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Phytochemical analysis and antimicrobial evaluation of *Ficus racemosa* and *Achyranthes aspera* from the Amravati Region

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Abstract

The rising threat of antimicrobial resistance has intensified the search for novel therapeutic agents from medicinal plants. This study aimed to scientifically validate the traditional use of *Ficus racemosa* and *Achyranthes aspera*, two wild edible plants (Ranbhajya) from the Amravati region, by evaluating their phytochemical constituents and antimicrobial efficacy. Leaf samples were collected, shade-dried, and extracted using ethanol and acetone via maceration. Qualitative phytochemical screening was performed, and the antimicrobial activity was assessed against clinically relevant pathogens (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi*, and *Candida albicans*) using the disc diffusion method. Phytochemical analysis revealed the presence of key bioactive compounds, including phenols, tannins, terpenoids, steroids, and carbohydrates, with variations between solvents and plant species. Antimicrobial assays demonstrated selective and significant inhibitory activity. The ethanol extract of *A. aspera* was most effective against *S. typhi* (15 mm) and *C. albicans* (14 mm), while its acetone extract showed broader activity against *S. aureus*, *K. pneumoniae*, and *S. typhi*. *F. racemosa* ethanol extract inhibited *S. aureus*, *E. coli*, *K. pneumoniae*, and *C. albicans*, whereas its acetone extract was effective against *S. aureus*, *K. pneumoniae*, and *C. albicans*. The findings confirm the antimicrobial potential of these plants and underscore the influence of extraction solvent on bioactivity. This study supports their traditional use and highlights their promise as sources of plant-based antimicrobials to combat drug-resistant infections.

Keywords: Medicinal Plants, *Ficus racemosa*, *Achyranthes aspera*, Phytochemicals, Antimicrobial Activity, Disc Diffusion, Ranbhajya.

1. Introduction

The global escalation of antimicrobial resistance (AMR) poses a severe threat to public health, diminishing the efficacy of conventional antibiotics and necessitating the discovery of novel therapeutic agents [1]. Medicinal plants, used for centuries in traditional medicine systems like Ayurveda, represent a rich and largely untapped reservoir of bioactive compounds with antimicrobial properties [2].

In India, a class of wild edible plants known as "Ranbhajya" holds significant ethnobotanical importance. These plants, consumed as food and medicine by rural and tribal communities, are renowned for their nutrient density and therapeutic potential [3]. *Achyranthes aspera* L. (Apamarga) and *Ficus racemosa* L. (Gular) are two such Ranbhajya plants widely distributed in the Amravati region of Maharashtra.

Achyranthes aspera (Amaranthaceae) is a perennial herb traditionally used to treat a spectrum of ailments, including skin diseases, asthma, cough, piles, and microbial infections [4]. Its pharmacological properties are attributed to a diverse phytochemical profile encompassing flavonoids, saponins, tannins, phenols, and alkaloids [5]. *Ficus racemosa* (Moraceae), a large tree, is extensively used in Ayurveda for managing diabetes, inflammation, diarrhea, and respiratory disorders [6]. Bioactive compounds such as tannins, flavonoids, terpenoids, and steroids have been isolated from its bark, leaves, and fruits, contributing to its documented antibacterial and antifungal activities [7].

While the medicinal properties of these plants are recognized in folklore, systematic scientific validation of their antimicrobial efficacy, particularly for specimens from the Amravati region, remains limited. Therefore, this study was designed to evaluate the phytochemical composition and in vitro antimicrobial activity of ethanol and acetone extracts of *A. aspera* and *F. racemosa* leaves against a panel of clinically significant bacterial and fungal pathogens.

2. Methodology

2.1. Collection and Authentication of Plant Material

Fresh, healthy leaves of *Achyranthes aspera* L. and *Ficus racemosa* L. were collected from forested areas in the Amravati region during the monsoon season (August–September 2023). The plants were identified and authenticated by a certified botanist. Voucher specimens were deposited in the departmental herbarium for future reference.

2.2. Preparation of Plant Extracts

The collected leaves were thoroughly washed with tap water followed by distilled water, and shade-dried at room temperature for 10-15 days. The dried leaves were pulverized into a fine powder using a mechanical grinder. Ten grams of each powdered sample were subjected to maceration with 100 mL of two different solvents (ethanol and acetone) for 72 hours at room temperature with occasional shaking. The extracts were filtered through Whatman No. 1 filter paper, and the solvents were evaporated under reduced pressure using a rotary evaporator. The resulting crude extracts were stored at -20°C in airtight containers until further use.

Fig. 1a *Achyranthes aspera Linn.*

Fig. 1B: *Ficus racemosa Linn.*

2.3. Phytochemical Screening

Qualitative phytochemical analysis of the ethanol and acetone extracts was performed using standard protocols [8, 9] to detect the presence of various bioactive constituents, including carbohydrates (Benedict's test and Fehling's test), proteins and amino acids (Ninhydrin test), glycosides, saponins (Foam test), quinones, steroids, terpenoids (Salkowski test), phenols, and tannins (Ferric chloride test).

2.4. Microbial Strains and Culture Conditions

The antimicrobial activity was tested against standard clinical isolates: Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923), Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhi* ATCC 14028), and a fungal strain (*Candida albicans* ATCC 90028). The cultures were obtained from the Department of Microbiology, Dr. Panjabrao Deshmukh Memorial Medical College, Amravati. The strains were maintained on Nutrient Agar (NA) slants at 4°C and sub-cultured in Nutrient Broth (NB) at 37°C for 24 hours before the assay.

2.5. Antimicrobial Susceptibility Testing

The antimicrobial activity was evaluated using the standard Kirby-Bauer disc diffusion method [10]. Briefly, the turbidity of the microbial suspensions was adjusted to 0.5 McFarland standard (~10^8 CFU/mL). A sterile cotton swab was used to lawn the inoculum uniformly onto the surface of Mueller-Hinton Agar (MHA) plates. Sterile 6 mm filter paper discs were impregnated with 20 µL of each plant extract (100 mg/mL concentration) and placed aseptically on the inoculated agar plates. Discs impregnated with the respective pure solvents (ethanol and acetone) served as negative controls. The plates were incubated at 37°C for 24 hours. The antimicrobial activity was determined by measuring the diameter of the zones of inhibition (ZOI) in millimeters (mm). All tests were performed in triplicate.

3. Results and Discussion

3.1. Phytochemical Analysis

The qualitative phytochemical screening of *A. aspera* and *F. racemosa* leaf extracts revealed a diverse profile of secondary metabolites, as summarized in Table 1. Both plants tested positive for carbohydrates, phenols, and tannins in most extracts. *A. aspera* acetone extract showed a richer profile, containing quinones, steroids, and terpenoids, which were absent in its ethanol extract. Conversely, for *F. racemosa*, steroids and terpenoids were detected only in the ethanol extract. Proteins, amino acids, cardiac glycosides, and saponins were not detected in any of the extracts.

Table 1: Qualitative Phytochemical Analysis of *A. aspera* and *F. racemosa* Leaf Extracts

Phytochemical Test	*A. aspera*		*F. racemosa*	
	Acetone	Ethanol	Acetone	Ethanol
Carbohydrates	+	+	+	+
Proteins/Amino acids	-	-	-	-
Cardiac Glycosides	-	-	-	-
Saponins	-	-	-	-
Quinones	+	-	-	-
Steroids	+	-	-	+
Terpenoids	+	-	-	+
Phenols	+	+	+	+
Tannins	+	+	+	+

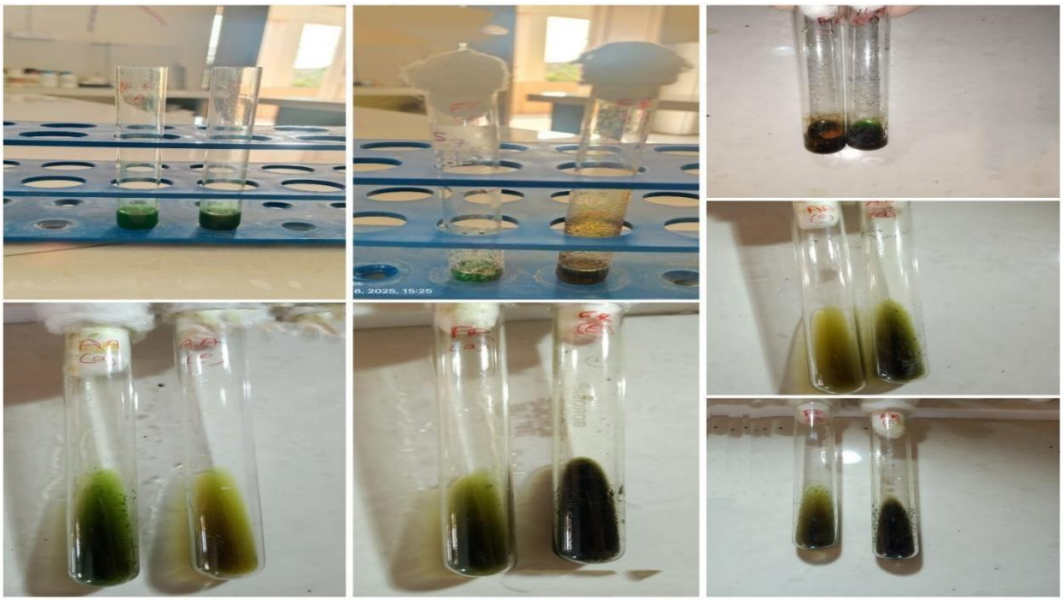

Fig. 2: . Phytochemical Analysis

Table 2: Antimicrobial Activity (Zone of Inhibition in mm) of Plant Extracts

Test Microorganism	*A. aspera*		*F. racemosa*	
	Acetone	Ethanol	Acetone	Ethanol
	-	10	14	11
S. aureus	-	-	10	-
E. coli	15	11	-	-
S. typhi	-	12	10	10
K. pneumoniae	11	11	-	-
P. aeruginosa	14	-	10	11
C. albicans	-	10	14	11

(–): No inhibition zone

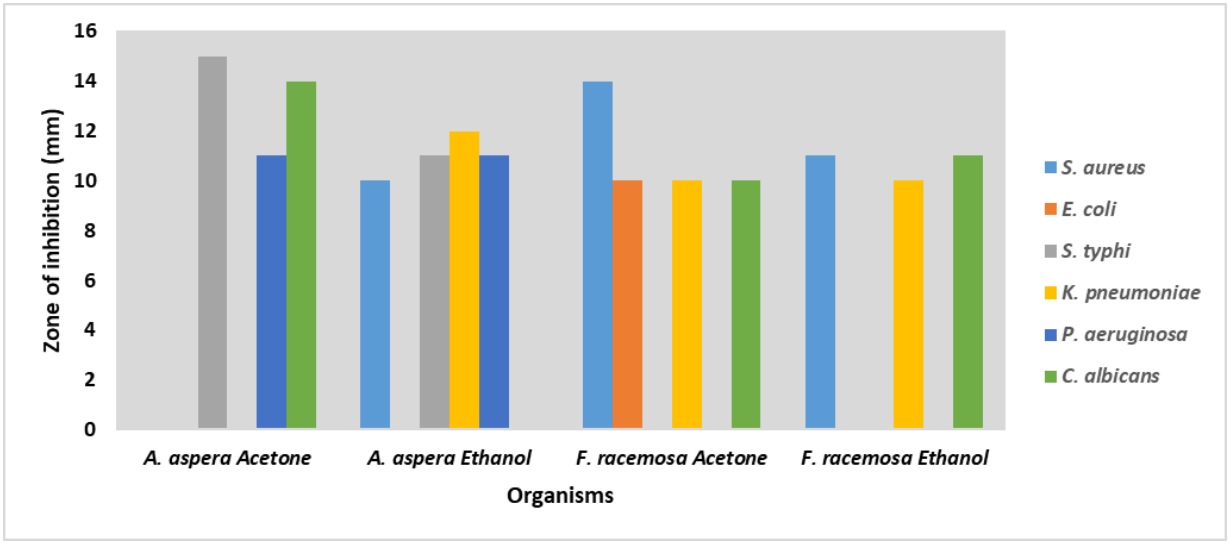

Fig. 3 : Antimicrobial Activity (Zone of Inhibition in mm) of Plant Extracts

Achyranthes aspera: The ethanol extract was most effective against *S. typhi* (15 mm ZOI) and *C. albicans* (14 mm ZOI), with moderate activity against *P. aeruginosa* (11 mm). The acetone extract showed activity against *S. aureus* (10 mm), *K. pneumoniae* (12 mm), *S. typhi* (11 mm), and *P. aeruginosa* (11 mm). Neither extract was active against *E. coli*.

Ficus racemosa: The ethanol extract exhibited activity against *S. aureus* (14 mm), *E. coli* (10 mm), *K. pneumoniae* (10 mm), and *C. albicans* (10 mm). The acetone extract was effective against *S. aureus* (11 mm), *K. pneumoniae* (10 mm), and *C. albicans* (11 mm). No activity was observed against *S. typhi* or *P. aeruginosa* with either extract.

The negative control discs (pure solvents) showed no zones of inhibition.

4. Discussion

The present study provides scientific evidence supporting the traditional use of *A. aspera* and *F. racemosa* as natural antimicrobial agents. The phytochemical screening confirmed the presence of several bioactive compounds known for their therapeutic properties. Phenols and tannins, which were detected in all extracts, are well-documented for their antimicrobial action through protein denaturation and enzyme inhibition [11]. The presence of terpenoids and steroids in specific extracts of both plants is significant, as these compounds can disrupt microbial cell membranes [12].

The antimicrobial results demonstrate that the efficacy of the plant extracts is highly dependent on the solvent used for extraction and the target microorganism. The superior extraction of certain antimicrobial compounds by ethanol (a polar solvent) and acetone (a solvent of intermediate polarity) aligns with their ability to dissolve a wide range of phytochemicals. For instance, the strong activity of *A. aspera* ethanol extract against *S. typhi* and *C. albicans*, and the broader spectrum of its acetone extract, suggest that different suites of antimicrobial compounds were solubilized by each solvent.

Similarly, the activity of *F. racemosa* ethanol extract against *E. coli*—an activity absent in the *A. aspera* extracts—highlights the species-specific nature of the bioactive compounds. The consistent activity of both plants against *S. aureus* and *K. pneumoniae* is promising, given the clinical importance of these pathogens. The antifungal activity against *C. albicans* further broadens the therapeutic potential of these plant extracts.

The absence of activity against certain pathogens, such as the lack of effect of *F. racemosa* on *P. aeruginosa*, could be attributed to the intrinsic resistance mechanisms of this bacterium, including its efflux pumps and low permeability outer membrane [13]. The results are in concordance with previous studies that have reported antimicrobial properties in these plants [5, 7], but also highlight the geographical and methodological variations that can influence bioactivity.

5. Conclusion

This study successfully demonstrates that leaf extracts of *Achyranthes aspera* and *Ficus racemosa* from the Amravati region possess significant antimicrobial properties against a range of pathogenic bacteria and fungi. The phytochemical profile confirmed the presence of antimicrobial compounds, and the bioactivity was found to be dependent on the extraction solvent. The findings validate the ethnomedicinal use of these plants and position them as promising candidates for the development of novel plant-based antimicrobials. Future work should focus on the bioassay-guided fractionation of the active extracts to isolate and characterize the specific bioactive compounds, determine their minimum inhibitory concentrations (MIC), and evaluate their safety and efficacy in in vivo models.

Conflicts of interest: The authors stated that no conflicts of interest.

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