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BIOACTIVE PHYTOCHEMICAL ANALYSIS AND THERAPEUTIC ACTIVITIES OF *UTLERIA SALICIFOLIA*

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ABSTRACT
Utleria salicifolia is an unexplored medicinal plant with potential pharmacological importance. In this study, an intensive phytochemical, antioxidant and antimicrobial activity of the ethanol extract was conducted. Qualitative phytochemical profiling indicated the existence of a wide spectrum of secondary metabolites represented by phenol, flavonoids, tannins, glycosides, saponins, anthraquinones and amino acids. These indicated a high quantity of bioactive components. The DPPH assay revealed concentration dependent antioxidant activities with an IC50 value of 370 µg/mL, suggesting a moderate antioxidant capacity that may be associated to the extracts phenolic and flavonoid content. Further with minimum inhibitory concentrations varying from 150 to 250 µg/mL, of the extract demonstrated broad spectrum antimicrobial activity against both Gram-positive and Gram-negative microorganisms. With significance was the maximum susceptibility obtained against *Bacillus subtilis* and *Escherichia coli*. Altogether, these findings offer the represent the first consistent evidence that *U. salicifolia* possesses numerous phytochemical constituents exhibiting noticeable antioxidant and antimicrobial properties on this plant, and hence provides an attractive possibility for the identification of novel therapeutic compounds.
Keywords: *Utleria salicifolia*, phytochemical, antioxidant, antimicrobial activity

INTRODUCTION
Many of the naturally occurring bioactive chemicals found in medicinal plants are used as lead molecules in the production modern medicines. The systematic identification of secondary metabolites, including alkaloids, flavonoids, tannins, terpenoids, glycosides, phenolic compounds, and saponins, is known as phytochemical investigation. It is considered a vital component in investigating the potentialities of traditionally used plants. Antimicrobial, antioxidant, anti-inflammatory, cytoprotective, and immunomodulatory properties are among the many biological activities that these secondary metabolites are known to play a major role in. The South Western Ghats of India are home to the ethnomedically important shrub *Utleria salicifolia* Bedd. ex Hook. f. (Periplocaceae), which has long been valued by the indigenous peoples, including the Malasar, Kadar, and Muthuvan tribes.
Traditionally called to as “Mahali kizhangu” the tubers of this plant are frequently used in traditional medicines and home remedies to treat several kinds of illnesses, especially those related to infections, digestive pain, and inflammatory diseases. Scientific research on the phytochemical components of the root and other medicinal potentials have remained limited and inadequately reported, despite the root's significant role in tribal medicine. The need to find new and potent antimicrobial agents from natural sources has increased recently due to the increasing prevalence of antimicrobial resistance worldwide. By focussing on important cellular functions, plant-derived chemicals have showed an incredible ability to prevent the growth of harmful pathogens, making them desirable alternative or supplements to synthetic medicines (Rastogi Subha, 2004).

Therefore, evaluating the antibacterial activity of *U. salicifolia* root extracts can provide crucial information on its traditional uses in the treatment of illnesses. Since oxidative stress, which results from an imbalance between the synthesis of free radicals and antioxidant defences, is linked to the pathophysiology of many chronic and degenerative diseases, the antioxidant properties of medicinal plants is equally important. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, an attractive and reliable technique for evaluating antioxidant capacity, can be used to measure the high free radical scavenging effects of phenolic and flavonoid-rich plant extracts. The potential contribution of plant extracts in preventing oxidative damage and protecting cellular integrity is reflected in their ability to reduce DPPH radicals (Salehi *et al.,* 2020).

Given *Utleria salicifolia's* ethnopharmacological significance and lack of significant scientific research on its chemical and medicinal properties, an in-depth examination of its phytochemical components and biological activities is essential. Thus, the current work is to conduct a thorough phytochemical investigation of the root extract, assess its antibacterial efficiency against specific pathogenic microorganisms, and determine its potential as an antioxidant using DPPH radical scavenging activity. It is expected that these findings will validate the plants traditional applications, raise awareness of its medical significance, and possibly reveal new natural compounds with therapeutic potential.

MATERIALS AND METHODS

Plant Collection

The Anamalai Hills, Coimbatore District, Tamil Nadu, India, which is known for its therapeutic flora and environment favourable to the growth of endemic species, provided fresh roots of *Utleria salicifolia*. immediately as the roots were collected, they were cleaned with running tap water and then distilled water to get free of any contaminants and attached soil. After being shade-dried for ten to fifteen days at room temperature (28 ± 2 °C), the roots were ground into a fine powder using a mechanical grinder and then sieved through a mesh screen to ensure uniform particle size. After that, the materials were kept in moisture-proof, airtight containers until they were extracted. (Adhikary *et al.,* 2025).

Plant Extract Preparation

Ethanolic extraction was used to maximise the recovery of polyphenols, flavonoids, and other ethanol-soluble bioactive metabolites. Soxhlet extraction was used on around 100 g of the dried root powder for eight hours under continuous hot-percolation conditions using analytical-grade ethanol as the solvent. To produce a thick crude extract, the extract was then filtered and concentrated under low pressure using a rotary evaporator at 40–45 °C. 12.07% (w/w) was determined to be the ethanolic extract's percentage yield. The dried extract was transferred to sealed glass vials and stored at 4 °C for subsequent phytochemical and bioactivity investigations. (Pooja *et al.,* 2025).

Preliminary Screening for Phytochemicals

The qualitative phytochemical screening was carried out to determine the presence of significant varieties of secondary metabolites. According to traditional phytochemical analysis guidelines, the tests were carried out. Standardised colorimetric and precipitation assays were used to identify alkaloids, anthraquinones, glycosides, flavonoids, phenolic compounds, saponins, tannins, fixed oils, carbohydrates, amino acids, and terpenoids in the crude ethanolic extract. All of the experiments stated above were performed in triplicate to ensure the reliability and consistency of phytochemical detection (Singh *et al.,*2022).

Determination of Antioxidant Activity-DPPH Radical Scavenging Assay

The ethanolic root extract's antioxidant potential was quantitatively assayed by the DPPH assay, which is one of the widely accepted methods for evaluating electron-donating and radical-neutralizing capacities. A freshly prepared methanolic DPPH solution (0.1 mM) was mixed with different concentrations of the extract. Mixtures with methanol and extract alone acted as the blank. Butylated hydroxyl anisole, BHA, was used as the standard antioxidant control. The reaction mixtures were incubated in the dark at room temperature for the stipulated reaction time to avoid the photodegradation of DPPH. Spectrophotometric measurement at 516 nm was performed to determine absorbance values, and subsequently, the radical-scavenging activity was calculated in % with respect to the control sample (Sundarraj *et al.,* 2011)

Determination of Antimicrobial Activity

Agar Disk Diffusion Assay

The antimicrobial potency of the ethanolic crude extract was evaluated for efficacy using the standard agar disc diffusion method outlined in standard microbiological protocols. Nutrient agar plates were appropriately inoculated with standardized microbial cultures using the spread plate method. The bacterial cultures chosen for this experiment include *Bacillus cereus, Bacillus subtilis, Escherichia coli, Salmonella typhimurium,* and *Staphylococcus aureus*, which are classified based on their gram positivity or negativity, consisting of both gram-negative and gram-positive bacteria. A set of sterile, 6mm disc filter papers soaked with different levels of plant extracts was aseptically placed on the inoculated nutrient agar surface. Chloramphenicol (C30, 30µg/disc) was used as the standard positive control material, whereas Dimethyl Sulfoxide (DMSO) was the negative control agent for this experiment. The agar plates were incubated for a standard 24-hour period of time at a temperature of 37 °C, and subsequent measurement of the inhibition zone was taken from the reverse side of each plate in millimeters, thereby indicating higher inhibition zones for higher antimicrobial activity levels (Dhif *et al.,*2025).

Minimum Inhibitory Concentration - MIC

The MIC of the ethanolic extract was determined by the broth dilution method described by Wiegand et al. (2008). Serial two-fold dilutions of the extract in nutrient broth were prepared to obtain a series of test concentrations. Each tube was then inoculated with bacterial cultures adjusted to 1×10^6 CFU/mL. The tubes containing the inoculum were then incubated at 37 °C, under an aerobic atmosphere, for 24 hours. Subsequently, the MIC was considered to be the lowest concentration of the extract at which visible microbial growth was completely inhibited (Hulankova, 2024). All assays were performed three times to maintain accuracy and reproducibility.

RESULT AND DISCUSSION

Phytochemical Analysis

The preliminary phytochemical screening of the ethanolic root extract from *Utleria salicifolia* indicated the presence of various major classes of secondary metabolites, as revealed from Table 1. The extract exhibited a strong positive result for carbohydrates, phenolic compounds, flavonoids, tannins, glycosides, saponins, anthraquinones, and amino acids, thus ensuring their presence in the root material in considerable quantity. On the other hand, alkaloids and fixed oils did not show any presence in the ethanolic extract.

The strong detection of phenols, flavonoids, tannins, and saponins suggests that the root of *U. salicifolia* is particularly rich in polar bioactive constituents known to contribute significantly towards antioxidant and antimicrobial activities. Phytochemical profiling is an important preliminary step in the process of understanding medicinal plants therapeutic potentials since the biological activity of the plant extracts is practically dependent on the secondary metabolite content of the plants. The current study identified that the ethanolic root extract of *Utleria salicifolia* represents a wide array of bioactive phytoconstituents, thus justifying its traditional medicinal uses. The strong presence of phenolic compounds and flavonoids is particularly noteworthy because various reports have identified these compounds as being well endowed with potent antioxidant activities through free radical scavenging, metal ion chelation, and inhibition of lipid peroxidation. Their abundance might directly be responsible for the detected antioxidant activity in subsequent DPPH radical scavenging assays (Shah *et al.*,2023).

Table:1. Preliminary Phytochemical Analysis of *Utleria salicifolia*

S.No	Phytochemical groups	Present/Absent
1.	Carbohydrates (Molish Test)	++
2.	Phenols (Phosphomolybdic acid test)	++
3.	Flavonoids (Lead acetate test)	++
4.	Tannins (Braemer's test)	++
5.	Alkaloids (Draggendorff's test)	-
6.	Glycosides (Legals test)	++
7.	Saponins (Foam test)	++
8.	Anthraquinones (Borntrager's test)	++
9.	Amino acids (Ninhydrin test)	++
10.	Fixed oils	-

+= Presence, - = Absence

The tannins detected in considerable amounts are known to affect antimicrobial and astringent activities by precipitating microbial proteins and affecting cell membrane integrity. Similarly, saponins act via surface-active properties that have been reported to increase the permeability of microbial cell membranes, hence enhancing their antimicrobial efficacy. The presence of glycosides further adds to the pharmacological relevance of the extract due to the fact that many glycosidic compounds have been reported to exert antimicrobial, cardioprotective, as well as anti-inflammatory effects (Brar *et al.,* 2025). Some compounds detected in this extract, like anthraquinones, have antimicrobial and antioxidant activities, providing for potential synergistic interactions with phenolic compounds toward enhanced bioactivity of the plant. The nutritional factors represented by amino acids might be associated with the nutritional value that could enhance metabolic functions and, as such, provide a venue for tissue repair, thus indirectly contributing to therapeutic effect(s). The absence of alkaloids and fixed oils would suggest that biological activity from *U. salicifolia* roots is probably expressed through non-alkaloidal, polar phytochemicals rather than lipidsoluble ones (Sun and Shahrajabian, 2023).

Antioxidant activity

Antioxidant potential of the ethanolic root extract of *Utleria salicifolia* was assessed by DPPH free radical scavenging assay and is represented in Table 2. Radical scavenging activity increased with increasing concentration of the extract in a dose-dependent manner.

The scavenging activity at the concentration 200 µg/mL was found to be 32.63%, which increased progressively to 47.07%, 61.08%, 72.28%, and 86.78% at the concentrations 300, 400, 500, and 600 µg/mL, respectively. The IC_{50} value of the ethanolic extract was estimated to be 370 µg/mL, which reveals the extracts' possession of moderate but effective antioxidant potential. The obtained trend depicts the ability of the extract to donate hydrogen atom or electrons in a dose-dependent fashion to neutralize DPPH radicals. Free radical–mediated oxidative stress is considered as the cause of several chronic and degenerative diseases. There arises an importance for the identification of appropriate and effective natural antioxidants. This study showed that the ethanolic root extract of *Utleria salicifolia* exhibits effective DPPH radical scavenging activity in a dose-dependent mode, with a greater efficacy at higher concentration. Accordingly, dose-dependent enhancement in scavenging activities reflects active principles of antioxidants that stabilize free radicals through an electron or hydrogen donation mechanism (Baliyan *et al.,* 2022).

Table:2. Antioxidant (DPPH) activity of *Utleria salicifolia*

Sample	Concentration (µg/ml)	Scavenging activity (%)	IC^{50} (µg/ml)
Ethanol Extract	200	32.63	370
	300	47.07	
	400	61.08	
	500	72.28	
	600	86.78	

With an IC_{50} value of 370 µg/mL, the antioxidant strength is moderate compared to the synthetic antioxidants; it is, however, quite appreciable for a crude plant extract. This property can be closely linked with the phytochemical content present in the extract, specifically the phenolic, flavonoid, and tannin content that was detected in the preliminary phytochemical screening. This is because the phenolic hydroxyl group is established to act as a primary functional component in the neutralization of free radicals, while the mechanism involves the redox character of the flavonoids. Tannins and anthraquinones could also increase the potential of the antioxidant (Ibrahim *et al.,* 2019). The scavenging activity exhibited at a higher concentration of 600 µg/mL with a value of 86.78% emphasizes the use of *U. salicifolia* as a source for obtaining antioxidants. The scavenging activity shown by the root extract indicates its use in preventing oxidative damage and aiding in the protection of cells against oxidative stress damage. In addition to this, the antioxidant activity can partially justify the use of *U. salicifolia* in the traditional medicinal practice for the treatment of inflammatory and ulcer conditions where oxidative stress contributes to the condition (Lalhminghlui and Jagetia.2018)

Antimicrobial Activity

The antimicrobial properties of the ethanolic root extract of *Utleria salicifolia* were tested against certain Gram-positive and Gram-negative bacteria using the agar disk diffusion and minimum inhibitory concentrations (MIC) method. As evident from Table 3, there was noticeable antibacterial activity against all the tested organisms and was dose-dependent. In a 1% solution, the zone of inhibition was between 8 and 13 mm, and in a 2% solution, there was a pronounced increase in the zone of inhibitions between 14 and 25 mm.

Table:3. Antimicrobial activity of *Utleria salicifolia*

Microorganisms	Ethanol Extract		Positive Control	MIC ((µg/ml)
	1%	2%		
Bacillus cereus	10	25	32	200
Bacillus subtilis	11	23	42	250
Escherichia coli	8	14	33	150
Salmonella typhimurium	13	18	36	200
Staphylococcus aureus	9	15	39	150

The maximum zone of inhibition was found for *Bacillus cereus* and *Bacillus subtilis* in a 2% solution of the extract, respectively. It also showed significant activity against Gram-negative bacteria, with inhibition zones of 14 mm against *Escherichia coli* and 18 mm against *Salmonella typhimurium* when a concentration of 2% was used. *Staphylococcus aureus* was moderately sensitive, producing inhibition zones of 9 mm and 15 mm using concentrations of 1% and 2%, respectively. The positive control antibiotic used in the test had larger inhibition zones than the plant extracts, making it a confirmation of a valid test. MIC was between 150-250 µg/mL, with both *Escherichia coli* and *Staphylococcus aureus* showing low MIC of 150 µg/mL. The antimicrobial data clearly indicate that the ethanolic root extract of *Utleria salicifolia* has broad-spectrum antibacterial potential against Gram-positive as well as Gram-negative bacteria. The concentration-dependent increase in the zones of inhibition suggests that the antimicrobial potential of the extract is directly proportional to the concentration of active constituents. The marked activity against *Bacillus* species such as *B. cereus* and *B. subtilis* can be explained on the basis of the relatively simpler cell wall complex of Gram-positive bacteria (Agada et al., 2023).

Although Gram-negative bacteria have shown greater resistance compared to other bacteria owing to the presence of a lipopolysaccharide outer membrane, the extract does show significant inhibition on *E. coli* and *S. typhimurium*. The lower values recorded for the MIC of *E. coli* and *S. aureus* show the antibacterial efficacy even at lower concentrations of the extract (Wang *et al.,* 2021). The observed antimicrobial activity in this study can be readily linked to the phytochemical compounds that have been identified in the preliminary screening of the extracts. Phenolic compounds and flavonoids have been shown to have antimicrobial activity through several different methods, including cell membrane disruption, inhibition of nucleic acid synthesis, and inhibition of microbial enzymes. Tannins have been noted to precipitate microbial proteins and disrupt cell walls, while saponins increase cell membrane permeability, resulting in the leakage of cell contents. Also contributing to the antimicrobial activity may be the anthraquinones in the extracts (Tiranakwit *et al.,* 2023). Despite the relatively small zone inhibition brought about by the plant extract compared with that of the standard antibiotic, this is already a significant finding for a plant extract. The presence of multiple compounds with bioactivity could indicate synergy between these compounds. This study scientifically confirms the folk use of *Utleria salicifolia* in the treatment of infections and its potential use as a source for antimicrobial drugs.

SUMMARY AND CONCLUSION

In the current study, the phytochemical content, antioxidant activity, and antimicrobial property of the ethanolic root extract of *Utleria salicifolia* were extensively investigated. Phytochemical screening showed the presence of various biologically active secondary compounds, such as phenolics, flavonoids, tannins, glycosides, saponins, anthraquinones, carbohydrates, and amino acids, relating to its medicinally rich nature. Antioxidant activity analysis using DPPH radical-scavenging activity showed that it had concentration-dependent activity, significantly nullifying the free radicals, thus having IC50 value of 370 µg/ml, explaining its moderate to good capacity to neutralize free radicals, predominantly contributed by its phenolic and flavonoid content. Antimicrobial analysis indicated that it had broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria, showing good inhibitory activity with low MIC range from 150 to 250 µg/ml. These biologically active properties can be attributed to combined effects of various biologically active components that bring about microbial cell integrity damage and reduction of oxidative stress. These data strengthen the traditional claims of using *U. salicifolia* medicinally and its potential role in serving as an active compound derived from natural sources of antioxidants, antimicrobial agents. Further detailed studies using chemical isolation of active components followed by validation of antifatigue activity using in vivo studies should be considered.

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