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Analysis of Antimicrobial Activity of *Woodfordia fruticosa* Plant Extract Against Bacterial and Fungal Human Pathogens

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Abstract

The present study investigates the antimicrobial effects of *Woodfordia fruticosa* (Dhataki) using crude methanolic extracts of its leaves and flowers against five bacterial and two fungal pathogens through disc diffusion assay. Both extracts exhibited varying degrees of inhibitory activity. The leaf extract was effective against all tested pathogens except *Aspergillus niger*, while the flower extract showed no inhibitory effect against Escherichia coli and Streptococcus pneumoniae. This research suggests that substituting synthetic antibiotics with medicinal bioactive compounds from *Woodfordia fruticosa* could eliminate the side effects of synthetic drugs, offering a safer, long-term alternative for treating infections.

***Key words*:** *Woodfordia fruticosa, Aspergillus niger,* Antimicrobial activity, Plant extract, Human pathogens

The field of herbal medicine holds significant importance in traditional medical practices worldwide. Intensified studies on medicinal plants, steeped in folklore reputation, are crucial not only to encourage their appropriate use but also to explore their potential as novel sources for drug discovery [1], [3], [7], [19]. In the last two decades, there has been a notable surge in interest toward investigating natural materials as reservoirs of fresh antibacterial agents. Various extracts derived from traditional medicinal plants have undergone rigorous testing to ascertain their therapeutic properties. Consequently, certain natural products have gained approval as new antibacterial medications. Nevertheless, there remains an imminent necessity to identify innovative compounds capable of combatting pathogens displaying heightened resistance levels [6], [17].

In recent times, the global escalation of multiple drug resistance has emerged due to the widespread and uncontrolled utilization of conventional antimicrobial drugs employed in treating various infectious diseases [20]. This mounting issue signifies a pressing concern, with microbial agents displaying reduced susceptibility to standard antibiotics and the proliferation of highly resistant strains during antibacterial treatment observed worldwide [21]. Consequently, there's an urgent demand for innovative approaches [21] to combat this challenge. Natural compounds derived from higher plants offer a promising avenue for the development of novel antimicrobial agents, potentially possessing distinctive mechanisms of action [4], [9], [13-14]. Unlike synthetic drugs, these plant-based antimicrobials are often associated with fewer side effects and demonstrate immense therapeutic potential in managing various infectious diseases [10]. Notably, several essential drugs such as vincristine for cancer treatment, digitalis as a heart regulator, and ephedrine for bronchodilation initially originated from plant-based research. For instance, salicylic acid, a precursor to aspirin, was originally derived from white willow bark and meadowsweet plants, while digoxin was sourced from foxglove. Cinchona bark provided quinine, a vital remedy against malaria. Vincristine, beneficial in specific cancer types, finds its origin in the periwinkle plant. Additionally, the opium poppy stands as a source for morphine, codeine, and paregoric, an effective remedy for diarrhea [8]. These examples underscore the substantial contributions of plant-derived compounds to modern medicine.

This study represents the inaugural exploration into the antimicrobial potential of the Indian medicinal plant, *Woodfordia fruticosa* (Lythraceae), against a spectrum of human pathogens. The extract demonstrated significant antibacterial properties, attributed to the high tannin content in the flowers. The research focused on assessing the crude methanol extract obtained from the flower of *Woodfordia fruticosa* for its potential antibacterial properties, prompted by its traditional medicinal use. Notably, the flowers of this plant are rich in tannins, known for their diverse properties including astringency, acridity, refrigeration, stimulation, styptic abilities, uterine sedation, anthelmintic qualities, constipation alleviation, antibacterial effects, wound healing, detoxification, and fever reduction [2]. Previously isolated constituents from the *Woodfordia fruticosa* flower include ellagitannin dimers [24], exhibiting astringent and haemostatic properties that influence histamine release, alongside Woodford in C [12], identified for its antitumor activity [5]. Investigations have indicated the plant extract's capacity to stimulate intestinal contractions, supporting its traditional use in managing bowel disorders. Moreover, the plant extract demonstrates antipyretic

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actions comparable to acetylsalicylic acid and finds application in powdered form for ulcers and wounds, reducing discharge and fostering granulation [11].

MATERIALS AND METHODS

Sterile conditions

Standard scientific laboratory practices were followed during the work. All the microbiological wok was performed in laminar air flow cabinet. Hands gloves, mouth cap and apron were used during handling of microbial cultures.

Isolation of microorganisms

Bacterial and fungal pathogens tested in this study were isolated from clinical samples of suspected symptomatic patients. five bacterial namely *Pseudomonas aeruginosa, Escherichia coli, Streptococcus pneumoniae, Staphylococcus aureus,* and *Bacillus subtilis* two fungal pathogens *Saccharomyces cerevisiae* and *Aspergillus niger* were isolated and confirmed by Staining, Morphological and Biochemical characteristics.

Preparation and maintenance of stock culture

The clinical isolates of bacterial and fungal pathogens were inoculated on nutrient agar slants and potato dextrose agar respectively and incubated overnight at 37 °C. These cultures were stored in a refrigerator at 4 °C. Fresh slopes cultures were prepared every 3-4 weeks until subjected to further antimicrobial study. For daily working the microbial colonies (Bacterial) were streaked on MH agar plates and the fungal pathogens were streaked on SDA in test tubes.

Collection and storage of plant materials

The samples of *Woodfordia fruticosa* for antibacterial and antifungal activity testing were collected from Bhandardara, Dist. Ahmednagar. The leaves and flower parts used in traditional medicines were collected in cotton bags by cutting it into small pieces. Authentication of plant material was done at Dept. of Botany, P.V.P. College, Pravaranagar, District Ahmednagar, Tal –Rahata. After collection of the plant material namely leaves, root, & fruits parts were cleaned (running tap water for 10 min and then with distilled water for 5 min), shade dried (10 to 15 days) and powdered by using mixer grinder. The powdered plant material was stored at 4oC until further use.

Preparation of plant extract

The plant extracts were obtained by using Soxhlet apparatus by passing 30 – 40 cycles of methanol as solvent. The excess solvent present in the extract were allowed to evaporate by incubating for 5 days at 40 ^{o}C in hot air oven. The remaining weight of extract was measured.

Preparation of inoculum culture

The inoculums for most of the microorganisms were prepared by transferring a loopful of culture of microorganisms from the nutrient media slant to a tube containing 5ml of liquid media (Muller and Hilton broth in case of bacteria and (0.85%) Saline solution in case of Fungi). The concentration (Total count) of overnight grown cultures was determined by using McFarland scale [17].

Inoculating the agar plates

Approximately 15ml of sterilized agar medium was poured in autoclaved petri-plate for base agar under sterilized condition. The overnight grown cultures of turbidity approximately $3x10^{8}$ bacterial and fungal were used to inoculate the Muller and Hilton agar (bacteria) and methylene blue agar (fungi) assay petri plates respectively [14]. The inoculums of bacteria were transferred into petri dish containing solid nutrient media of agar using sterile swab for making lawn culture [15].

Preparation of paper disc for antimicrobial for disc diffusion assay

From the 1 g/ml extract stock solution, sterilized paper disc of 6mm diameter prepared from Whatman filter paper and were impregnated with plant extract to get concentration on disc as 5mg, 10 mg, 20 mg, 30 mg and kept for drying in hot air oven at 40^{o}C for overnight for evaporating the solvents.

Placing extracts discs and incubation

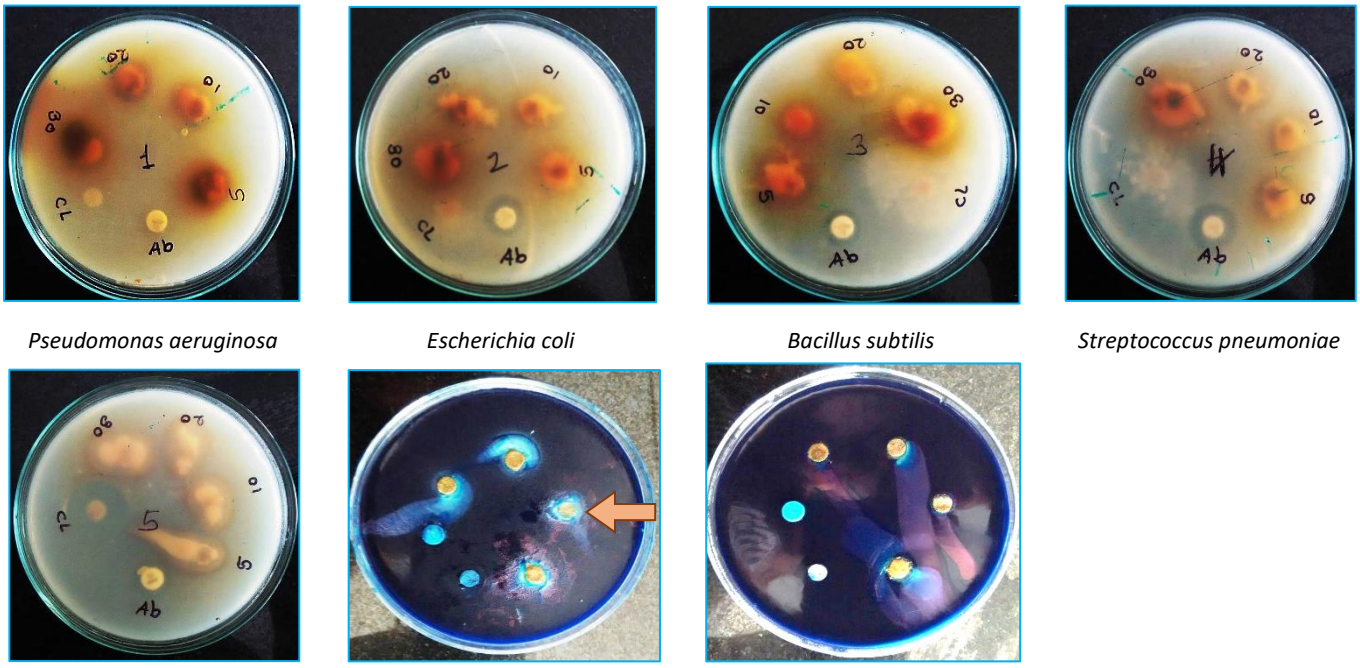
Dried discs containing extracts of plant material and control discs were then aseptically placed on the bacterial or fungal smear in Muller & Hilton agar and Methylene blue agar assay dish by using flame sterilized forceps for observation of inhibition, in case of disc diffusion method. Plates were then incubated upside down for 18-24 h at 37°C. For preparation of negative & positive control disc negative control discs were prepared by imprinting the discs with methanol. Similarly, standard positive control discs (containing antibiotics) were used in the study. Resulting zones of inhibition were observed and recorded as positive and negative. The inhibitory zone around test paper discs indicated absence of bacterial growth and that was recorded as positive and absence of zone as negative.

Table 1 Comparative results of the inhibition formed by the methanol extract prepared from different plant parts of *Woodfordia fruticosa* against bacterial and fungal opportunistic pathogens tested by disc diffusion assay

Plant name	Extraction solvent	Plant part	Concentration of extract mg/ml	Inhibition of pathogens against respective extract						
				1	2	3	4	5	6	7
				Pseudomonas aeruginosa	*Escherichia coli*	*Bacillus subtilis*	*Streptococcus pneumoniae*	*Staphylococcus aureus*	*Saccharomyces cerevisiae*	*Aspergillus niger*
Woodfordia fruticosa	Methanol	Leaf	5	+	+	+	+	+	+	-
			10	+	+	+	+	+	+	-
			20	+	+	+	+	+	+	-
			30	+	+	+	+	+	+	-
		Flower	5	+	-	+	-	+	+	+
			10	+	-	+	-	+	+	+
			20	+	-	+	-	+	+	+
			30	+	-	+	-	+	+	+
Disc with methanol			-Ve control	-	-	-	-	-	-	-
Antibiotic disc			+Ve control	+	+	+	+	+	+	+

RESULTS AND DISCUSSION

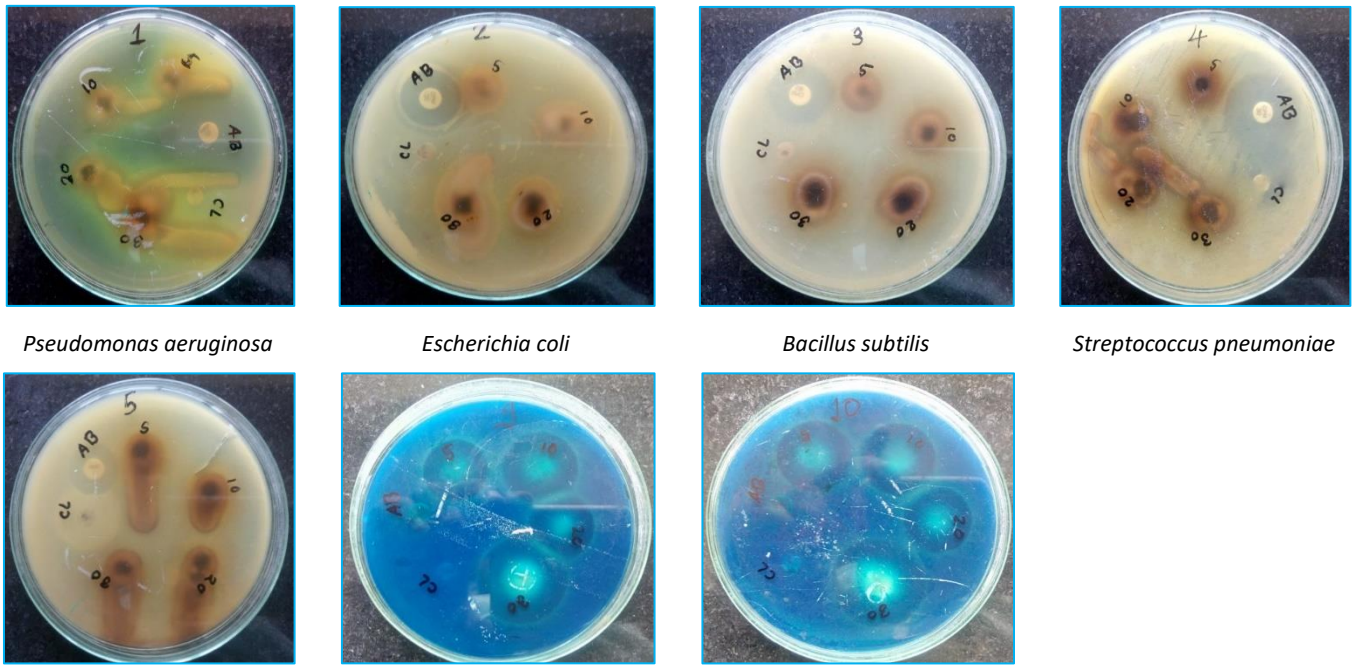
Woodfordia fruticosa plant parts i.e. leaf and flower extracts were prepared with organic solvents such as methanol by using Soxhlet apparatus. 1g/ml stock solutions of the respective extract were prepared with methanol and were used to prepare the discs of four different concentrations i.e. 5, 10, 20, 30 mg/ml. The antimicrobial activities of the extracts were tested on 5 bacterial and 2 fungal human pathogens by using disc diffusion method. It was found that leaf and flower methanolic extract exhibited significant antimicrobial activity against both bacterial and fungal pathogens.



Pseudomonas aeruginosa *Escherichia coli* *Bacillus subtilis* *Streptococcus pneumoniae*

Staphylococcus aureu *Saccharomyces cerevi* *Aspergillus niger*

Plate 4.1 Antibacterial and Antifungal activity of different concentration of *Woodfordia fruticosa* leaves extract with Methanol as a solvent



Pseudomonas aeruginosa *Escherichia coli* *Bacillus subtilis* *Streptococcus pneumoniae*

Staphylococcus aureu *Saccharomyces cerevi* *Aspergillus niger*

Plate 4.2 Antibacterial and antifungal activity of different concentration of *Woodfordia fruticosa* flower extract with Methanol as a solvent

Paper disc impregnated with different concentration i.e. (5, 10, 20 and 30mg/ml) of methanolic extract of leaves exhibited antimicrobial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Saccharomyces cerevisiae* except *Aspergillus niger* were found to be resistant. Methanolic flower extract also found to be effective against *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Saccharomyces cerevisiae* and *Aspergillus niger* except *Escherichia coli* and *Streptococcus pneumoniae* were shown resistance at all the concentration on the extract impregnated paper disc. Research screened the antimicrobial acvitity of six extracts (two each of methanol, chloroform and hexane) prepared from leaf and flower samples of *Woodfordia fruticosa* against 14 microorganisms by disk diffusion method. Among six extracts examined, 66% extracts showed antimicrobial property against Bacillus subtilis; 50% extracts against *Staphylococcus aureus*, *Salmonella typhi*, *Salmonella*

paratyphi, Citrobacter frendii each; 33% extracts against *Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Shigella dysenteriae* each, and 16% extracts against *Enterobacter spp*., *Acenitobacter spp*., each. It concluded that extracts were more likely to inhibit Gram-positive bacteria with respect to Gram-negative bacteria [12]. Similar study was performed for evaluation of antimicrobial property of the *Woodfordia fruticose* leaves aqueous, ethanol and methanol extract though agar well diffusion assay against eight Grams negative and positive bacteria. The highest zone of inhibition was observed in Methanolic extract in *Shigella* is 23.0mm, In Ethanolic extract; it was 22.0 mm in *Shigella.* He concluded that Methanolic shows good zone of inhibition in almost bacteria and Ethanolic and Methanolic extracts can be used in the treatment of infection caused by these bacterial strains used during this study [4].

CONCLUSION

Our investigation validates the therapeutic effectiveness attributed to *Woodfordia fruticosa* in traditional medicine. Furthermore, these findings provide a strong rationale for selecting this plant for more extensive exploration in phytochemical and pharmacological studies. The outcomes of this research not only affirm the traditional usage of the investigated plant but also indicate the presence of specific constituents within the plant extract that exhibit antibacterial properties. These constituents hold potential for application as antimicrobial agents in the development of novel drugs intended for treating infectious diseases caused by pathogens. The most promising extracts showing high activity merit isolation to identify and evaluate the therapeutic antimicrobial compounds further in pharmacological studies.

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