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Preliminary Phytochemical and Antibacterial Studies of *Combretum ovalifolium* Roxb.

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ABSTRACT:
Objective: The present study aims were recognized for Pharmacological analysis and phytochemical screening of the medicinal plant *Combretum ovalifolium* Roxb.
Methods: As a portion of the pharmacognostic study the transverse section of the leaf was investigational and Histochemical localization studies were also done. The dried leaf powder was subjected to solvent extraction for preliminary secondary metabolites examination and a fluorescence study was done with the leaf powder.
Results: Phytochemical screening of *Combretum ovalifolium* Roxb. revealed the presence of steroids, triterpenoids, reducing sugars, sugars, alkaloids, phenolic compounds, flavonoids, catechins, saponins, tannins, and amino acids. Antibacterial activity was observed against six bacterial strains: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Enterococcus aerogenes*, and *Staphylococcus aureus*. Ethyl alcohol extracts from the leaf showed broad-spectrum activity, while stem, root, and bark extracts exhibited selective inhibition against various bacteria. These results support the potential use of *Combretum ovalifolium* as a natural antibacterial agent
Conclusion: This study will support the authentication and standardization of the plant *Combretum ovalifolium*. The present discoveries of this study show that the *Combretum ovalifolium*l eaves have the great latent to act as a source of positive drugs because of the presence of a diversity of secondary metabolites such as alkaloids, flavonoids, phenol, terpenoids, saponin, and carbohydrates. These phytoconstituents may act as a source of pharmacologically dynamic agents and also natural antioxidants.

Keywords: *Combretum ovalifolium*, Phytochemical Screening, Antibacterial Activity, Bioactive Compounds, Medicinal Plants

INTRODUCTION

From the very beginning of existence, people turned to nature and natural resources to keep themselves healthy. A healthy life is the most important wealth. Biological diversity is a foundation for human health. Biological diversity is the inconsistency among living organisms from all sources. This includes genetic diversity within species, diversity between species of fauna and flora, and the diversity of ecosystems.
Pharmacognosy is a significant relation between pharmacology and medicinal chemistry. It is a dynamic link between Ayurvedic and Allopathic systems of remedy. Pharmacognosy is the study of natural crops and their application in the enhancement of health. The opportunity of Pharmacognosy has extended from the outdated morphological description of plants and other organisms to include modern aspects of molecular science relating to the exploration of naturally occurring bioactive compounds, their mode of action, and eventually, their application in all viable and social activities. The search for new therapeutics was originally absorbed on plant species used in traditional medicine, but the growth of specific and sensitive bioassays and efficient methodologies for the isolation and structural determination of bioactive constituents has facilitated the high throughput screening of the enormous molecular diversity found in plants, microorganisms, and animals (Massuo Kato & John Pezzuto, 2000). New Pharmacognosy includes all aspects of drug development and discovery, where biotechnology-driven applications will play an important role (Kalpana Joshi *et al.*, 2004). Recent advancements in the field further emphasize the critical role of plant-based bioactive compounds in combating emerging diseases and promoting human health (Sharma *et al.*, 2024).

The identification of biologically active compounds is an important necessity for quality control and dosage determination of plant-based drugs. A medicinal herb can be regarded as a synthetic workshop as it produces and contains a quantity of chemical compounds. Those compounds which are responsible for the medicinal activity of the herb are the secondary metabolites. Ample phytochemical investigations of most of the medicinally important herbs of India have not been carried out so far. This would be valuable in the standardization and dosage determination of herbal drugs. Furthermore, there should be a quality control test for the entire preparation to ensure the quality of the drug (Dubey *et al.*, 2004). Current work has highlighted the potential roles of secondary products at the cellular level as plant growth regulators, modulators of gene expression, and in signal transduction (Kaufman *et al.*, 1999). Recent studies have also demonstrated that advancements in analytical techniques, such as high-throughput screening, are now enabling more efficient identification

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and characterization of bioactive compounds from medicinal plants, significantly enhancing the development of new therapeutic agents (Ghosh *et al*., 2023).

MATERIALS AND METHODS:

PLANT COLLECTION:

Combretum ovalifolium Roxb. was collected from Tirunelveli District. The mature and healthy plant was collected naturally from different locations after the rainy season (February, March and April). The different specimens were identified referring to the Flora of Presidency of Madras (Gamble, 1915-1936); Flora of Tamil Nadu Carnatic (Mathew, 1983), and Bulletin, Madras Government Museum (Gravely and Mayuranathan, 1931). Voucher specimens were documented in the herbarium of St. Xavier's College (Autonomous), Palayamkottai (XCH), Tamil Nadu, India.

Extraction of Plant Materials-Cold Extraction Method:

Shade-dried coarsely powdered material was extracted with different solvents and the extracts were used for the preliminary phytochemical screening.

Figure 1: Collection of Plant

PHYTOCHEMICAL ANALYSIS

Alkaloids - Mayer's Test

Reagents: 2N HCl, Mayer's reagent (potassium mercuric iodide solution). Procedure: Mix 1 mL of plant extract with 2N HCl. Add 1-2 drops of Mayer's reagent.

Steroids - Salkowski Test

Reagents: Acetic anhydride, chloroform, concentrated H_2SO_4. Procedure: Add 0.5 mL acetic anhydride to 1 mL of extract. Add chloroform and layer with concentrated H_2SO_4.

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Flavonoids - Shinoda Test
Reagents: Magnesium ribbon, concentrated HCl. Procedure: Add a small piece of magnesium ribbon to 2 mL extract. Add a few drops of concentrated HCl.
Tannins - Ferric Chloride Test
Reagents: 0.1% $FeCl_3$ solution. Procedure: Add a few drops of $FeCl_3$ solution to the aqueous extract.
Saponins - Foam Test
Reagents: Distilled water. Procedure: Shake 10 mL of plant extract vigorously. Observe froth formation.
Reducing Sugars - Fehling's Test
Reagents: Fehling A and Fehling B solutions. Procedure: Mix equal parts of Fehling A and Fehling B. Add the plant extract and heat.
Phenolics - Ferric Chloride Test
Reagents: 1% $FeCl_3$ solution. Procedure: Add a few drops of $FeCl_3$ solution to the alcoholic extract.
Anthraquinones - Borntrager's Test
Reagents: Benzene, ammonia. Procedure: Hydrolyze extract with dilute HCl. Extract with benzene and add ammonia.
Amino Acids - Ninhydrin Test
Reagents: 1% Ninhydrin solution. Procedure: Add a few drops of Ninhydrin solution to the extract. Heat gently.

RESULTS:
Table: 1.Preliminary Phytochemical Analysis of *Combretum ovalifolium* Roxb.

S No	Test for	Petroleum Ether	Benzene	Chloroform	Ethyl Alcohol	Distilled Water
1	Steroids	+	+	-	+	-
2	Triterpenoids	+	+	+	+	+
3	Reducing Sugars	-	+	+	+	-
4	Sugars	+	+	+	+	-
5	Alkaloids	-	-	+	-	-
6	Phenolic Compounds	-	-	-	+	+
7	Flavonoids	+	+	+	+	+
8	Catechins	+	-	+	+	+
9	Saponins	+	+	+	+	-
10	Tannins	+	+	-	+	-
11	Anthro quinones	-	-	-	-	-
12	Aminoacids	-	+	-	-	+

+Present, -Absent

Phytochemical Analysisof*Combretum ovalifolium* were analysed by extracts of different solvents like, Petroleum ether, benzene, chloroform, ethyl alcohol, and distilled water showed that different types of chemical constituents and the results are presented in (Table 1). Sugar, catechins, and saponins are predominantly present in leaf samples. Amino acids are present in the water extract and benzene extract of the plant. Tannins are present in petroleum ether, benzene, and ethanol extracts. Reducing sugars is observed in benzene, chloroform, and ethanol extracts of the plant. This compound has been shown to have immense significance as antihyperchole sterol, hypotensive and cardiac depressant properties (Trease and Evans, 1983 and Prince *et al.*, 1987). Hence, these plants could be suitable for these purposes. Plants rich in saponins 73 generally have immunity-boosting and anti-inflammatory properties (Kenner and Requena, 1996). Phenolics, alkaloids, terpenoids, and cardiac glycosides detected in the extracts and have been documented to possess medicinal properties and health-promoting effects (Okwu, 2004 and Liu, 2004). Earlier studies showed that the anti-dysenteric and anti-diarrheal properties of medicinal plants were due to tannins, alkaloids, saponins, flavonoids, and sterols (Galvez *et al.*, 1991Logang*aet al.*, 2000).These phytoconstituents may act as a source of pharmacologically active agents and also natural antioxidants. The present evaluation of various biochemical parameters will be helpful while standardizing the drug for its various pharmacological potentials and checking the adulteration in natural valuable drugs at the time of consumption for desired pharmacological effect.

Antibacterial studies in *Combretum ovalifolium* Roxb.
The antibacterial activity of various extracts of *Combretum ovalifolium* has been presented in Table: 4.36. The activity of the herbal decoction on the test organisms is not uniform.

A) Leaf
The petroleum ether leaf extracts do not show any activity against the bacterial strains *Pseudomonas aeruginosa* and *Enterococcus aerogenes.* Ethyl alcohol extract shows the broad spectrum of activity against all tested organisms. The

inhibition zones range from 3 mm to 18 mm at different extracts. Benzene extract exhibits an inhibition zone against *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Bacillus cereus, Enterococcus aerogenes,* and *Staphylococcus aureus.* The chloroform extract inhibits the growth of *Klebsiella pnuemoniae, Bacillus cereus, Enterococcus aerogenes,* and *Staphylococcus aureus.* (Plate: 1). The distilled water extracts overpower the growth of all the selected bacterial strains.

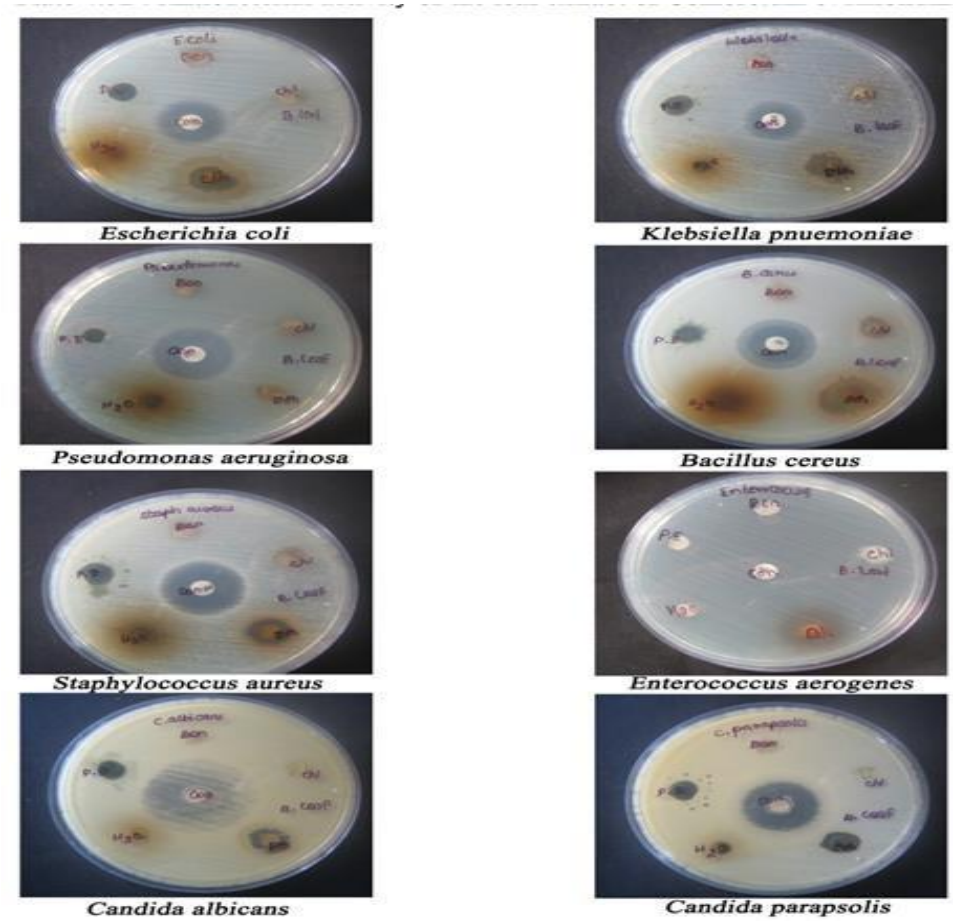


Plate: 1. Anitibacterial activity of Leaf extract of Combretum ovalifolium

B) Stem

The petroleum ether extract displays an inhibition zone against *Klebsiella pnuemoniae, Bacillus cereus, Enterococcus aerogenes* and *Staphylococcus aureus* selected strains only. The benzene extract displays an inhibition zone against one of the selected strains only. The chloroform extract shows an inhibition zone against *Staphylococcus aureus* and *Enterococcus aerogenes.* Ethyl alcohol extract is found to be the most effective among all the extracts. The inhibition zones range from 7 mm to 13 mm at different extracts (Plate: 2). In this investigation, the ethyl alcohol extract of the stem recorded significant antibacterial activities against all the tested bacterial strains. All the chosen organic extracts were found to be effective against *Staphylococcus aureus.*

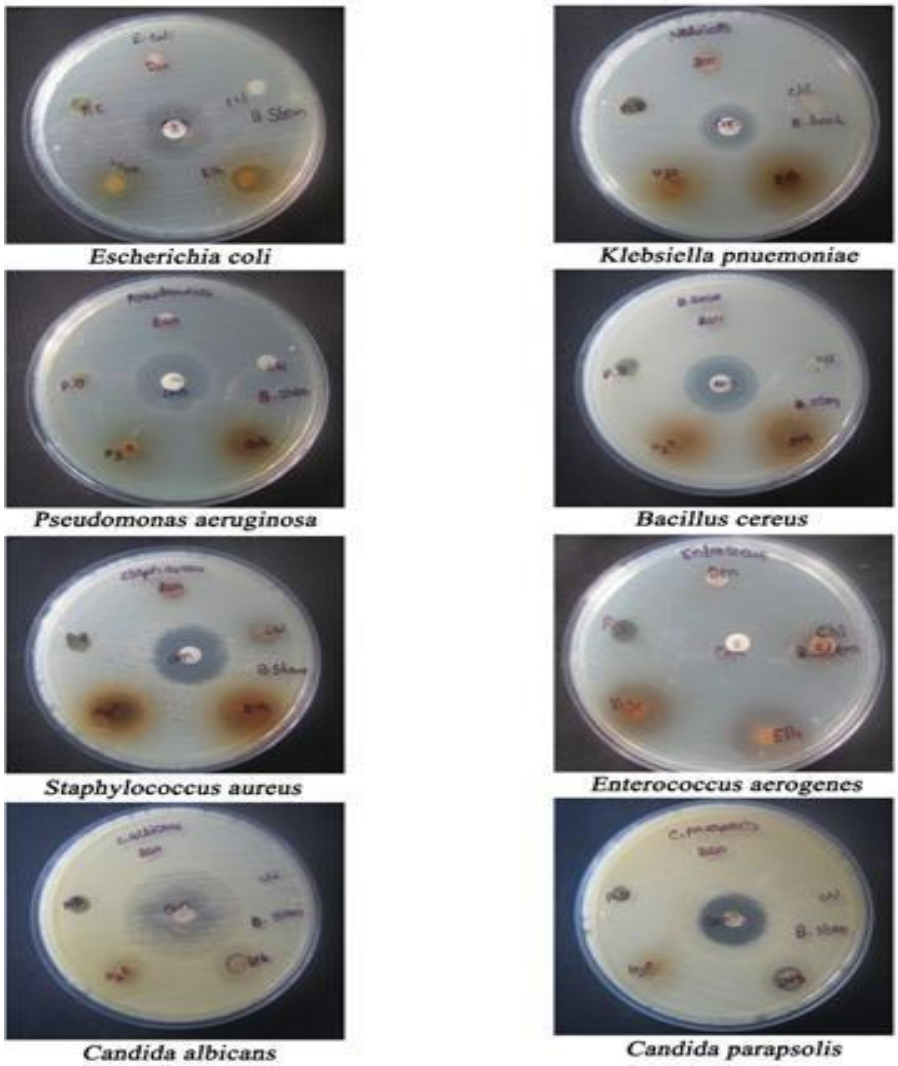
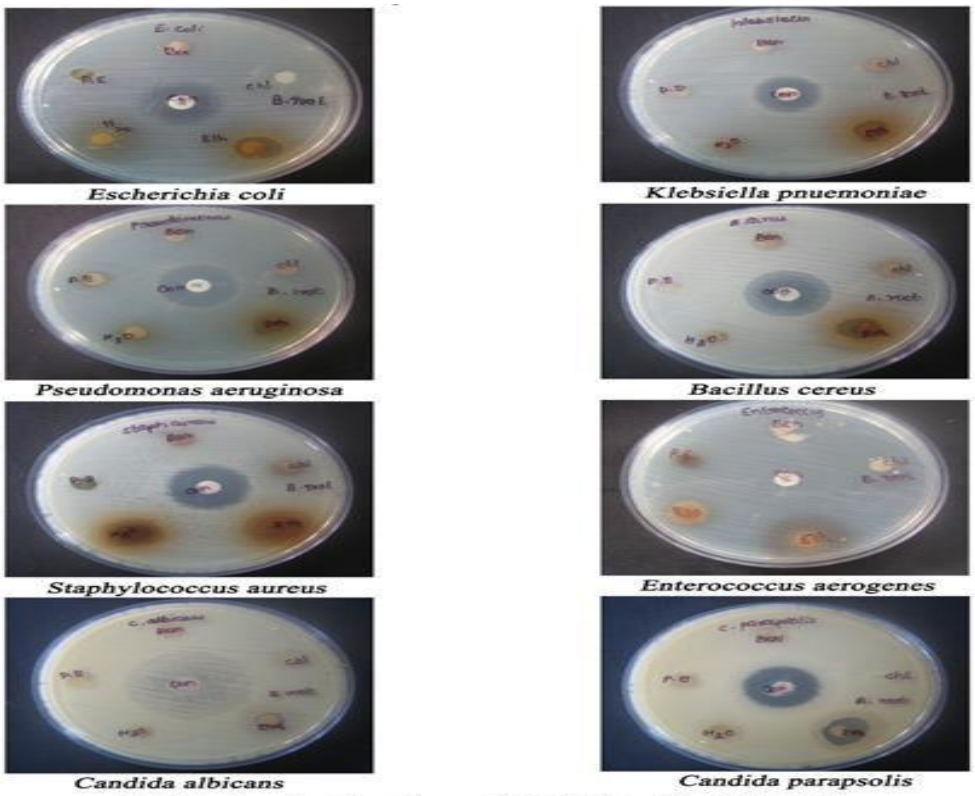


Plate: 2. Anitibacterial activity of Stem extract of *Combretum ovalifolium*

C) Root

Petroleum ether extracts of the root produce inhibition against bacteria *Escherichia coli, Staphylococcus aureus* and *Enterococcus aerogenes*. The benzene extract exhibits antibacterial activity by inhibiting the growth of *Bacillus cereus* and *Staphylococcus aureus.* Chloroform extracts of the root show inhibition against bacteria *Klebsiella pneumonia, Bacillus cereus and Staphylococcus aureus.* Ethyl alcohol extract shows high sensitivity against *Escherichia coli and Bacillus cereus* and *Escherichia coli* (12 mm) and *Enterococcus aerogenes* (7 mm) is the least sensitive. Distilled water extracts of the root exhibits inhibition against all the chosen bacteria. (Plate: 3).

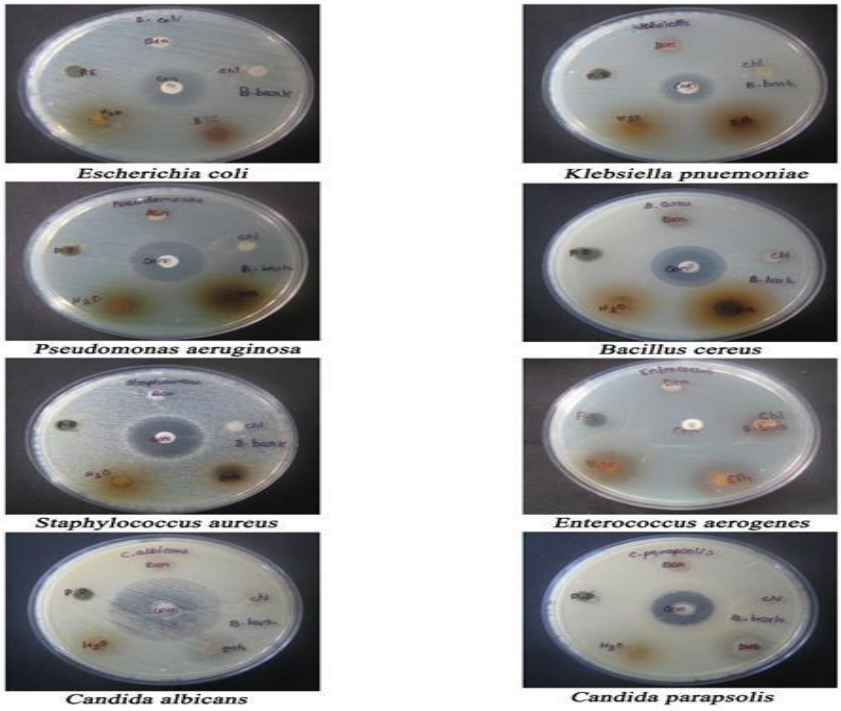


P.E - Petroleum ether, Ben - Benzene, Chl - Chlorform, Eth - Ethyl alcohol
H_2O - Distilled water, Con - Control

Plate: 3. Anitibacterial activity of Root extract of Combretum ovalifolium

D) Bark

The petroleum ether extract of the bark shows inhibition against all chosen bacteria. The ethyl alcohol extract of the bark produces larger inhibition zone (9 mm) against *Bacillus cereus*. Benzene extract does not show any inhibition zone against *Escherichia coli*, *Pseudomonas aeruginosa*,*Enterococcus aerogenes* and *Staphylococcus aureus* (Plate: 4). The distilled water extract of the leaf shows inhibition against all chosen bacteria.



P.E - Petroleum ether, Ben - Benzene, Chl - Chlorform, Eth - Ethyl alcohol
H_2O - Distilled water, Con - Control

Plate: 4. Anitibacterial activity of Bark extract of *Combretum ovalifolium*

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Table: 2.Antibacterial studies on Leaf, Stem, Root, and Bark extracts of *Combretum ovalifolium* Roxb.

S.No	Name of the bacteria	Part	Diameter of inhibition zone (mm)					
			Pet. ether	Benzene	Chloroform	Ethyl alcohol	Dis. water	Control
1.	*Escherichia coli*	Leaf	5 ± 0.6	-	-	12 ± 1.0	9 ± 0.5	19 ± 0.5
		Stem	-	-	-	11 ± 1.1	3 ± 0.6	16 ± 1.1
		Root	2 ± 0.5	-	-	12 ± 1.1	3 ± 0.9	15 ± 1.0
		Bark	-	-	-	3 ± 0.7	7 ± 0.8	18 ± 1.3
2.	*Klebsiellapnuemoniae*	Leaf	6 ± 0.7	-	-	11 ± 1.3	8 ± 0.6	18 ± 0.9
		Stem	2 ± 0.5	-	-	12 ± 1.3	5 ± 0.9	18 ± 0.5
		Root	-	-	2 ± 1.3	9 ± 1.0	2 ± 0.6	17 ± 0.8
		Bark	3 ± 0.8	2 ± 0.8	-	7 ± 0.8	3 ± 1.0	15 ± 0.6
3.	*Pseudomonas aeruginosa*	Leaf	-	-	-	2 ± 0.9	6 ± 1.1	20 ± 0.7
		Stem	-	-	-	9 ± 0.5	6 ± 0.7	20 ± 0.8
		Root	-	-	-	9 ± 0.5	2 ± 0.5	19 ± 1.0
		Bark	3 ± 0.6	-	-	7 ± 0.9	5 ± 0.7	21 ± 0.6
4.	*Bacillus cereus*	Leaf	5 ± 1.0	-	2 ± 1.2	18 ± 1.3	16 ± 0.6	22 ± 0.8
		Stem	3 ± 1.1	-	-	13 ± 1.0	4 ± 0.5	22 ± 0.7
		Root	-	2 ± 0.7	2 ± 1.0	12 ± 0.9	2 ± 0.6	22 ± 0.7
		Bark	3 ± 0.5	2 ± 0.5	-	9 ± 0.6	2 ± 0.9	23 ± 0.5
5.	*Enterococcus aerogenes*	Leaf	-	-	-	6 ± 0.7	-	-
		Stem	2 ± 0.7	-	4 ± 1.1	7 ± 1.2	8 ± 0.8	-
		Root	2 ± 0.7	-	-	7 ± 0.9	2 ± 0.4	-
		Bark	3 ± 0.5	-	3 ± 1.1	4 ± 0.6	5 ± 0.6	-
6.	*Staphylococcus aureus*	Leaf	5 ± 0.9	-	2 ± 0.6	11 ± 1.1	13 ± 1.3	22 ± 0.6
		Stem	3 ± 0.8	2 ± 1.3	3 ± 0.8	7 ± 1.3	8 ± 1.2	21 ± 0.7
		Root	2 ± 0.6	2 ± 1.0	2 ± 0.9	9 ± 0.6	8 ± 0.6	25 ± 0.9
		Bark	2 ± 1.0	-	-	5 ± 1.2	6 ± 0.7	16 ± 1.3

Values are means of three independent analyses of the extract ± standard deviation (n=3)

CONCLUSION

The preliminary phytochemical analysis of *Combretum ovalifolium* Roxb. reveals the presence of several bioactive compounds, such as steroids, triterpenoids, sugars, saponins, flavonoids, and catechins, across various solvent extracts.

Ethyl alcohol extracts of both leaf and stem showed broad-spectrum antibacterial activity, demonstrating potential therapeutic applications for combating bacterial infections. The leaf extract exhibited significant activity against *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*, while the stem extract displayed the most consistent antibacterial effects across all tested strains. Additionally, the root and bark extracts of *Combretum ovalifolium* showed selective inhibition against various pathogenic bacteria, with the root extract in particular demonstrating notable antibacterial effects in *Escherichia coli* and *Bacillus cereus.*

These findings support the ethnomedicinal use of *Combretum ovalifolium* as a source of natural antimicrobial agents, particularly in the treatment of bacterial infections. The plant's rich composition of secondary metabolites, including saponins, flavonoids, and tannins, may contribute to its antibacterial properties, with saponins recognized for their immunity-boosting and anti-inflammatory effects. Further studies on the isolation and characterization of these bioactive compounds could enhance our understanding of their mechanisms of action and pave the way for the development of novel antimicrobial therapies.

In conclusion, *Combretum ovalifolium* demonstrates promising phytochemical and antibacterial potential, warranting further investigation for its possible incorporation into herbal medicine and pharmacological applications.

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