name
1	13.533	253945	1.04	95	93.05	87-44-5	Caryophyllene
2	17.732	823040	3.33	86	91.05	137695-18-0	(E)-2-((8R,8aS)-8,8a-Dimethyl-3,4,6,7,8,8a-hexahydro-1,3,4,6,7,8,8a-hexahydro-1,
3	18.942	984246	3.99	85	131.10	61847-19-4	4a,5-Dimethyl-3-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydro-1,2,3,4,4a,5,6,7-octahydronaphthyl
4	21.771	429281	1.74	67	98.05	19407-28-4	Phenanthrene, 1,2,3,4,4a,9,10,10a-octahydro-1,1,4a-trimethyl-
5	30.895	356545	1.44	86	55.05	213738-77-7	Glycidyl palmitoleate
6	33.973	186978	7.57	83	55.05	141-08-2	1-Glyceryl ricinoleate
7	34.648	287432	1.16	66	143.10	0-00-0	Dihydromonolin dicasitate
8	35.366	8095816	32.79	84	109.10	0-00-0	6 beta.Bicyclo[4.3.0]nonane, 5 beta.-iodomethyl-1.beta.-isopr
9	36.072	5375020	21.77	83	81.10	0-00-0	9,19-Cyclolanost-25-ene-3,24-diol
10	36.229	2047363	8.29	85	109.10	1617-68-1	Lup-20(29)-en-3-ol, acetate, (3 beta.)-
11	38.607	1424787	5.77	82	83.10	83-48-7	Stigmasterol

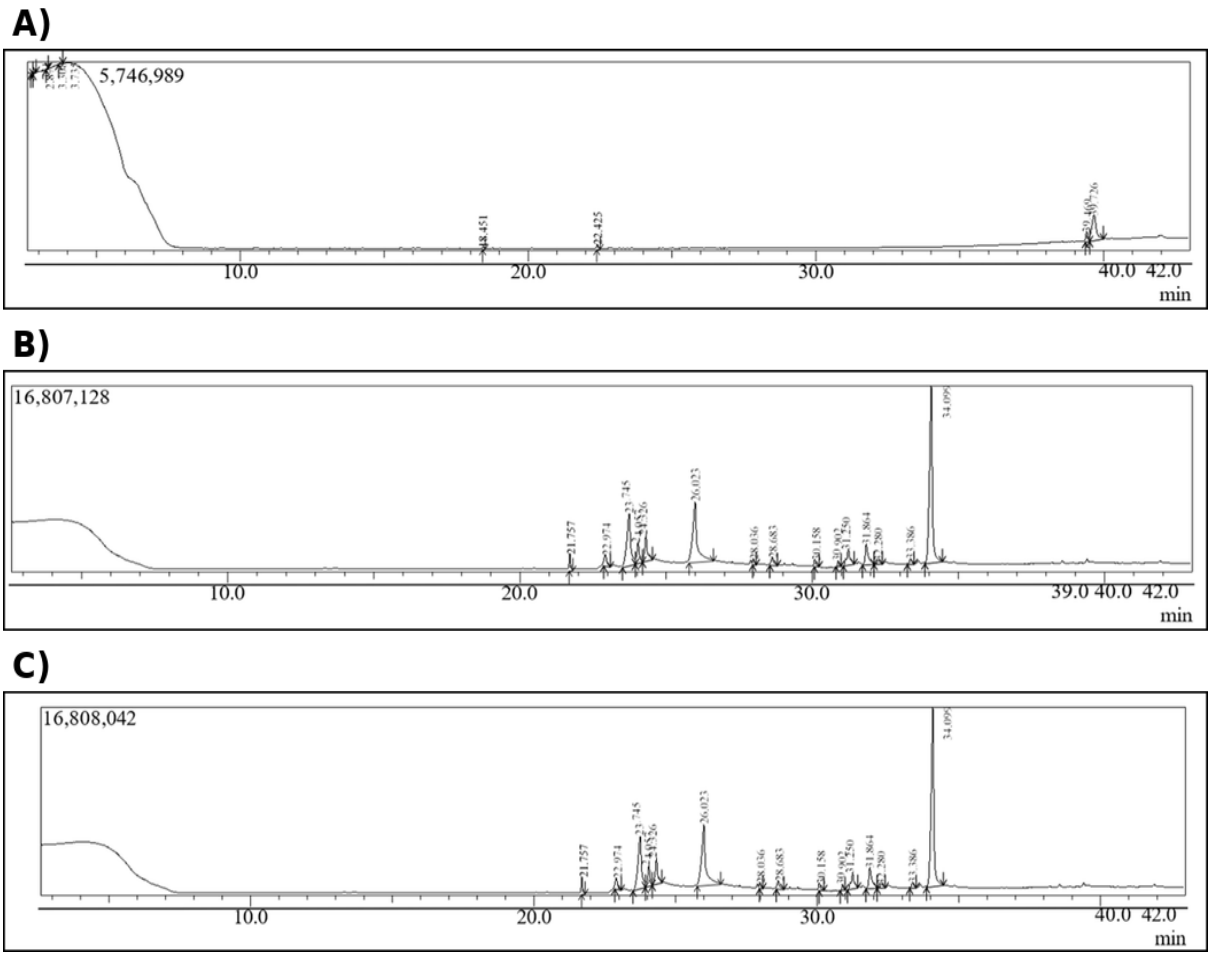


Fig. 3: Chromatograph of methanolic extract of K. pinnata by GC-MS A) leaf B) Bark and C) Root

Table 5: Phytoconstituents identified from methanolic leaf extract of *K. pinnata* by GC-MS analysis

Peak#	R.Time	Area	Area%	Similarity	Base m/z	CAS#	Name
1	22.425	372170	3.63	95	81.05	102608-53	- 3,7,11,15-Tetramethyl-2-hexadecen-1-ol
2	39.469	1221217	11.90	80	145.10	0-00-0	Stigmast-5-en-3-ol, oleate
3	39.726	7892240	76.91	85	67.05	53950-59-7	Phytyl linoleate

Table 6: Phytoconstituents identified from methanolic bark extract of *K. pinnata* by GC-MS analysis

Peak#	R.Time	Area	Area%	Similarity	Base m/z	CAS#	Name
1	21.758	4224745	1.53	96	98.05	127062-51	- 13-Hexyloxacyclotridec-10-en-2-one
2	23.975	5039883	1.84	92	67.05	60-33-3	9,12-Octadecadienoic acid (Z,Z)-
3	23.745	41553257	15.08	92	67.05	544-71-8	(9E,11E)-Octadecadienoic acid
4	24.057	11457272	4.16	93	67.05	544-71-8	(9E,11E)-Octadecadienoic acid
5	24.326	13899701	5.04	93	67.05	544-71-8	(9E,11E)-Octadecadienoic acid
6	26.024	54814382	19.9	89	55.05	80060-76-0	(Z)-18-Octadec-9-enolide
7	28.683	3366886	1.22	88	67.05	2277-28-3	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester
8	31.250	9624845	3.49	89	67.05	3443-82-1	9,12-Octadecadienoic acid
							(Z,Z)-, 2-hydroxy-1-(hydroxymeth
9	31.864	12637746	4.59	89	67.05	2277-28-3	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester
10	34.099	109725239	39.82	82	98.05	141-22-0	Ricinoleic acid

Table 7: Phytoconstituents identified from methanolic root extract of *K. pinnata* by GC-MS analysis

Peak#	R.Time	Area	Area%	Similarity	Base m/z	CAS#	Name
1	21.757	4224745	1.53	96	98.05	127062-51	- 13-Hexyloxacyclotridec-10-en-2-one
2	22.974	5039882	1.83	91	67.05	60-33-3	9,12-Octadecadienoic acid (Z,Z)-
3	23.745	41553257	15.08	92	67.05	544-71-8	(9E,11E)-Octadecadienoic acid
4	24.057	11457272	4.16	93	67.05	544-71-8	(9E,11E)-Octadecadienoic acid
5	24.326	13899701	5.04	93	67.05	544-71-8	(9E,11E)-Octadecadienoic acid
6	26.023	54814380	19.89	88	55.05	80060-76-0	(Z)-18-Octadec-9-enolide
7	28.683	3366886	1.22	88	67.05	2277-28-3	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester
8	31.250	9624845	3.49	89	67.05	3443-82-1	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymeth
9	31.864	12637746	4.59	89	67.05	2277-28-3	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester
10	34.099	109725239	39.82	82	98.05	141-22-0	Ricinoleic acid

4.2. **Anti-bacterial studies**

The *P. vettiveroides* and K. pinnata extracts showed concentration-dependent activity across all the tested bacterial strains. In *P. vettiveroides*, the leaf extract exhibited the significant activity against Bacillus cereus (10.17 mm at 100 µL), followed by Pseudomonas aeruginosa (5.00 mm) and Staphylococcus aureus (4.43 mm), with comparatively lower efficacy against Escherichia coli (4.07 mm). While the stem extract showed only mild activity against S. aureus (2.35 mm), and the root extract acted exclusively against B. cereus (6.57 mm), with no inhibition recorded against the remaining strains (Plate 1; Fig. 4). Whereas in K. pinnata extracts, the bark extract exhibited the highest potential activity with maximum zones of inhibition against B. cereus (11.23 mm), P. aeruginosa (10.47 mm), and E. coli (8.63 mm), followed by the leaf extract (B. cereus – 10.04, E. coli – 8.93 and P. aeruginosa - 7.15 mm), respectively (Plate 3 & 4). The root extract exhibited the weakest activity of all the samples, being only marginally effective against B. cereus (5.36 mm) and negligible against the remaining bacteria (Plate 2; Fig. 4). Similar results were reported by Jeyachandran and Mahesh (2007), where the maximum inhibition zone was observed in the methanolic bark extract of *K. africana.* Our results revealed that bark and leaf extracts of K. pinnata, along with the leaf extract of *P. vettiveroides*, showed potent antibacterial activity, suggesting the role of potent phytoconstituents present in these plant parts.

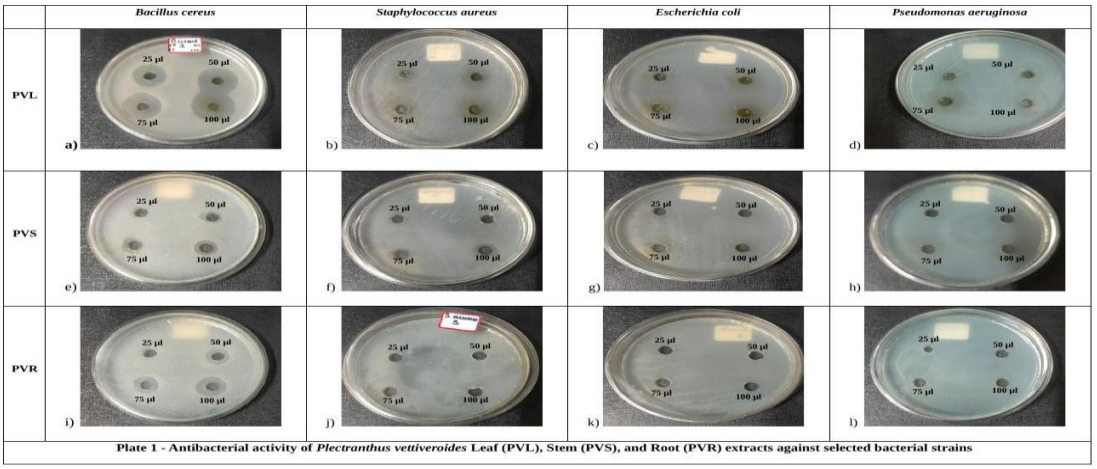


Plate 1 - Antibacterial activity of *Plectranthus vettiveroides* Leaf (PVL), Stem (PVS), and Root (PVR) extracts against selected bacterial strains

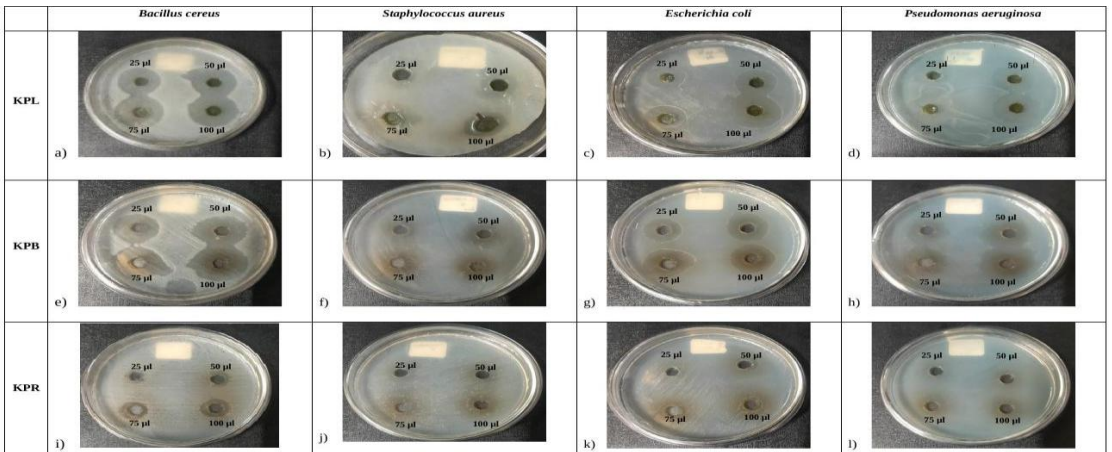


Plate 2 - Antibacterial activity of *Kigelia pinnata* Leaf (KPL), Bark (KPB), and Root (KPR) extracts against selected bacterial strains

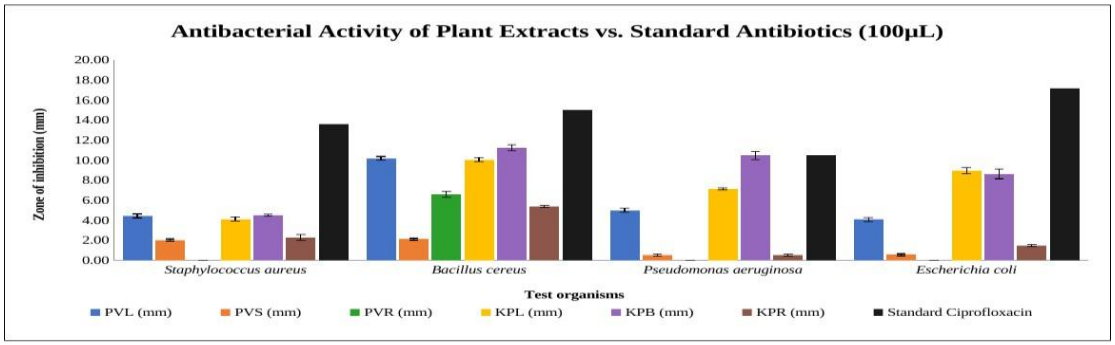


Fig 4: Antibacterial activity of *P. vettiveroides, K. pinnata* extracts and standard against selected bacterial strains

CONCLUSION

The present study reveals a comprehensive phytochemical and pharmacological evaluation of *P. vettiveroides* and *K. pinnata* as a valuable source of pharmacologically related bioactive compounds. The phytochemical screening revealed distinct metabolite distribution patterns with plant extract of *P. vettiveroides* and *K. pinnata*, recording the maximum secondary metabolites. GC-MS studies further revealed a diverse array of triterpenoids, phytosterols, and fatty-acid derivatives. The leaf and root extracts of *P. vettiveroides* and the bark and leaf extracts of *K. pinnata* revealed the most potent antibacterial activity, against *Bacillus cereus* and *Escherichia coli*, due to the synergistic effect of phytochemicals. Our results showed broad-spectrum anti-bacterial potential, thus lending scientific support to the traditional therapeutic practices of these plants. Further studies on the active phytoconstituents and in vivo validation are needed to elucidate their therapeutic potential.

Declaration

The author declares no conflict of interest

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