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Qualitative and quantitative phytochemistry and antimicrobial activity of *Blumea membranacea* DC *and Blumea oxyodonta* DC

Neha P Kopare, Dipak K Koche
Department of Botany, Shri Shivaji College of Arts, Commerce and Science, Akola, Maharashtra, India

Abstract
Blumea membranacea DC. and *Blumea oxyodonta* DC. (Asteraceae) are traditionally used medicinal plants; however, systematic scientific validation of their phytochemical composition and antimicrobial potential remains limited. The present investigation aimed to qualitatively and quantitatively characterize the phytochemical constituents of root, stem, leaf, and inflorescence and to evaluate the antimicrobial activity of leaf and inflorescence extracts. Plant materials were extracted using a Soxhlet apparatus with solvents of increasing polarity (n-hexane, ethanol, methanol, and distilled water). Preliminary phytochemical screening revealed the presence of phenolics, tannins, alkaloids, flavonoids, cardiac glycosides, coumarins, phytosterols, and saponins, while carbohydrates, proteins, and fatty acids were detected in trace amounts. Methanolic extracts exhibited the highest extraction efficiency and were therefore selected for quantitative estimation and antimicrobial assays. Quantitative analysis demonstrated significantly higher concentrations of alkaloids, phenolics, flavonoids, tannins, and coumarins in leaf tissues compared with roots, stems, and inflorescences. Antibacterial activity of methanolic leaf extracts was evaluated using the agar well diffusion method against selected Gram-positive and Gram-negative bacterial strains, revealing pronounced inhibitory effects, whereas no appreciable antifungal activity was observed against *Aspergillus niger* and *Candida albicans*. Inflorescence extracts exhibited comparatively weak antibacterial activity and lacked antifungal effects. Gentamicin and ciprofloxacin were used as standard antibacterial controls, while itraconazole and amphotericin B served as antifungal standards. Minimum inhibitory concentration (MIC) assays confirmed significant antibacterial efficacy at low extract concentrations. Collectively, these findings substantiate the ethnomedicinal relevance of *B. membranacea* and *B. oxyodonta* and highlight their potential as promising sources of bioactive compounds for the development of novel antimicrobial agents.

Keywords: Coumarins, cardiac glycosides, flavonoids, alkaloid, MIC

Introduction

Traditional medicinal plants constitute a vital component of primary healthcare systems worldwide and continue to serve as indispensable sources of therapeutic agents. According to the World Health Organization, a substantial proportion of the global population relies on plant-based remedies for the prevention and treatment of diseases. Medicinal plants are rich reservoirs of structurally diverse bioactive compounds, including alkaloids, phenolics, flavonoids, tannins, terpenoids, and glycosides, which exhibit a wide range of pharmacological properties. The isolation, characterization, and biological evaluation of these phytoconstituents provide a scientific basis for the development of novel, safe, and effective drug molecules, thereby bridging traditional knowledge with modern pharmaceutical research (Caius, 1986 and Warrier *et al*, 1996) [21].

The family Asteraceae (Compositae) is one of the largest families of angiosperms, comprising more than 32,000 species distributed across approximately 1,900 genera. Members of this family are widely recognized for their medicinal, nutritional, and ecological significance. The genus *Blumea*, belonging to this family, encompasses nearly 80 species, many of which are extensively employed in traditional medicinal systems. In Ayurvedic literature, *Blumea* species are described as bitter, astringent, acrid, thermogenic, anti-inflammatory, styptic, ophthalmic, digestive, anthelmintic, hepatoprotective, expectorant, febrifuge, antipyretic, diuretic, deobstruent, and stimulant in nature, highlighting their broad therapeutic spectrum (Cock *et al*., 2017 and Anand *et al*., 2019) [3, 8].

Blumea membranacea DC. and *Blumea oxyodonta* DC. are two medicinally important species traditionally used by rural and tribal communities for the management of various ailments. In ethnomedicinal practices, these plants are commonly employed for the treatment of infections, inflammatory conditions, gastrointestinal disorders, and febrile illnesses. Such traditional knowledge, preserved by indigenous and ethnic groups with limited access to modern healthcare facilities, underscores the critical role of medicinal plants in sustaining community health and wellbeing (Anand *et al*., 2019) [3].

The emergence and rapid spread of antimicrobial resistance have intensified the global demand for alternative therapeutic agents, particularly those derived from natural sources. Plant-based antimicrobials represent a largely unexplored reservoir of novel bioactive molecules with significant therapeutic potential. Several studies have demonstrated that phytochemicals not only exhibit potent antimicrobial activity but also reduce the adverse effects and resistance associated with conventional synthetic antibiotics. In this context, species of the genus *Blumea*, including *B. balsamifera* and *B. lacera*, have been scientifically validated for their antimicrobial properties and phytochemical richness, particularly with respect to alkaloids, flavonoids, tannins, and cardiac glycosides (Jahan *et al*., 2014 and Ismail *et al*., 2022) [10, 11].

Despite their ethnomedicinal relevance, comprehensive phytochemical profiling and antimicrobial evaluation of *B. membranacea* and *B. oxyodonta* remain scarce. Considering their traditional usage and the promising pharmacological attributes reported in related *Blumea* species, it is hypothesized that these plants may harbor diverse bioactive

compounds with significant antimicrobial potential, including novel chemical entities. Therefore, the present study was undertaken to systematically investigate the qualitative and quantitative phytochemical composition of different plant parts and to evaluate the antimicrobial efficacy of leaf and inflorescence extracts, thereby providing a robust scientific foundation for their potential pharmaceutical applications.

a. b.

Fig 1: Morphology of a. *Blumea membranacea* and b. *Blumea oxyodonta*

Material and methods

Collection of plant materials

The plant *Blumea membranacea* was collected from forest like area at Lava Dhaba Road, Nagpur and *Blumea oxyodonta* was collected from Botanic Garden of department of botany RTMNU Nagpur. The plants were identified by using flora of Maharashtra (Singh and Karthikeyan, 2000) and was confirmed by Dr. N.M. Dongarwar, Professor and Head of the Department of Botany, RTMNU Nagpur. The habit of both the plant species is presented in fig. 1 (a and b).

Preparation of extracts

Collected plants were washed thoroughly under tap water, root, stem leaf and inflorescence was separated and dried in shade. After drying, fine powder of root, stem, leaf and inflorescence was made separately and packed in Ziplock bags and labelled.

Crude sample extract was prepared by Soxhlet extraction method. About 15 g of powdered sample material was packed into a thimble and extracted with 250 mL of different solvents n-hexane, Acetone, Methanol and distilled water separately.

The process of extraction has to be continued for 24 hrs or till the solvent in siphon tube of extractor become colourless. After the extract was evaporated till volume becomes 100 ml and stored in airtight amber bottles for future use in refrigerator at 4°C.

Qualitative Phytochemical screening

Preliminary phytochemical analysis was carried out for *Blumea membranacea and Blumea oxyodonta* root, stem leaf and inflorescence's n-hexane, acetone, methanol and distilled water extracts as per standard methods ((Harborne, 1973, Koche *et al*., 2010 and Sahira Banu *et al*. 2015) [9, 13, 15].

Quantitative analysis of Major Phytochemicals:

Alkaloid

Total alkaloids in the plant samples was estimated by method adopted from (Ajanal *et al*., 2012) [1] with some modification. For alkaloid quantification, 10 mL of 2N HCl was added to the 10 ml of plant extract (1 mg/mL) and the mixture was transferred in separating funnel and washed with 10 mL of chloroform. The washing process was repeated three times. Later the pH of the washed sample in HCl was brought to neutral with 0.1 N NaOH. Now the sample (1 mg/mL) is ready for analysis. 1 mL of this sample was added to the separating funnel followed by addition of 5 mL of phosphate buffer (71.6 gm Na_2HPO_4) in 1 L distilled water and pH was adjusted to pH 4.7 with 0.2 M citric acid) and 5 mL of 0.03% BCG. The mixture was vigorously shaken and then 1 mL of chloroform was added, again the mixture was shaken and the lower chloroform layer containing alkaloid was collected in test tube. Then the mixture is again added with 2 mL, 3 mL and 4 mL of chloroform and the lower chloroform layer was collected in the same test tube. The volume of collected chloroform layer was made up to 10 mL with chloroform and the absorbance of the sample was noted at 470 nm. The similar procedure was repeated with standard atropine (0.1mg/ml working stock solution) with different concentrations (5, 10, 15, 20, 25, 30 up to 50 µg) and the final quantification of alkaloid was done from the graph equation ($Y=0.0029x+0.0008$, $R^2=0.9962$).

Phenol

Total Phenol Content (TPC) was estimated by Folin-ciocalteu method (Ordonez *et al*., 2006; Chandran and Indira, 2016) [7]. In brief 2.5 mL of 10% Folin-ciocalteu reagent and 2 mL of 7.5% sodium carbonate were added to 100 µg of extract. The reaction mixture was incubated at 45°C for 45 minutes and the blue coloured

phosphomolybdic/phosphotungstic acid complex was measured at 760 nm. The similar procedure was repeated with standard Gallic acid (0.1mg/ml working stock solution) with different concentrations (5, 10, 15, 20, 25, 30 up to 50 µg) and the final quantification of phenol was done from the graph equation (Y=0.0195x+0.0912, R2=0.9968) and presented as mg GAE/g of extract.

Flavonoid

Total Flavonoid Content (TFC) was determined by aluminium chloride method (Wolfe *et al*., 2003; Chandran and Indira, 2015) [23] with slight modification. 200 µL of 5% sodium nitrite was added to 100 µg of extract and allowed to react for 5 minutes. 200 µL of 10% aluminium chloride was added to the mixture and after 5 minutes, 2 mL of 1M NaOH was added and the absorbance of the orange-red aluminium complex was taken at 510 nm.

The similar procedure was repeated with standard quercetin (0.1mg/ml working stock solution) with different concentrations (5, 10, 15, 20, 25, 30 up to 50 µg) and the final quantification of flavonoid was done from the graph equation (0.0011x) and presented as mg QE/g of extract.

Coumarins

Total Coumarins Content (TCC) was determined by (Buragohain R. (2015) [5]. method. 500 µL of plant extract, 200 mL distilled water and 500 µL lead acetate (5% w/v) solution were added in a test tube. After shaking thoroughly 7 mL distilled water was added and mixing well. 2 mL of this solution was taken in another test tube and 8 mL of 0.1 M (v/v) HCl solution was added. The solution was kept for 30 minutes at room temperature and absorbance was recorded at 320 nm using UV-Spectrophotometer. The similar procedure was repeated with standard coumarin (0.1mg/ml working stock solution) with different concentrations (5, 10, 15, 20, 25, 30 up to 50 µg) and the final quantification of coumarin was done from the graph equation (Y=0.0102x+0.0006, R2=0.9984). The TCC was expressed as mg of Coumarins equivalent/g of sample extract (mg CE/g).

Tannins

The tannins content was determined by Folin-Ciocalteu method (Chandran K and Indira G 2016) [7] with some modification. In brief, 500µl of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin Ciocalteu phenol reagent, 1 ml of 35% sodium carbonate solution and was diluted to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 minutes. Formation of blue colour due the reduction of tungsto molybdic acid by tannin compound in alkaline medium. Absorbance of the blue coloured solution was measured at 700nm. The similar procedure was repeated with standard Tannic acid (0.1mg/ml working stock solution) with different concentrations (5, 10, 15, 20, 25, 30 up to 50 µg) and the final quantification of tannin was done from the graph equation (Y=0.0033x+0.0129, R2=0.9982). The TTC was expressed as mg of tannic acid equivalent/g of sample extract (mg TAE/g).

Determination of Antimicrobial activity

Antibacterial activity

The Antibacterial activity was performed by Kirby-Baur well diffusion method (Bauer et.al.,1966 and) [4]. 24 hours freshly grown (0.5 McFarland turbidity Standard) cultures of S. *aureus* (Gram positive), E. *coli (*Gram negative), *Pseudomonas aeruginosa* (Gram negative), *serratia marcescens (*Gram negative), *Klebsiella pneumoniae* (Gram negative) in nutrient broth medium were inoculated by lawn culture technique on sterile solidified Muller Hinton Agar (HIMEDIA-M173) plates with the help of sterile cotton swabs.

Leaf and inflorescence extract *of Blumea membranacea* and *Blumea oxyodonta* were taken for study. The stock solutions of different concentrations from 0 to 200 mg/ml were made in DMSO 25µl of different concentration in each well. Wells were bored on the MHA media plates with the help of sterile cork borer. Each well loaded with solution of following concentration 25µg, 50µg, 100µg respectively for leaf extract and higher concentration 312.5 µg, 625 µg,1250 µg,2500 µg,5000 µg for inflorescence. Then allowed to diffuse at room temperature for 2-3 hours. The plates were incubated in the upright position at 37 °C for 24 hours. Then the zone of inhibition was observed.

The control plates were kept in which DMSO was added as negative control and Antibiotic disc as a positive control Gentamicin 10µg and ciprofloxacine 3µg was used.

Antifungal activity

The Antifungal activity of Leaf and inflorescence extracts *of Blumea membranacea* and *Blumea oxyodonta* was performed by Kirby-Baur well diffusion method (Bauer et.al.,1966) [4]. 24 hours freshly grown (0.5 McFarland turbidity Standard) cultures of Strain Strain CA15-*Candida albicans*, AS10-*Aspergillus niger* in Potato dextrose broth medium were inoculated by lawn culture technique on sterile solidified Potato Dextrose Agar (Himedia – MH096) plates with the help of sterile cotton swabs.

The stock solutions of concentration 5mg/ml were prepared in DMSO solvent. Three wells were bored on the MHA media plates with the help of sterile cork borer. Each well loaded with solution following concentration, 25µl, 50µl,100µl respectively for leaf extract and higher concentration 312.5 µg, 625 µg,1250 µg,2500 µg,5000 µg for inflorescence. Then allowed to diffuse at room temperature for 2-3 hours. The plates were incubated in the upright position at 37 °C for 48 hours. Then the zone of inhibition was observed.

The control plates were kept in which DMSO was added as negative control and Antifungal disc of Itraconazole 30 mcg and Amphotericin B (300µg) were used as positive control For *Candida albicans* and *Aspergillus niger.*

Determination of MIC (Minimum inhibitory concentration)

MIC is defined as the lowest concentration of the antimicrobial agent that inhibits the microbial growth after 24 hours of incubation was studied with some modified method from (Wiegand et. al. 2008) [22].

In the present study sample was made as concentration 5mg/ml i. e, 5000µg/1000µl. Here the seven concentrations were tested for MIC as 175µg, 150µg, 125µg, 100µg,75µg, 50µg and 25µg were tested. In control set bacterial growth was used as standard pre grown on nutrient broth and whose O. D. taken as standard and if the final bacterial/fungal growth O.D. is less than the original O.D. of control bacterial/ fungal growth noted as MIC in µl concentration.

Results

Qualitative Phytochemical Analysis

The qualitative phytochemical screening of acetone, methanol, and distilled water extracts of root, stem, leaf, and

inflorescence revealed the differential distribution of various secondary metabolites in different plant parts (Table-1).

Alkaloids were detected in all plant parts, with methanolic extracts exhibiting stronger reactions compared to acetone and aqueous extracts. Leaf and inflorescence tissues showed comparatively higher alkaloid abundance, as evidenced by pronounced positive responses in Mayer's and Wagner's tests, whereas Dragendorff's test confirmed alkaloid presence predominantly in leaf extracts. Root and stem exhibited moderate alkaloid content, indicating differential metabolite distribution among tissues.

Flavonoids were consistently detected in all plant parts across all solvent extracts, as indicated by strong positive reactions in the alkaline reagent test. The absence of positive reactions in the ammonia test suggests the predominance of specific flavonoid subclasses. Notably, leaves and inflorescences demonstrated higher flavonoid abundance relative to roots and stems.

Saponins were detected exclusively in methanolic extracts of root and stem, whereas leaf and inflorescence extracts showed negative responses, indicating tissue-specific accumulation and solvent-dependent extractability of these compounds.

Protein presence was variable among tissues and solvents. Ninhydrin and biuret tests revealed trace to moderate protein content, predominantly in leaf and inflorescence methanolic extracts, while roots and stems exhibited comparatively lower protein levels.

Tannins exhibited strong positive reactions, particularly in leaf and inflorescence methanolic extracts, as evidenced by intense coloration in both Braymer's and gelatin tests. Roots and stems displayed weak or negative responses, indicating lower tannin concentration in these tissues.

Phenolics were prominently detected in methanolic and acetone extracts of leaves and inflorescences, with intense blue–green coloration in the ferric chloride test, confirming high phenolic abundance. In contrast, root and stem extracts showed weak or negative reactions, suggesting limited phenolic accumulation in underground and structural tissues.

Coumarins were detected in acetone and methanolic extracts of all plant parts, with relatively stronger responses observed in leaves and inflorescences, highlighting their enrichment in reproductive and photosynthetic tissues.

Carbohydrates were primarily detected in methanolic and aqueous extracts, particularly in leaves and inflorescences, as indicated by positive Benedict's test results, reflecting active metabolic and photosynthetic processes.

Oils and Fats were detected only in acetone and methanolic extracts of inflorescences, suggesting localized accumulation in reproductive tissues.

Cardiac glycosides were detected in all plant parts, with strong positive reactions particularly in root and stem methanolic extracts. Anthraquinone glycosides were predominantly detected in leaf and inflorescence tissues, as confirmed by Borntrager's and sulfuric acid tests, indicating functional specialization in secondary metabolite biosynthesis.

Phytosterols were abundantly detected in acetone and methanolic extracts of all plant parts, with particularly strong reactions in roots and inflorescences, suggesting their structural and regulatory significance.

Table 1: shows Preliminary Phytochemical Screening of *Blumea membranacea*

Sr. No.	Phytochemical Evaluation for	Name of Test	Root			Stem			Leaf			Inflorescence		
			A	M	DW	A	M	DW	A	M	DW	A	M	DW
1	Alkaloids	Mayers test	-	+	+	+	+	+	+	++	+	+	+	+
		Wagners test	+	+	-	-	+	-	++	++	+	++	++	+
		Dragendroffs test	-	-	-	-	-	-	+	++	-	-	+	-
2	Flavonoids	Alkaline reagent test	++	++	+	+	+	+	+	+	+	+	+	+
		Ammonia test	-	-	-	-	-	-	-	-	-	-	-	-
3	Saponins	Foam test	-	+	-	-	+	-	-	-	-	-	-	-
4	Proteins	Ninhydrin test	-	-	-	-	-	-	-	+	-	-	+	-
		Biuret test	++	-	+	+	-	+	-	-	+	+	-	-
5	Tannins	Braymers test	-	-	-	-	-	-	+	+++	+	+	+++	+
		Gelatin test	+	++	+	++	++	+	+	+++	+	+	++	+
6	Phenols	Ferric chloride test	-	++	-	-	++	-	+++	+++	++	+++	++	+
7	Caumarins	Buragohain test	+	++	-	+	+	-	+	+	-	+	+	-
8	Carbohydrates	Benedict test	-	+	++	-	+	-	-	++	++	+	+++	+
9	Oils and fat	Spot test	-	-	-	-	-	-	-	-	-	+	+	-
10	Cardiac Glycosides	Keller killani Test	+++	++	-	++	+	-	+	+	-	+	+	-
11	Anthraquinone	Borntragers test	-	+	-	-	-	-	+	+	-	+	+	-
		Sulphuric acid test	+++	+++	-	++	++	-	+	++	-	++	+	-
12	Phytosterol	Salkowaski test	++	+++	-	++	++	-	+	+	-	++	++	-

(**Abbreviations:** A- Acetone, M- Methanol, DW- Distilled water)

Table 2: shows Preliminary Phytochemical Screening of *Blumea oxyodonta*

Sr. No.	Phytochemical Evaluation for	Name of Test	Root			Stem			Leaf			Inflorescence		
			A	M	DW	A	M	DW	A	M	DW	A	M	DW
1	Alkaloids	Mayers test	-	+	+	+	+	+	+	++	+	+	+	+
		Wagners test	-	+	-	+	+	-	++	++	-	++	++	-
		Dragendroffs test	-	-	-	-	+	-	+	++	-	-	+	-
2	Flavonoids	Alkaline reagent test	-	+	-	+	+	+	+	+	+	+	+	+
		Ammonia test	+	++	+	-	-	-	-	-	-	+	++	-
3	Saponins	Froth test	-	-	-	-	-	-	--	-	-	-	-	-
4	Proteins	Ninhydrin test	-	-	-	-	-	-	+	++	-	-	-	-

		Biuret test	-	+	-	++	+	+	+	++	-	+	+	+
5	Tannins	Braymers test	-	-	-	-	+	-	+	+++	+	-	+++	-
		Gelatin test	+	+	-	+	+++	+	+	+++	+	+	+++	+
6	Phenols	Ferric chloride test	-	+	-	+	+	-	+++	+++	+	+	+++	-
7	Caumarins	Buragohain test	-	+	-	+	+	-	+	+	-	+	+	-
8	Carbohydrates	Benedict test	-	+	-	-	+	-	-	+	+	+	++	+
9	Oils and fat	Spot test	-	-	-	-	-	-	-			+	+	-
10	Cardiac glycosides	Keller killani Test	+++	+	-	+	+	-	+	++	-	+	++	-
11	Anthraquinone	Borntragers test	++	+++	-	+	+	-	+	+	-	+	+	-
		Sulphuric acid test	++	+	-	++	++	-	++	+++	-	+++	+++	-
12	Phytosterol	Salkowaski test	+++	++	-	++	++	-	-	+	-	++	++	-

(**Abbreviations:** A- Acetone, M- Methanol, DW- Distilled water)

Table 2 is showing the preliminary phytochemical analysis of B. oxydonta. The details are presented below.

Alkaloids were variably present in all parts, with stronger reactions generally observed in methanolic extracts, particularly in the leaf and inflorescence, indicating a higher accumulation of alkaloids in aerial parts. Acetone and aqueous extracts showed comparatively weaker or negative reactions.

Flavonoids showed prominent presence in methanolic extracts of all plant parts, while acetone and aqueous extracts exhibited moderate to weak reactions. The leaf and inflorescence displayed comparatively higher flavonoid content, suggesting their richness in these compounds.

Saponins were found to be absent in all the tested extracts of root, stem, leaf, and inflorescence, indicating their negligible or complete absence in the studied plant material.

Proteins exhibited variable presence, with positive reactions mainly in methanolic extracts, especially in the leaf. The biuret test showed stronger reactions than the ninhydrin test, suggesting moderate protein content in aerial parts.

Tannins were strongly detected in leaf and inflorescence, particularly in methanolic extracts, as evidenced by intense reactions in Braymer's and gelatine tests. Root and stem showed comparatively lower tannin content.

Phenolic compounds were prominently present in leaf and inflorescence, especially in methanol extracts, showing strong positive reactions with ferric chloride test, indicating high phenolic content in these parts.

Coumarins were moderately present in all plant parts, with relatively stronger reactions in methanolic extracts of stem, leaf, and inflorescence.

Carbohydrates were detected mainly in methanolic and aqueous extracts of leaf and inflorescence, whereas root and stem exhibited weak or negative reactions, indicating higher carbohydrate accumulation in reproductive and photosynthetic tissues.

Oils and fats were largely absent in root and stem, while a weak to moderate presence was recorded in the leaf and inflorescence, suggesting limited lipid content in the plant.

Cardiac glycosides showed strong positive reactions in root acetone extract and moderate reactions in methanolic extracts of stem, leaf, and inflorescence, indicating their presence across different plant parts.

Anthraquinones were strongly detected in root extracts, particularly in methanol, and moderately present in stem, leaf, and inflorescence, suggesting a higher concentration in underground parts.

Phytosterols were prominently present in root and stem extracts, especially in acetone and methanol, while leaf and inflorescence exhibited comparatively weaker reactions.

Qualitative phytochemical screening of acetone, methanol, and aqueous extracts of root, stem, leaf, and inflorescence of the plants revealed a diverse spectrum of secondary metabolites with marked tissue-specific and solvent-dependent variations (Table 1.1 and 1.2).

From the above results, methanolic extracts demonstrated the highest diversity and intensity of phytochemical constituents, followed by acetone extracts, while aqueous extracts showed comparatively lower detection. Among plant parts, the leaf and inflorescence were found to be richer in secondary metabolites, particularly flavonoids, tannins, phenols, and alkaloids, indicating their potential medicinal and pharmacological significance.

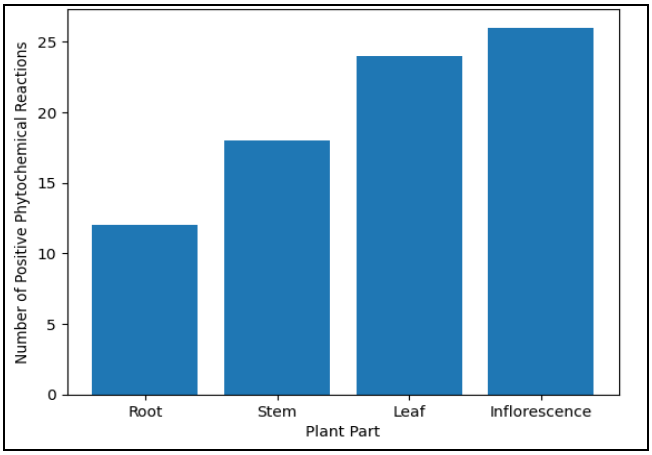


Fig 2: Comparative phytochemical distribution in different plant parts of *Blumea membranacea* and *B. oxydonta*

The qualitative phytochemical profiling demonstrated that methanol was the most efficient solvent, followed by acetone, for the extraction of diverse secondary metabolites. Leaves and inflorescences exhibited greater phytochemical diversity and abundance compared to roots and stems, highlighting their metabolic activity and therapeutic relevance. The pronounced presence of alkaloids, phenolics, flavonoids, tannins, coumarins, and glycosides in leaf tissues supports their selection for subsequent quantitative analysis and antimicrobial assays (Table- 1, 2 and Fig. 2).

Quantitative Phytochemical Analysis

The quantitative estimation of major phytochemicals in different plant parts (root, stem, leaf, and inflorescence) of *Blumea membranacea* and *Blumea oxyodonta* revealed significant variation in the distribution and concentration of secondary metabolites among species as well as among plant organs (Table 3 and 4).

Alkaloids

In *B. membranacea*, alkaloid content ranged from 13.86 ± 0.69 to 17.31 ± 0.91, with the highest concentration recorded in the root (17.31 ± 0.91), followed by leaf (17.19

± 1.30) and stem (16.16 ± 1.55), while the lowest was observed in the inflorescence (13.86 ± 0.69). In contrast, *B. oxyodonta* exhibited comparatively higher alkaloid levels in the leaf (22.94 ± 1.74), followed by inflorescence (15.93 ± 1.24), stem (15.47 ± 1.21), and root (14.78 ± 0.72). Overall, *B. oxyodonta* showed a higher accumulation of alkaloids, particularly in the leaf.

Phenolics

A markedly higher phenolic content was recorded in *B. oxyodonta* compared to *B. membranacea*. In *B. membranacea*, phenolic content ranged from 10.49 ± 4.15 (stem) to 103.66 ± 3.13 (leaf), with leaf and inflorescence showing notably higher values (103.66 ± 3.13 and 95.28 ± 5.77, respectively). In *B. oxyodonta*, phenolics ranged from 39.89 ± 2.24 (stem) to 115.79 ± 3.09 (leaf), with leaf (115.79 ± 3.09) and inflorescence (80.58 ± 1.07) being the richest sources. This indicates that aerial parts, particularly leaves, are major reservoirs of phenolic compounds in both species.

Flavonoids

Flavonoid content followed a similar trend, with *B. oxyodonta* showing consistently higher values. In *B. membranacea*, flavonoids ranged from 13.36 ± 0.91 (stem) to 48.81 ± 2.40 (leaf), while in *B. oxyodonta*, they varied from 25.79 ± 7.62 (stem) to 82.45 ± 0.91 (leaf). The highest flavonoid concentration was observed in the leaves of *B. oxyodonta*, followed by inflorescence, highlighting their pharmacological potential.

Tannins

In *B. membranacea*, tannin content ranged from 6.93 ± 1.39 (root) to 34.20 ± 3.85 (leaf), with comparatively higher levels in leaf and inflorescence. Similarly, *B. oxyodonta* exhibited maximum tannin content in the leaf (30.77 ± 0.69), followed by inflorescence (17.03 ± 4.20), stem (12.59 ± 1.26), and root (10.16 ± 0.69). Both species demonstrated a preferential accumulation of tannins in aerial parts.

Coumarins

Coumarin content in *B. membranacea* ranged from 6.81 ± 0.11 (stem) to 20.73 ± 0.11 (inflorescence), whereas in *B. oxyodonta*, it varied from 10.73 ± 0.11 (stem) to 30.79 ± 0.11 (leaf). Notably, *B. oxyodonta* exhibited substantially higher coumarin levels in both leaf and inflorescence, indicating a stronger biosynthetic capacity for coumarins compared to *B. membranacea*.

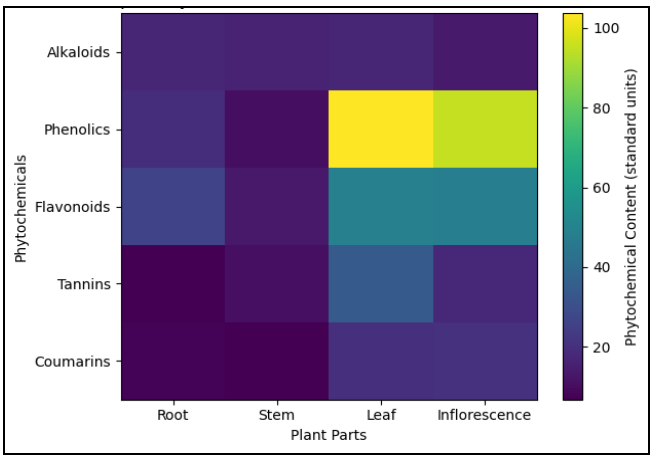


Fig 3: Heat map of Phytochemical distribution in *Blumea membranacea*

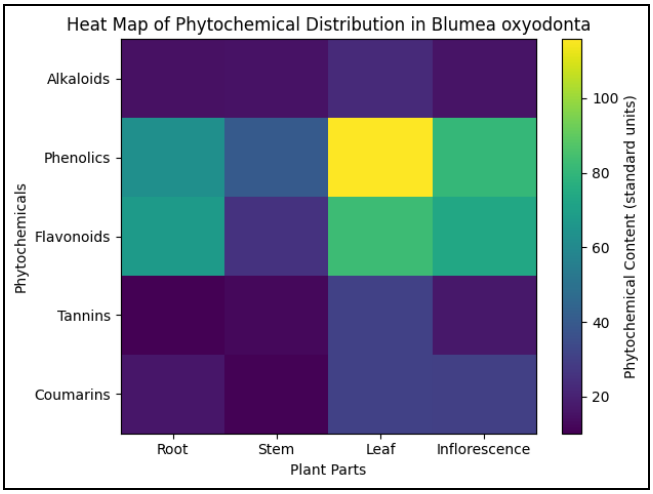


Fig 4: Heat map of Phytochemical distribution in *Blumea membranacea*

The comparative analysis clearly indicates that *Blumea oxyodonta* possesses a higher phytochemical richness than *Blumea membranacea*, particularly in terms of phenolics, flavonoids, tannins, and coumarins. Among the plant parts, leaf and inflorescence consistently showed maximum accumulation of phytochemicals, suggesting that these organs are the most metabolically active and pharmacologically important. The high levels of phenolics and flavonoids further indicate a strong antioxidant potential, especially in *B. oxyodonta* leaves (Fig. 3 and 4).

Antimicrobial Analysis

Table 3 presents the antimicrobial activity of methanolic leaf extracts of *Blumea membranacea* and *Blumea oxyodonta* against selected Gram-negative and Gram-positive bacteria, as well as two fungal strains, evaluated in terms of zone of inhibition (mm). Gentamicin (10 µg) and itraconazole (30 µg) served as positive controls for antibacterial and antifungal activity, respectively, while DMSO was used as the negative control.

The methanolic leaf extract of *Blumea membranacea* exhibited moderate to appreciable antibacterial activity against all tested bacterial strains. The highest inhibitory effect was observed against *Staphylococcus aureus* (Gram-positive), with a zone of inhibition of 18 ± 0 mm at 125 µg, indicating strong sensitivity of this organism to the extract. Among Gram-negative bacteria, *Klebsiella pneumoniae* showed a zone of 18 ± 0.70 mm at 500 µg, followed by *Escherichia coli* with 15 ± 0 mm at 250 µg and *Pseudomonas aeruginosa* with 14 ± 0 mm at 250 µg. Although the activity of the extract was lower than that of the standard antibiotic gentamicin, which produced zones ranging from 13 to 32 mm, the results clearly demonstrate the broad-spectrum antibacterial potential of *B. membranacea* leaf extract.

Similarly, the methanolic leaf extract of *Blumea oxyodonta* showed notable antibacterial activity, particularly against *Escherichia coli*, which recorded the highest zone of inhibition of 18 ± 0.35 mm at 500 µg. The extract also showed considerable activity against *Staphylococcus aureus* (19 ± 0 mm at 250 µg) and *Pseudomonas aeruginosa* (16 ± 0 mm at 500 µg). In contrast, *Klebsiella pneumoniae* exhibited comparatively lower sensitivity, with a zone of 15 ± 0 mm at 125 µg. Overall, *B. oxyodonta* demonstrated slightly higher antibacterial efficacy than *B. membranacea*, particularly against *E. coli* and *S. aureus*.

In contrast to their antibacterial effects, both plant extracts failed to exhibit antifungal activity against *Aspergillus niger* and *Candida albicans*, as indicated by the absence of any inhibition zones. The positive control itraconazole showed clear inhibition zones (11–12 mm), confirming the validity of the assay, while DMSO did not produce any inhibitory effect, validating its role as a negative control.

Table 3: shows the antimicrobial activity (zone of inhibition in mm) of methanolic extracts from *Blumea membranacea leaf* and *Blumea oxyodonta* leaf

	Blumea membranacea leaf		***Blumea oxyodonta* leaf**	
Bacteria / Fungi	Effective amount in microgram	Average Zone at Effective Amount (in mm)	Effective amount in microgram	Average Zone at Effective Amount (in mm)
Escherichia coli (Gram -ve)	Gentamicin 10 µg	13 ± 0	Gentamicin 10 µg	13± 0
	DMSO	NI	DMSO	NI
	LE 250 µg	15 ± 0	LE 500 µg	18±0.35
Pseudomonas aeruginosa (Gram -ve)	Gentamicin 10 µg	16 ± 0	Gentamicin 10 µg	16 ± 0
	DMSO	NI	DMSO	NI
	LE 250 µg	14 ± 0	LE 500 µg	16 ± 0
Klebsiella pneumoniae (Gram -ve)	Gentamicin 10 µg	25 ± 0	Gentamicin 10 µg	25 ± 0
	DMSO	NI	DMSO	NI
	LE 500 µg	18±0.70	LE 125 µg	15 ± 0
Staphylococcus aureus (Gram +ve)	Gentamicin 10 µg	32 ± 0	Gentamicin 10 µg	32 ± 0
	5% DMSO	NI	5% DMSO	NI
	LE 125 µg	18 ± 0	LE 250 µg	19 ± 0
Aspergillus niger	Itraconazole 30 µg	12 ± 0	Itraconazole 30 µg	12 ± 0
	DMSO	NI	DMSO	NI
	LE	NI	LE	NI
Candida albicans	Itraconazole 30 µg	11 ± 0	Itraconazole 30 µg	11 ± 0
	DMSO	NI	DMSO	NI
	LE	NI	LE	NI

(+ve control- Gentamicin, -ve control - DMSO, +ve control- Itraconazole, Abbreviation: - LE- Leaf extract, NI- No inhibition)

The observed antibacterial activity of both *Blumea* species may be attributed to the presence of bioactive phytochemicals such as alkaloids, flavonoids, phenolics, and terpenoids, which are known to disrupt microbial cell walls, interfere with enzyme systems, and inhibit nucleic acid synthesis. The comparatively higher susceptibility of *Staphylococcus aureus* suggests that Gram-positive bacteria are more sensitive to these extracts, possibly due to differences in cell wall structure.

The findings indicate that methanolic leaf extracts of *B. membranacea* and *B. oxyodonta* possess promising antibacterial properties, while their antifungal potential appears negligible under the tested conditions (Table 3 and 4). These results support the traditional use of *Blumea* species in treating bacterial infections and suggest their potential as sources of novel antibacterial agents.

Table 4 summarizes the antimicrobial activity of methanolic inflorescence extracts of *Blumea membranacea* and *Blumea oxyodonta* against selected Gram-negative and Gram-positive bacterial strains, as well as two fungal pathogens, expressed as zone of inhibition (mm). Ciprofloxacin (3 µg) and amphotericin B (300 µg) were used as positive controls for antibacterial and antifungal assays, respectively, while DMSO served as the negative control.

The methanolic inflorescence extracts of both *B. membranacea* and *B. oxyodonta* exhibited limited antibacterial activity. No inhibitory effect was observed against *Escherichia coli* and *Pseudomonas aeruginosa*, indicating complete resistance of these strains to the inflorescence extracts at the tested concentrations. In contrast, a weak inhibitory response was recorded against *Serratia marcescens* and *Staphylococcus aureus*. The inflorescence extract of *B. membranacea* produced a zone of inhibition of 9.33 ± 0.57 mm against *S. marcescens* at 2500 µg and 9 ± 0 mm against *S. aureus* at 1250 µg. Similarly, the inflorescence extract of *B. oxyodonta* exhibited inhibition zones of 6 ± 5.1 mm against *S. marcescens* at 2500 µg and 9 ± 0 mm against *S. aureus* at 1250 µg. These inhibition zones are considerably lower than those produced by the standard antibiotic ciprofloxacin (26–29 mm), indicating relatively weak antibacterial potency of the inflorescence extracts.

Table 4: shows the antimicrobial activity (zone of inhibition in mm) of methanolic extracts from *Blumea membranacea* inflorescence and *Blumea oxyodonta* inflorescence

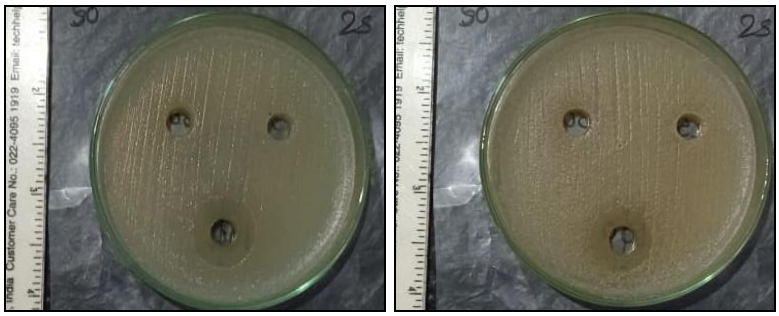
	Blumea membranacea inflorescence		***Blumea oxyodonta* inflorescence**	
Bacteria / Fungi	Effectiveamountin microgram	Average Zone at Effective Amount (in mm)	Effective amount in microgram	Average Zone at Effective Amount (in mm)
Escherichia coli (Gram -ve)	Ciprofloxacin 3 µg	28 ± 0	Ciprofloxacin 3 µg	28 ± 0
	DMSO	NI	DMSO	NI
	IE	NI	IE	NI
Pseudomonas aeruginosa (Gram -ve)	Ciprofloxacin 3 µg	29 ± 0	Ciprofloxacin 3 µg	29 ± 0
	DMSO	NI	DMSO	NI
	IE	NI	IE	NI
Serratia marcescens (Gram -ve)	Ciprofloxacin 3 µg	28 ± 0	Ciprofloxacin 3 µg	28 ± 0
	DMSO	NI	DMSO	NI

	IE 2500 µg	9.33 ±0.57	IE 2500 µg	6 ± 5.1
Staphylococcus aureus (Gram +ve)	Ciprofloxacin 3 µg	26 ± 0	Ciprofloxacin 3 µg	26 ± 0
	DMSO	NI	DMSO	NI
	IE 1250 µg	9 ± 0	IE 1250 µg	9 ± 0
Aspergillus niger	Amphotericin B 300 µg	18 ± 0	Amphotericin B 300 µg	18 ± 0
	DMSO	NI	DMSO	NI
	IE	NI	IE	NI
Candida albicans	Amphotericin B 300 µg	12 ± 0	Amphotericin B 300 µg	12 ± 0
	DMSO	NI	DMSO	NI
	IE	NI	IE	NI

(+ve control- Ciprofloxacin, -ve control - DMSO, +ve control- Amphotericin B, Abbreviation: - IE- Inflorescence extract, NI- No inhibition)

Regarding antifungal activity, neither of the inflorescence extracts demonstrated inhibitory effects against *Aspergillus niger* or *Candida albicans*, as no zones of inhibition were detected. In contrast, the positive control amphotericin B showed clear and significant inhibition zones of 18 ± 0 mm against *A. niger* and 12 ± 0 mm against *C. albicans*, confirming the reliability of the experimental assay. The absence of activity in the extracts suggests that the bioactive constituents present in the inflorescence may not be effective against the tested fungal strains or may be present in insufficient concentrations (Fig. 5).

The results indicate that methanolic inflorescence extracts of *B. membranacea* and *B. oxyodonta* possess only weak antibacterial activity, limited to *Serratia marcescens* and *Staphylococcus aureus*, and lack antifungal potential under the experimental conditions. In comparison to the leaf extracts (Table 4), the inflorescence extracts exhibit markedly reduced antimicrobial efficacy, suggesting that antimicrobial phytoconstituents are more abundantly accumulated in the leaves than in the inflorescences. These findings highlight the importance of plant part selection in the exploration of plant-based antimicrobial agents.



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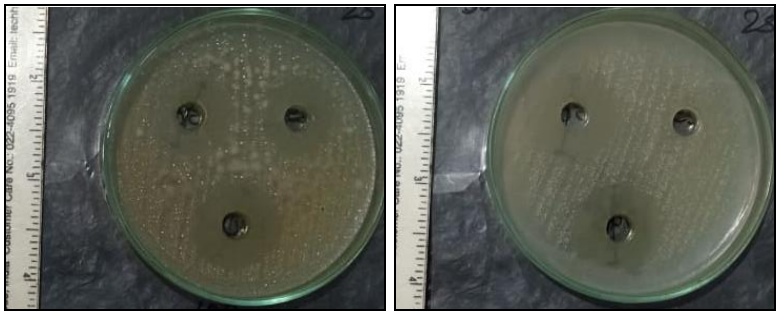

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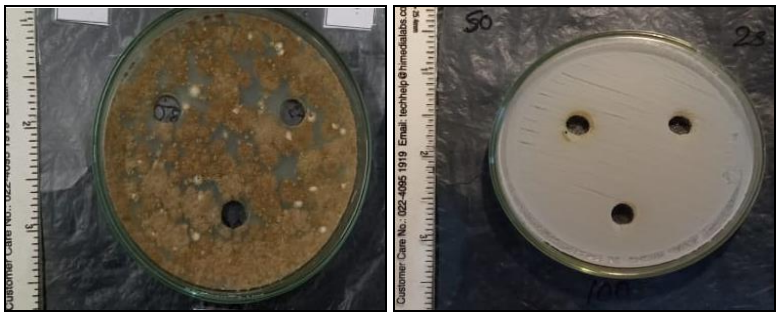

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Table 5: MIC *Blumea membranacea* leaf extract against bacteria and fungi

Concentra tion	Microbial (Fungal/ Bacterial) Strains/ Species					
	Staphylococcus aureus	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***	***Escherichia coli***	***Aspergillus Niger***	***Candida Albicans***
Control	3.587	1.522	1.749	1.731	1.484	1.761
(A)35µl	2.271	1.926	1.773	1.689	1.687	1.460

(175µg)						
(A)30µl (150 µg)	1.690	1.803	1.876	1.688	1.558	1.396
(A)25µl (125µg)	1.733	1.756	1.692	1.663	1.296	1.396
(A)20µl (100 µg)	1.427	1.660	1.608	1.612	1.459	1.236
(A)15µl (75 µg)	1.172	1.339	1.621	1.491	1.379	1.188
(A)10µl (50 µg)	0.980	1.179	1.490	1.076	1.161	1.320
(A)5µl (25 µg)	1.317	1.036	1.680	1.121	0.8745	1.210

Table 6: MIC *Blumea oxyodonta* leaf extract against bacteria and fungi

Concentra tion	Microbial (Fungal/ Bacterial) Strains/ Species					
	Staphylococcus aureus	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***	***Escherichia coli***	***Aspergillus Niger***	***Candida Albicans***
Control	2.6205	1.490	1.675	1.6675	1.3475	2.74825
(A)35µl (175µg)	0.938	1.362	0.968	1.607	0.806	1.104
(A)30µl (150 µg)	0.791	1.051	0.939	1.518	0.902	0.874
(A)25µl (125µg)	0.770	1.206	0.903	1.062	0.798	0.758
(A)20µl (100 µg)	0.734	0.922	0.872	0.928	0.704	0.686
(A)15µl (75 µg)	0.613	0.886	0.669	0.924	0.703	0.628
(A)10µl (50 µg)	0.474	0.778	0.654	0.835	0.585	0.616
(A)5µl (25 µg)	0.81	0.475	0.958	0.801	0.417	0.451

MIC showing good inhibition at lower concentration against bacteria and satisfactory inhibition against fungi, in well diffusion method inhibition was seen in very higher concentration against bacteria and no inhibition was seen against fungi (Table 5 and 6).

In well diffusion method it is possible that some chemical compounds may not diffuse through agar medium properly, MIC is performed in liquid (Broth) medium so it shows good antimicrobial activity.

In *Blumea membranacea,* the MIC (Minimum Inhibitory Concentration) value of leaf's extract against *Staphylococcus aureus* is 50µg concentration showing good inhibition compared to control, value against *Klebsiella pneumoniae* is 25µg concentration showing satisfactory inhibition compared to the control, value against *Pseudomonas aeruginosa* is 50µg concentration showing satisfactory inhibition compared to the control,) value against *Escherichia coli* is 25µg concentration showing satisfactory inhibition compared to the control, value against *Aspergillus Niger* is 25µg concentration showing good inhibition compared to the control, value against *Candida Albicans* is 75µg concentration showing satisfactory inhibition compared to the control.

In *Blumea oxyodonta*, the MIC (Minimum Inhibitory Concentration) value of leaf's extract against *K. pneumoniae* is 25µg concentration showing good inhibition compared compared to the control, value against *Pseudomonas aeruginosa* is 25µg concentration showing good inhibition compared to the control, value against *Staphylococcus aureus* is 50µg showing good inhibition concentration compared to the control, value against *Escherichia coli* is 25µg concentration showing good inhibition compared to the control,) value against *Aspergillus Niger* 25µg concentration showing good inhibition compared to the control, value against *Candida Albicans* is 25µg concentration showing good inhibition compared to the control.

Discussion

The present study evaluated the phytochemical composition and antimicrobial potential of *Blumea membranacea* and *Blumea oxyodonta*. The qualitative phytochemical screening revealed the presence of several secondary metabolites such as alkaloids, flavonoids, phenolics, tannins, coumarins, glycosides, phytosterols, carbohydrates, proteins, and lipids. These compounds were unevenly distributed among different plant parts and solvent extracts, indicating both tissue-specific accumulation and solvent-dependent extractability. Methanolic extracts generally exhibited stronger phytochemical reactions than acetone and aqueous extracts, which may be attributed to the higher polarity of methanol and its efficiency in extracting a wide range of bioactive metabolites from plant tissues. Similar findings have been reported for several Indian medicinal plants where methanol extracts contained the highest number of phytoconstituents and showed stronger biological activity (Pande and Tripathi, 2014 and Buragohain, 2015) [5].

Among the plant parts, leaves and inflorescences showed comparatively higher abundance of phytochemicals, particularly phenolics, flavonoids, and tannins. This observation suggests that aerial tissues act as primary sites of secondary metabolite biosynthesis and storage. Such patterns have also been reported in studies on Indian medicinal plants where leaves were found to contain higher

concentrations of phenolic and flavonoid compounds due to their protective role against environmental stress and microbial attack.

Quantitative phytochemical analysis further confirmed that *Blumea oxyodonta* possesses comparatively higher levels of phenolics, flavonoids, tannins, and coumarins than *Blumea membranacea*, particularly in the leaves. Phenolic and flavonoid compounds are widely recognized for their antioxidant and antimicrobial activities, as they can neutralize reactive oxygen species and inhibit microbial growth through disruption of cellular structures and enzyme systems. Studies on Indian medicinal plants have similarly demonstrated that high phenolic and flavonoid content is often associated with strong antioxidant and antimicrobial potential (Sulaiman *et al*., 2012, Shah *et al*., 2017 and Vaou *et al*., 2021) [16, 19, 20].

The antimicrobial assay revealed that methanolic leaf extracts of both species possess notable antibacterial activity against selected pathogenic bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. The extracts exhibited moderate to strong inhibition zones, although the activity was lower than that of standard antibiotics. The relatively higher susceptibility of *S. aureus* suggests that Gram-positive bacteria may be more sensitive to plant extracts due to the absence of an outer membrane barrier that is typically present in Gram-negative bacteria. The antibacterial activity observed in this study may be attributed to the synergistic action of phytochemicals such as alkaloids, phenolics, and flavonoids, which are known to disrupt microbial cell membranes and interfere with metabolic processes (Kapoor, 2021 and Soodoo *et al*., 2026) [12, 18].

In contrast, inflorescence extracts showed relatively weak antibacterial activity and no antifungal activity in the agar well diffusion method. However, the MIC assay demonstrated inhibition of microbial growth at comparatively lower concentrations in liquid medium. This difference may be due to the limited diffusion of certain phytochemicals in agar medium, whereas broth systems allow better interaction between antimicrobial compounds and microbial cells (Shah *et al*., 2016; Singh *et al*., 2016 and Amandine *et al*., 2025) [2, 17].

Conclusion

Present study concludes that the plant *Blumea membranacea* and *Blumea oxyodonta* both are rich in phytochemicals, and has potential antimicrobial activity. the comparative analysis indicates that *Blumea oxyodonta* possesses greater phytochemical richness and slightly stronger antimicrobial activity than *Blumea membranacea*. Furthermore, leaves were consistently found to be the most phytochemically active plant part. The higher accumulation of phenolics and flavonoids in leaf extracts may contribute significantly to their antibacterial activity. These findings support the traditional medicinal use of *Blumea* species and highlight their potential as natural sources of antimicrobial agents. Further research focusing on the isolation, characterization, and pharmacological evaluation of the active compounds is required to fully explore their therapeutic applications.

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Conflict of Interest

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Authors Contribution

DK has conceptualized the work; Experimental design, work and primary transcript was made by NK and Manuscript was reviewed by DK and both authors approved this version for publication in this journal.

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