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Investigation of Phytochemical, Physicochemical, Antioxidant and *In-Vitro* Antibacterial Activity of *Salacia fruticosa* Aerial Parts

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ABSTRACT

This study's goal was to investigate the phytochemical composition, physicochemical characteristics, antioxidant properties, and antibacterial effects of *Salacia fruticosa* aerial parts. Four extracts, namely aqueous, ethanolic, chloroform, and acetone, were assessed for their antioxidant potential through the DPPH radical scavenging assay. Additionally, their antibacterial activity against a range of bacterial species, including *Staphylococcus lentus, Staphylococcus albus, Staphylococcus aureus, Bacillus subtilis, Bacillus lentus, Vibrio cholerae, Salmonella enterica, Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*, was determined using the agar disc diffusion method. Analysis of phytochemicals uncovered numerous active constituents, such as alkaloids, carbohydrates, flavonoids, phenols, triterpenoids, glycosides, proteins, amino acids, and saponins. Notably, the ethanolic extract exhibited the highest antioxidant activity at 73.81% ± 1.21. Furthermore, the ethanolic extract displayed superior antibacterial activity against all tested bacterial species with 15-29 mm inhibition zone and 62.5 µg/ml minimum inhibition concentration, followed by the aqueous, chloroform, and acetone extracts. In conclusion, the phytochemical analysis of *Salacia fruticosa*'s ethanolic extract indicated the existence of different natural chemical components and demonstrated very impressive antioxidant and antibacterial properties.

Keywords: *Salacia fruticosa*, physicochemical, phytochemicals, DPPH, disc diffusion, antibacterial activity.

1. INTRODUCTION

At present, one of the most significant challenges is the growing issue of antimicrobial resistance. No antibacterial agent is now 100% effective against torpid microbes. Since people realized that the lifespan of antibiotics is limited and that overuse and abuse of conventional antibiotics are leading to microbial resistance, the use of plant extracts for medicinal medications has gained tremendous popularity. Considering the rise in drug-resistant microorganisms and the need to develop more effective antibacterial agent, clinical microbiologists and plant pathologists are screening medicinal plants for antibacterial activities as possible underutilized treatments.[1,2]

The abundance of unique molecules that plants produce has aided in the continued search for effective anti-microbials. A possible source of antimicrobial compounds could be the growing interest in separating anti-microbials from plants. It is possible to find an important, naturally occurring antibacterial agent in a common, well-developed green plant. The visible pieces of evidence are determined to confirm plant materials at the species level. Plant metabolic profiling by TLC, HPLC, IR, NMR, and X-ray crystallography are influenced by both genetic and natural factors apart from morphologically recognizable evidence.[3]

Salacia fruticosa (Hippocrateaceae) is a woody climbing shrub with elliptic-ovate leaves that grows in evergreen and semi-evergreen forests. They are mainly found in the Kerala and Tamil Nadu in India. In traditional medicine, *Salacia fruticosa* serves

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various purposes, acting as a bitter and pungent agent with thermogenic properties. It is employed for its anti-diabetic, diuretic, astringent, pain-relieving, anti-inflammatory, blood-purifying, wound-healing, and liver and digestive benefits. This versatile plant is valuable in addressing hemorrhoids, skin disorders, menstrual abnormalities, and wound care. In Kerala, psoriasis is treated by gluing the *Salacia fruticosa* root part.[4-6]

The present study aims to evaluate the physico-chemical, phytochemical, antioxidant, and *in vitro* antibacterial activity of extracts of aerial parts of *Salacia fruticosa* against both human and plant pathogenic microscopic organisms.

2. MATERIAL & METHODS

2.1 Chemicals and Reagents

Distilled water, DPPH, BHT, as well as various chemicals employed for phytochemical screening, such as ethanol, acetone, methanol, petroleum ether, and chloroform, were procured from HIMEDIA laboratory located in Mumbai, India.

2.2 Collection & Physicochemical Evaluation

Fresh aerial part components of *Salacia fruticosa* were harvested at the Botanical Herbal Garden located in Ranga Reddy District, Telangana, India. Standard procedures were followed to assess various physicochemical parameters such as moisture content, total ash content, acid insoluble ash, water-soluble ash, sulphated ash, and extractive values.[7,8]

2.3 Extraction of Plant Material

For easy separation and more solvent absorption to dissolve the active components contained inside the cell, the fresh plant material was air dried at ambient room temperature and ground into a coarse powder. Using the Soxhlet apparatus, the powdered material was extracted with water (E1), ethanol (E2), chloroform (E3), and acetone (E4). The extracts were then concentrated to dryness and kept in desiccators for further research.[9]

2.4 Phytochemical Screening

The aerial parts of *Salacia fruticosa* were treated with various extraction methods, and a phytochemical assessment was conducted. This examination identified the existence of diverse phytochemical components such as carbohydrates, alkaloids, saponin, tannin, phytosterols, phenols, flavonoids, proteins, glycosides, amino acids, and triterpenoids using established standard method.[10]

2.5 Antioxidant Activity

2.5.1 Qualitative Analysis

The antioxidant activity of different extracts of *Salacia fruticosa* aerial parts was determined using the method described by Lee et al., 2003.[11] 50 µL of each extract of *Salacia fruticosa* were placed in a microtiter plate. 100 µL of 0.1% methanolic DPPH was added to the samples, which were then incubated for 30 minutes in a dark environment. When the samples' shade changed from purple to yellow or pale pink, it was recorded. A strong favorable result is shown by a yellow tint, whereas a weak positive outcome is denoted by a pale pink color.

2.5.2 Quantitative Analysis

To quantitatively determine the antioxidant activity, we employed 2,2-diphenyl-1-picryl hydrazyl (DPPH) as a free radical.[12] Initially, 100 µL of each extract of *Salacia fruticosa* was mixed with 2.7 ml of methanol, followed by the addition of 200 µL of a 0.1% methanolic DPPH solution. The resulting supernatant was incubated for 30 minutes in a dark environment. To establish a control, the initial absorption of a blank sample containing an equivalent amount of methanol and DPPH solution was prepared and measured. Subsequently, a UV double-beam spectra scanner was used to quantify the absorption maxima of the solution at 5-minute intervals. The sample's antioxidant activity was compared to standard 0.16% butylated hydroxy toluene (BHT). The formula below was used to determine the antioxidant activity:

$$\text{Antioxidant Acitivy (\%)} = \frac{(\text{Control Absorbance} - \text{Test Sample Absorbance})}{\text{Control Absorbance}} \times 100$$

2.6 Acute Toxicity Studies

The acute toxicity studies for the different extracts (E1, E2, E3, E4) of *Salacia fruticosa* aerial parts were conducted in accordance with OECD guideline number 423. Each extract was given orally to the animals at doses of 5, 50, 300, and 2000 mg/kg. After receiving the dosages, they were then watched for 24 hours, 48 hours, and 14 days to look for any indications of mortality. The results indicated that all the extracts of *Salacia fruticosa* aerial part were found to be safe at all dosage levels. There were no reported instances of mortality when the rats were

administered aqueous, ethanol, chloroform, and acetone extract orally.[13]

2.7 Anti-bacterial study of *Salacia fruticosa*

2.7.1 Microorganisms

A total of ten bacterial cultures (*Staphylococcus lentus*, *Staphylococcus albus*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus lentus*, *Vibrio cholerae*, *Salmonella enterica*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) were used in this study. The National Collection of Industrial Microorganisms (NCIM), located in Pune, India, provided all the cultures. Before being subjected to antimicrobial testing, the cultures were sub-cultured on nutrition agar medium and kept on nutrient agar slants at 4°C.

2.7.2 Disc Diffusion Assay

The antibacterial activity of different extracts of *Salacia fruticosa* aerial parts was assessed using the standard disc diffusion assay method.[14,15] To conduct this test, all bacterial strains were first cultured in Muller Hinton Broth medium (Himedia) for 24 hours at 37°C. Subsequently, they were plated on Muller Hinton Agar (Himedia) to prepare them for the agar diffusion experiments.

For the actual assay, 0.1 ml of each bacterial culture was evenly distributed across the surface of an agar plate. Sterile discs, each with a diameter of 6 mm, were then placed onto the agar medium. These discs were loaded with 20 µL of various concentrations of different extracts of *Salacia fruticosa* aerial parts and incubated for 24 hours at 37°C. To serve as a negative control, solvent-only loaded blank discs were examined for antibacterial activity. Following the incubation period, the inhibition diameters were measured to determine the antibacterial effects of the different extracts of *Salacia fruticosa* aerial parts as well as to establish the baseline for the negative control discs.

2.7.3 Minimum Inhibitory Concentration (MIC)

The assessment of the antimicrobial efficiency of diverse extracts and combined fractions obtained from the aerial parts of *Salacia fruticosa* was carried out by establishing MIC values via a microdilution broth procedure. In this process, the leftover materials from the extracts and combined fractions were initially dissolved in DMSO and then diluted using broth at a 1:9 ratio, resulting in a concentration of 100 mg/ml for the extracts and 1 mg/ml for the combined fractions.

Subsequent serial dilutions within the broth were executed to generate a concentration range spanning from 1000 to 1 mg/ml. In a set of microtubes, 50 ml of bacterial suspension were blended with 50 ml of these serially diluted residues. A control microtube, similarly inoculated, contained 50 ml of broth diluted with 10% DMSO.

Following incubation, which lasted 24 hours at 37°C for aerobic bacteria and 48 hours for anaerobic strains, the MIC values were identified as the lowest concentration of antimicrobial residue that effectively impeded visible bacterial growth. To ensure precision, each test was conducted a minimum of two times.[14,15]

3. RESULTS

3.1 Physicochemical Evaluation

The physicochemical analysis of the aerial parts of *Salacia fruticosa* is presented in Table 1. The moisture content of 8.21% ± 1.23 in the powder's weight was found. The overall value of the ash was 7.18% ± 0.18. From the studies, it was evident that most of the components are soluble in alcohol (12.45 ± 1.34). Hence, ethanol was used as the extraction solvent based on this value. The powder sample was negative for microorganisms according to microbiological investigations.

S. No.	Phytochemical Compound	Result
1	Total Moisture Content	8.21 ± 1.23
2	Total Ash Value	7.18 ± 0.18
3	Water Soluble Ash Value	1.34 ± 0.25
4	Alcohol Soluble Extractive	12.45 ± 1.34
5	Acid Insoluble Ash Value	3.18 ± 0.27
6	Sulphated Ash Value	7.18 ± 1.67
7	Water Soluble Extractive	8.73 ± 0.12

Table 1: Physicochemical properties of aerial parts of *Salacia fruticosa*

3.2 Phytochemical Evaluation

Different extracts of *Salacia fruticosa* aerial parts from distinct accessions were subjected to phytochemical screening assays using various solvents. The E2 exhibited a higher presence of positive chemical constituents, including alkaloids, carbohydrates, flavonoids, phenols, triterpenoids, glycosides, proteins, amino acids, and saponins (Table 2) followed by E1, E3 and E4. The existence of these chemical components can yield advantageous

S. No.	Phytochemical Compound	Solvent of Extract			
		Aqueous	Ethanol	Chloroform	Acetone
1	Alkaloid	++	++	+	+
2	Carbohydrates	++	++	+	+
3	Phytosterols	+	++	++	-
4	Saponin	++	++	+	+
5	Tannin	+	++	-	-
6	Phenol	+	++	+	+
7	Proteins	++	+	+	+
8	Amino Acids	++	+	+	+
9	Flavonoids	++	++	++	++
10	Triterpenoids	++	++	++	+
11	Glycosides	+	++	+	+
12	Lignin	-	+	+	-

(+) = positive; (++) = strong positive; (-) = negative

Table 2: Phytochemical properties of aerial parts of *Salacia fruticosa*

health outcomes. Natural substances provide boundless prospects for pharmaceutical advancement because of their chemical variability. The remedial characteristics of botanical flora can be ascribed to the existence of secondary metabolites.

3.3 Estimation of Antioxidant Activity

The qualitative evaluation of the antioxidant properties of different extracts of *Salacia fruticosa* aerial parts is presented in Table 3. In comparison to the other solvents, the ethanolic extract showed an additional positive reaction in terms of free antioxidant activity. The data revealed that the ethanolic extract (73.81% ± 1.21) demonstrated the highest radical scavenging activity, followed by the aqueous (68.43% ± 0.35), chloroform (61.42 ± 1.43) and acetone (58.01 ± 0.46) extracts. These findings were contrasted with the BHT standard's antioxidant activity (91.72%).

S. No.	Extract	Response of DPPH assay	
		Qualitative	Quantitative (%)
1	Aqueous	++	68.43 ± 0.35
2	Ethanol	+++	73.81 ± 1.21
3	Chloroform	++	61.42 ± 1.43
4	Acetone	++	58.01 ± 0.46

(++) = positive; (+++) = strong positive; Each value represents mean ± SD of three replicated experiments

Table 3: Antioxidant activity of aerial parts of *Salacia fruticosa*

3.4 Anti-bacterial study of *Salacia fruticosa*

The antimicrobial action of the tested extracts (E1, E2, E3, and E4) of *Salacia fruticosa* aerial parts against various bacterial strains were assessed by measuring the diameters of the inhibition zones and determining the Minimum Inhibitory Concentration (MIC) (Table 4).

Aqueous extract demonstrates substantial inhibitory effects, with impressive inhibition zone sizes ranging from 11 to 20 mm and sensitivity ranging from 125 to 500 µg/ml MIC value. In contrast, the ethanolic extract consistently yields larger bacterial inhibition zones 15-29 mm, indicating robust antimicrobial activity against *Staphylococcus albus*, *Bacillus lentus*, *Escherichia coli*, and *Pseudomonas aeruginosa* with MIC values ranging from 62.5 to 250 µg/ml. Chloroform extract shows moderate inhibition zones between 8-17 mm with MIC values between 125 and 250 µg/ml, with varying effectiveness against different strains. Acetone extract, however, potential effectiveness primarily against *Staphylococcus aureus* and *Staphylococcus albus*, with MIC values of 125 µg/ml, but demonstrates reduced sensitivity against *Staphylococcus lentus* and *Klebsiella pneumoniae*, where MIC values exceed 500 µg/ml.

4. DISCUSSION

Amid the global threat of antimicrobial resistance, traditional antibiotics are losing their efficacy against resilient microbial strains, emphasizing the need for potent antibacterial treatments. In response, clinical microbiologists are exploring medicinal plants as promising sources of antibacterial agents for the development of novel antibacterial agents.[16] *Salacia fruticosa*, a traditional medicinal plant, is among those studied for its potential antibacterial properties, given its diverse applications as anti-inflammatory, anti-diabetic, anti-psoriatic, astringent, and liver tonic attributes.[6]

S. No.	Organisms	Diameters of the Inhibition Zones (mm)				MIC (µg/ml)			
		E1	E2	E3	E4	E1	E2	E3	E4
1	*Staphylococcus lentus*	15	21	16	11	125	125	125	>500
2	*Staphylococcus albus*	11	23	8	5	125	62.5	250	>500
3	*Staphylococcus aureus*	11	25	9	5	125	125	125	125
4	*Bacillus subtilis*	18	26	14	7	125	500	125	500
5	*Bacillus lentus*	17	27	13	11	500	125	125	500
6	*Vibrio cholerae*	18	18	17	12	500	250	250	500
7	*Salmonella enterica*	17	21	12	9	500	125	125	250
8	*Escherichia coli*	18	23	15	12	125	125	250	500
9	*Klebsiella pneumoniae*	18	15	13	8	>500	250	500	>500
10	*Pseudomonas aeruginosa*	20	29	15	11	500	125	125	500

MIC= Minimum Inhibitory Concentration; E1: Aqueous; E2: Ethanol; E3: Chloroform; E4: Acetone

Table 4: Antimicrobial activity of aerial parts of *Salacia fruticosa*

The present study involved a comprehensive assessment of the antibacterial properties of plant extracts derived from the aerial parts of *Salacia fruticosa* and the resultant phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, triterpenoids, and glycosides, among others, suggests the potential for these compounds to have antimicrobial effects. Natural products, like those found in medicinal plants, hold immense potential for drug development due to their diverse chemical structures and therapeutic properties.[17]

The study investigates the antioxidant properties of *Salacia fruticosa*, examining various solvents. Antioxidants are pivotal in countering free radicals, which are linked to diseases and aging. The ethanolic extract (E2) of *Salacia fruticosa*'s aerial parts stands out for its robust free radical scavenging activity when compared to the other extracts. It contains phenolic compounds, including flavonoids and phenolic acids, which act as potent electron donors, protecting cellular components and combatting oxidative stress. Furthermore, it addresses various free radicals and lipid peroxidation, chelates pro-oxidant metal ions, enhances endogenous antioxidant enzymes, and mitigates ROS generation due to its anti-inflammatory properties.[18,19] This multifaceted approach underscores its potential as a natural remedy against oxidative damage and its associated health concerns.

The variations in the diameters of inhibition zones observed for different bacterial strains signify significant differences in the antimicrobial potential of these extracts. The aqueous extract exhibits significant inhibitory effects, resulting in notable inhibition zones and sensitivity. In contrast, the ethanolic extract consistently produces larger bacterial inhibition zones, indicating strong antimicrobial activity against majority of the microorganisms, with very least MIC values of 62.5 µg/ml. The chloroform extract shows moderate inhibition zones displaying varying effectiveness against different strains whereas the acetone extract exhibits reduced inhibition zones and sensitivity against all strains.

The ethanolic extract's efficacy might be due to its rich phytochemical composition, involving flavonoids, phenolic compounds, and bioactive molecules. These compounds employ multiple mechanisms to disrupt bacterial cells, inhibit key enzymes, and induce oxidative stress, leading to bacterial cell death. Additionally, the ethanolic solvent might likely enhance intracellular penetration, further boosting its antimicrobial actions. The aqueous, chloroform, and acetone extracts do not exhibit the same level of efficacy. This divergence in effectiveness can be linked to variations in the solvents' ability to extract the bioactive phytochemicals responsible for these properties.[20,21]

This study underscores the potential of *Salacia fruticosa* as a valuable source of bioactive compounds with diverse applications in health and medicine, with the ethanolic extract emerging as the most promising option due to its exceptional performance in both antimicrobial and antioxidant activities.

5. CONCLUSION

In conclusion, this study's focus on plant extracts' antibacterial effects offers hope in the fight against drug-resistant bacteria. By examining the

antibacterial abilities of medicinal plants like *Salacia fruticosa*, the present research contributed to the exploration of effective ways to combat bacterial infections. These results highlight the many advantages of plant-based medicine, which can not only help with bacterial infections but also potentially provide antioxidant benefits. As antibiotic resistance becomes a growing problem worldwide, tapping into the antibacterial properties of natural resources becomes even more critical in our search for novel treatment conclusions.

Conflict of Interest: None.

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