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Preliminary Phytochemicals And Antimicrobial Activity Of Leaves Of Petrea Volubilis L.

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ABSTRACT
This study aimed to evaluate the antimicrobial activity and conduct a phytochemical analysis of leaf extracts from *Petrea volubilis L*., a plant belonging to the Verbenaceae family, using four solvents: Hexane, Acetone, Ethanol, and Aqueous. Antimicrobial testing was performed using the agar disc diffusion method against four pathogens: *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus flavus*. Among the extracts, the ethanol extract exhibited the strongest antibacterial activity against *E. coli* with an 18 mm zone of inhibition, while *P. aeruginosa* showed the smallest zone in the acetone extract (17 mm). For fungal pathogens, the ethanol extract demonstrated the highest antifungal activity against *A. flavus* (16 mm), and the acetone extract showed significant inhibition against *C. albicans* (12 mm). The hexane extract showed moderate antimicrobial activity, while the aqueous extract did not exhibit effective inhibition against the tested pathogens. Phytochemical screening revealed the presence of several bioactive compounds, including alkaloids, tannins, saponins, flavonoids, glycosides, quinones, reducing sugars, amino acids, essential oils, and carbohydrates. Additionally, *Petrea volubilis* has traditionally been used in herbal medicine for its purported anti-inflammatory, analgesic, and antimicrobial properties. The leaves are believed to aid in the treatment of various ailments, such as wounds, infections, and digestive disorders. The results underscore the potential of *Petrea volubilis* as an effective herbal remedy and a promising source of antimicrobial agents for the treatment of various infectious diseases.

Keywords: *Petrea volubilis,* Verbenaceae, Antimicrobial Activity, Phytochemical Screening, Microorganisms, Traditional Uses.

INTRODUCTION

Medicinal and aromatic plants have been integral to human life for centuries, serving both nutritional and therapeutic purposes. The utilization of plant products as therapeutic and pharmacological alternatives is both affordable and widely accepted in various cultures. Forests are recognized as rich centers of biological diversity, incorporating a wide variety of bioresources that contribute to human health (J.W.A. Bezerra *et al.,* 2017; DK Patel, 2017). Dense vegetation characterizes these ecosystems, providing essential habitats for numerous species and necessary conditions for their survival and interaction with the environment, leading to unique evolutionary adaptations.

Petrea volubilis L., commonly known as the purple wreath or sandpaper vine, belongs to the Verbenaceae family and is celebrated for its ornamental flowers. However, its leaves have long been employed in traditional medicine due to purported therapeutic effects, with local communities utilizing them for various ailments ranging from minor cuts to chronic diseases. The plant's traditional use encompasses treatments for ailments such as infections, inflammation, and gastrointestinal disorders. In particular, many communities have leveraged *Petrea volubilis* for its potential anti-inflammatory and antimicrobial properties, sparking interest among researchers looking for new leads to combat microbial infections (Nguyen Thanh Chung *et al.,* 2020; Kavita Sharma and Kanika Dabahadker, 2020).

Investigation into the phytochemical constituents of *Petrea volubilis* reveals a wealth of bioactive compounds, including alkaloids, flavonoids, tannins, and saponins that are known for their antimicrobial effects (Khan *et al.,* 2018; Ferreira *et al.,* 2020). These compounds have been associated with various health benefits and are thought to contribute to the plant's efficacy in traditional medicine. Phytochemical screening of *Petrea volubilis* extracts has demonstrated significant antimicrobial activity against a range of pathogenic microorganisms, thus validating its ethnomedicinal applications (Okwu *et al.,* 2016; Ali *et al.,* 2021).

Given the pressing challenge of antibiotic resistance, plant-derived antimicrobials present a promising avenue for alternative treatment strategies. The rise in antibiotic-resistant strains necessitates urgently exploring natural products, which can offer novel antimicrobial properties (Newton *et al.,* 2019; Adesina *et al.,* 2020). Several studies have highlighted the increasing interest in herbal medicines as potential solutions to combat resistant infections, emphasizing the need for rigorous scientific research to validate their effectiveness (Duggal *et al.,* 2020; Mursalim *et al.,* 2019).

This study aims to evaluate the antimicrobial efficacy of leaf extracts from *Petrea volubilis*, utilizing various solvents including Hexane, Acetone, Ethanol, and Aqueous extracts against common pathogens such as Escherichia coli, Pseudomonas aeruginosa, Candida albicans, and *Aspergillus flavus*. Through this research, we seek to highlight the ethnomedicinal value of *Petrea volubilis* and provide insights into its pharmacological potential, which may lead to the development of effective herbal remedies. The outcomes of this study are expected to contribute to the understanding of *Petrea volubilis* as a reliable source of natural antimicrobials, reinforcing the importance of preserving and utilizing plant biodiversity for health care.

OBJECTIVES

1. To analyze the phytochemical profile of various leaf extracts from *Petrea volubilis*.
2. To perform a thorough phytochemical screening of *Petrea volubilis L*eaf extracts using different solvents (Hexane, Acetone, Ethanol, and Aqueous) to identify significant bioactive compounds.
3. To assess the antimicrobial activity of *Petrea volubilis L*eaf extracts against selected pathogens, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus flavus* utilizing the agar disc diffusion method.

METHODOLOGY

Materials

The plant material used for this study was *Petrea volubilis L*., collected from the premises of Sri Venkateswara University, Tirupati, Andhra Pradesh, India. The species identification was confirmed through references from "Flora of the Presidency of Madras" (Gamble, 1928).

Extraction Procedure:

1. Fresh leaves of *Petrea volubilis* were washed, dried, and ground into a fine powder.
2. A 30g sample of the powdered plant was subjected to Soxhlet extraction using Hexane, Acetone and Ethanol.
3. The aqueous extract was prepared by boiling the plant powder with distilled water.
4. Extracts were concentrated and stored for further Phytochemical analysis.

Phytochemical Screening:

1. Alkaloids: Mayer’s reagent was used to detect alkaloids by the formation of a white precipitate.
2. Flavonoids: Appearance of Red or Pink colour indicates the presence of flavonoids.
3. Reducing Sugars: Brick-red precipitation upon heating with Fehling’s solution indicated reducing sugars.
4. Tannins: Brownish-green coloration after ferric chloride treatment indicated the presence of tannins.
5. Saponins: Persistent frothing and emulsion formation with olive oil indicated saponins.
6. Glycosides: To observe a brick red precipitate as an indication for the presence of glycosides.
7. Quinones: The Appearance of Blue green or red colour indicates the presence of Quinone.
8. Amino acids: The appearance of Blue or violet colour indicates the presence of amino acids.

9. Essential Oils: The Soap Formation of solution indicates the presence of essential oils.
10. Carbohydrates: The Solution turned brown on heating indicates the presence of carbohydrates.

Antibacterial Activity

The antibacterial activity of the leaf extracts was tested using the agar disc diffusion method (Bauer *et al.,* 1996) against the following bacterial strains: *Escherichia coli* and *Pseudomonas aeruginosa*, The test was conducted as follows:

Plant Extract Preparation:

- 30 grams of powdered *Petrea volubilis L*eaves were extracted sequentially using 250 mL of Hexane, Acetone, Ethanol and Double distilled water.
- The extracts were concentrated and dissolved in their respective solvents to prepare 1/10 solutions for testing.

Agar Disc Diffusion Method:

- Bacterial strains were cultured on Muller Hinton Agar plates.
- Filter paper discs (5 mm) were soaked with 200-500 µL of plant extract and placed on the agar surface.
- The plates were incubated at 37°C for 24 hours, and the zone of inhibition around the discs was measured.

Antifungal Activity:

- The fungal strains *Aspergillus flavus* and *Candida albicans* were cultured on SDA.
- The plant extract was applied to sterile filter paper discs, which were then placed on the fungal inoculated agar plates.
- The plates were incubated at 37°C, and the zone of inhibition was measured at 24 and 48 hours.

RESULTS AND DISCUSSION

Analysis of Qualitative Phytochemical Screening

The qualitative analysis of phytochemical constituents in the leaves of *Petrea volubilis L*. revealed varying profiles across different solvent extracts (Table No.1, 2). The Hexane extract contained 4 phytochemicals: Tannins, Flavonoids, Glycosides, and Quinones, while Alkaloids, Reducing sugars, Saponins, Essential oils, Amino acids, and Carbohydrates were absent. The Acetone extract yielded 5 phytochemicals: Alkaloids, Tannins, Reducing sugars, Glycosides, and Quinones, but lacked Flavonoids, Saponins, Amino acids, and Essential oils. The Ethanol extract also identified 5 phytochemicals: Alkaloids, Flavonoids, Glycosides, Essential oils, and Carbohydrates, absent were Saponins, Reducing sugars, and Amino acids. The Aqueous extract showed 5 phytochemicals present: Reducing sugars, Tannins, Flavonoids, Glycosides, and Carbohydrates, while Alkaloids, Saponins, Amino acids, and Quinones were absent. Overall, the results indicate that *Petrea volubilis L*. leaves are rich in phytochemicals, particularly Glycosides and Tannins, across all solvent extracts, suggesting their potential therapeutic applications.

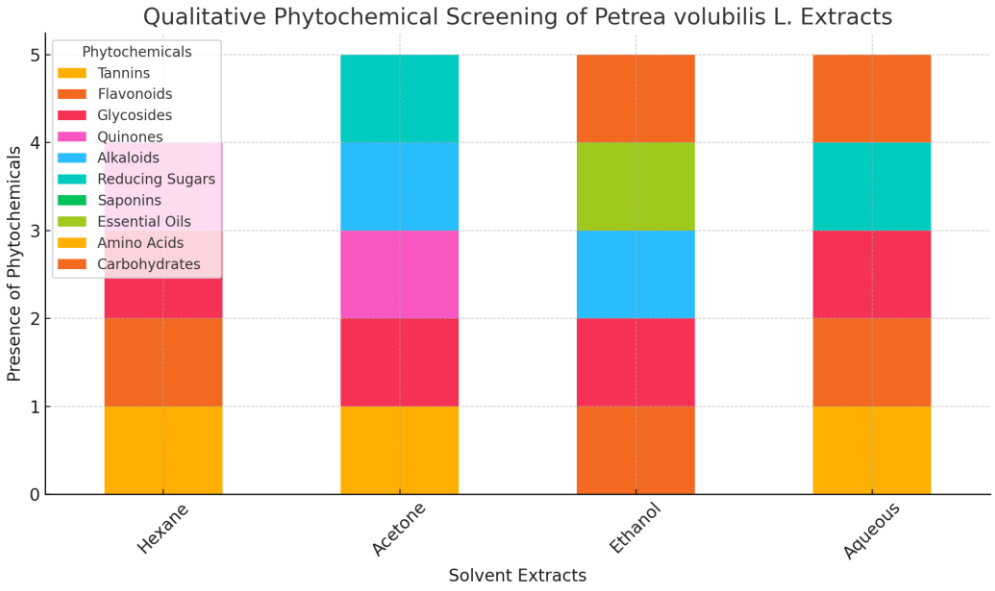
Table No. 1: The details of Selected Solvents and Soxhlet unit running Parameters of Leaves of *Petrea volubilis L*.

S.No.	Solvent Name	Boiling Point	Polarity	Total No. of Cycles	Soxhlet Total Running Hours
1.	Hexane	69^0 C	0.1	17	3
2.	Acetone	56^0 C	5.1	13	3
3.	Ethanol	78^0 C	4.3	09	3
4.	Aqueous	100^0 C	10.2	03	3

Table No. 2: Qualitative Analysis of Phytochemicals Constituents of Leaves of *Petrea volubilis L.*

S.NO	TEST NAME	HEXANE	ACETONE	ETHANOL	AQUEOUS
1.	Alkaloids	-	+	+	-
2.	Reducing sugars	-	+	-	+
3.	Tannins	+	+	-	+
4.	Saponins	-	-	-	-
5.	Flavinoids	+	-	+	+
6.	Glycosides	+	+	+	-
7.	Amino acids	-	-	-	-
8.	Essential oils	-	-	+	-
9.	Carbohydrates	-	+	+	+
10.	Quinones	+	-	-	+
Total No. of Phytochemicals		**4**	**5**	**5**	**5**

Note: (+) Present; (-) Absent



Antibacterial Activity Screening

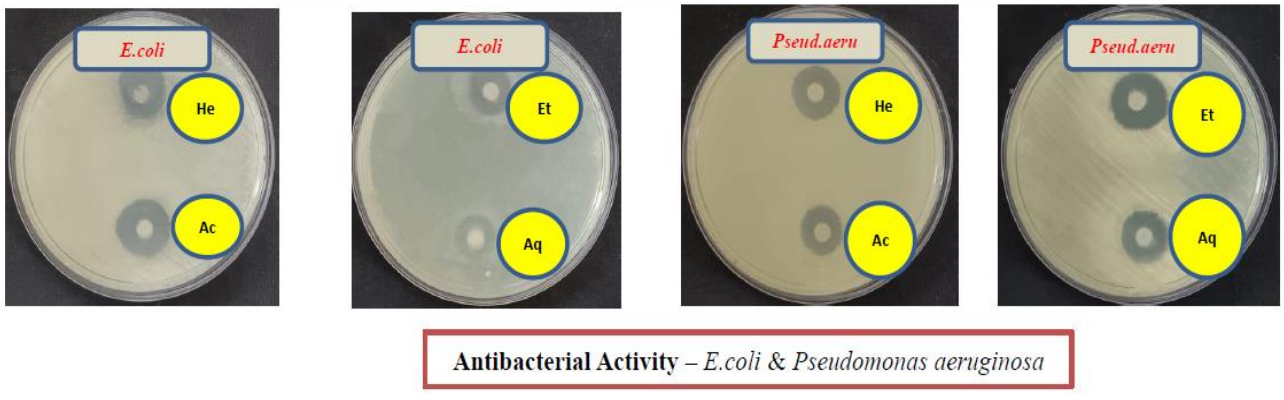
The antimicrobial activity of leaf extracts from *Petrea volubilis L.* was assessed using the disc diffusion method against key pathogens, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus flavus*, and *Candida albicans* (Table No. 3). The ethanol extract exhibited the strongest antibacterial activity against *E. coli*, with a zone of inhibition measuring 18 ± 0.4 mm, closely followed by the acetone extract at 17 ± 0.3 mm. The Hexane extract showed moderate activity with a zone of 15 ± 0.4 mm, while the aqueous extract had the lowest effectiveness against *E. coli*, yielding a zone of 13 ± 0.3 mm. For *Pseudomonas aeruginosa*, the ethanol extract again demonstrated significant inhibition at 16 ± 0.4 mm, while the Hexane extract showed a zone of 13 ± 0.3 mm, with aqueous extracts providing a zone of 14 ± 0.5 mm. Regarding antifungal activity, the ethanol extract was most effective against *Aspergillus flavus*, presenting an inhibition zone of 16 ± 0.3 mm, with the Acetone extract showing 14 ± 0.5 mm. The Hexane extract had a

zone of 15 ± 0.5 mm, while the aqueous extract exhibited lower activity with a zone of 12 ± 0.6 mm. For *Candida albicans*, the Acetone extract was most effective at 15 ± 0.2 mm, while the aqueous extract showed minimal inhibition at 10 ± 0.3 mm. Overall, the results highlight the notable antibacterial and antifungal properties of *Petrea volubilis L.* leaf extracts, illustrating their potential as natural antimicrobial agents in traditional medicine.

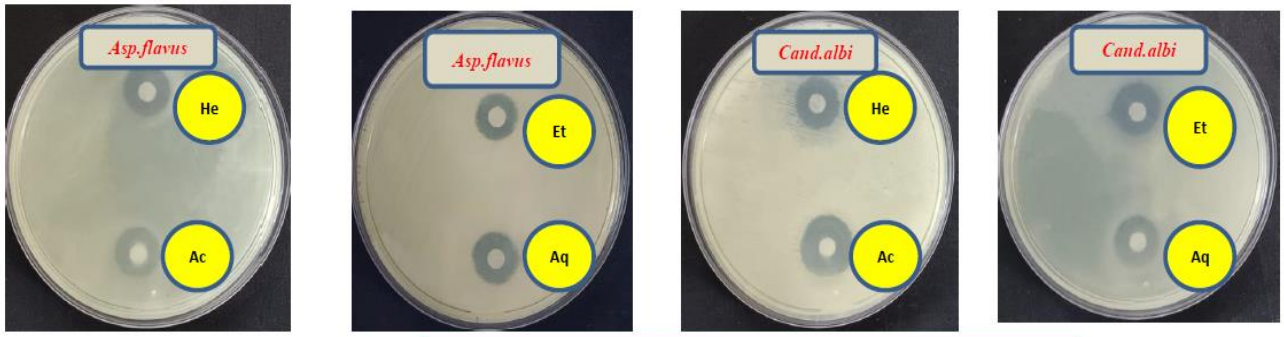
Table No. 3. Antimicrobial activity of Leaves of *Petrea volubilis L.* using Disc diffusion assay

S. No	Pathogens	Antibacterial activity - Zone of Inhibition (mm)			
		Hexane	Acetone	Ethanol	Aqueous
1.	*Escherichia coli*	15 ± 0.4	17 ± 0.3	18 ± 0.4	13 ± 0.3
2.	*Pseudomonas aeruginosa*	13 ± 0.3	15 ± 0.2	16 ± 0.4	14 ± 0.5
S. No	**Pathogens**	**Antifungal activity - Zone of Inhibition (mm)**			
		Hexane	**Acetone**	**Ethanol**	**Aqueous**
1.	*Aspergillus flavus*	15 ± 0.5	14 ± 0.5	16 ± 0.3	12 ± 0.6
2.	*Candida albicans*	12 ± 0.2	15 ± 0.2	14 ± 0.4	10 ± 0.3

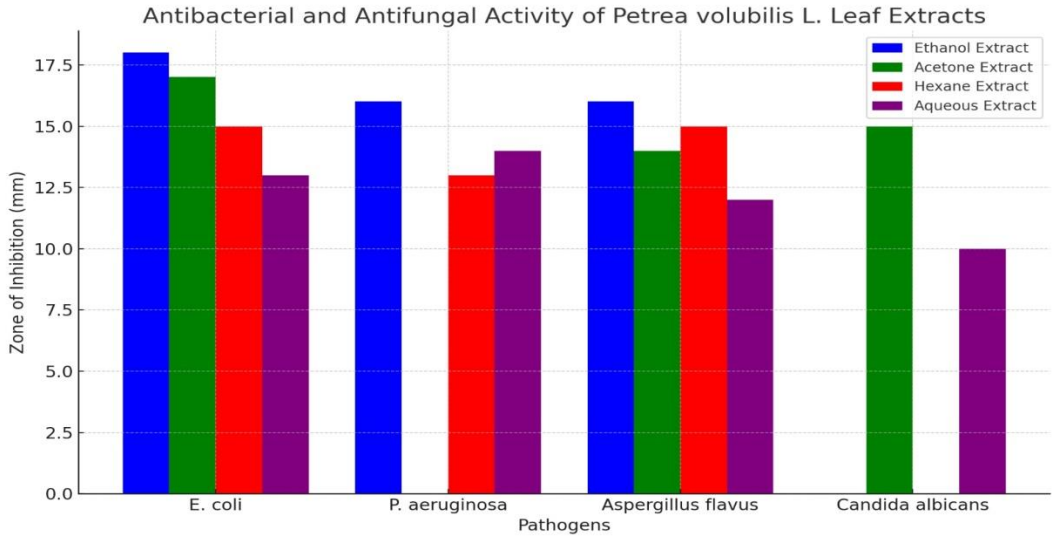
Plate: 1. Antimicrobial Activity of Leaves of *Petrea volubilis* L. (Zone of Inhibition)



Antibacterial Activity – *E.coli & Pseudomonas aeruginosa*



Antifungal Activity - *Aspergillus flavus & Candida albicans*



CONCLUSION

This study demonstrates that leaf extracts from *Petrea volubilis L*. possess significant antimicrobial properties, particularly in Ethanol extracts against *Escherichia coli* and *Aspergillus flavus.* The Ethanol extract also exhibited notable antifungal activity against *Candida albicans*, further emphasizing its potential for natural antimicrobial applications. The diverse range of bioactive phytochemicals identified suggests that *Petrea volubilis* can be a valuable source of herbal remedies. While Hexane and Acetone extracts proved effective against some pathogens, the Aqueous extract showed limited activity, highlighting the importance of solvent selection in the extraction process. These results support the traditional use of *Petrea volubilis* in herbal medicine for treating infections and inflammatory conditions, warranting further investigation into individual bioactive compounds for therapeutic development and their mechanisms of action against both bacterial and fungal pathogens.

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