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ANTI BACTERIAL ACTIVITY OF THE FLOWERS OF *WOODFORDIA FRUTICOSA* ON DIFFERENT MICRO-ORGANISM

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ABSTRACT: Use of plants as a source of traditional healing systems around the world that utilize herbal remedies is an important source for the discovery of new antimicrobials against resistant strains of bacteria**.** It is being used as a source of medicinal agents for antibacterial, antihelminthic, astringent, emetic, sedative and stimulant. A 10 g dried leaves powder of *Woodfordia fruticosa* was extracted separately in each different solvent i.e., aqueous, ethanol and methanol. The extract was stored at 4ºC in airtight bottles until further uses. Total 8 strains including gram positive and gram negative bacteria were selected to assess the susceptibility test against the different drug extract. These are *Escherichia coli* (ATCC-25922), *Pseudomonas aeroginosa* (ATCC-27853), *Salmonella paratyphi* (ATCC-9150), *Salmonella typhimorium* (ATCC-25241), *Shigella sonnei* (ATCC-25931), *klebseilla pneumonia* (ATCC-27736), *Staphylococcus aureus* (ATCC-25923) and *Proteus vulgaris* (ATCC-6380). The antibacterial activity was performed in vitro using Agar well diffusion assay and diameter of zone of inhibition was measured. The Methanolic shows good zone of inhibition in almost 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. The results suggest that Ethanolic and Methanolic extracts can be used in the treatment of infection caused by these bacterial strains used in this study.

INTRODUCTION: Use of plants as a source of traditional healing systems around the world that utilize herbal remedies is an important source for the discovery of new antimicrobials against resistant strains of bacteria [1].

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Many medicinal plants have anti-microbial properties uses in traditional Indian system of medicine mostly in Ayurveda & Unani, one of them is *Woodfordia fruticosa* Kurz, The English name of *Woodfordia fruticosa* is Gul-e-dhawa or Fire flame bush and as a Dhawa known in Unani System of Medicine. Leaves are also uses for therapeutic purpose. It is being used as a source of medicinal agents for antibacterial, antihelminthic, astringent, emetic, febrifuge, sedative and stimulant. The decoction of the flower is used for hemorrhage, burns, diabetes, leprosy and skin diseases [2, 3].

Natural products either as pure compounds or as standardized plant extracts provide unlimited opportunities for new drug. Therefore, researchers are increasingly turning their attention to traditional and folk medicine to develop better drugs against microbial infections [4]. The flowers of this plant possess high content of tannins and they have astringent, refrigerant, stimulant, uterine sedative, constipating, and antibacterial properties [5, 6].

The previously isolated classes of constituents from *Woodfordia fruticosa* flower are ellagitannin dimmers [7], with astringent and haemostatic properties that affect histamine release. It is used in menorrhagia, leucorrhoea [8] and antitumor activity [9]. The dried flowers are powdered and sprinkled over ulcers and wounds to diminish discharge and promote granulation [10]. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action [11].

MATERIALS AND METHODS:

Plant material: *Woodfordia fruticosa* (Gul-e-Dhawa) were procured from the local market of Hyderabad and was properly identified by the classical and botanical literature available and then further confirmation of the flower by Dr. V.C. Gupta, Department of Botany, Central Research Institute of Unani Medicine Erygadda, Hyderabad. The dried flowers were homogenized to fine powder and further subjected to extraction**.**

Crude Extraction: A 10 gm. dried leaves powder of *Woodfordia fruticosa* was extracted separately in each different solvent i.e. Aqueous, Ethanol and Methanol. Now the 10 gm. leaves powder was taken in 50 ml. of solvent in 100 ml beaker and kept on rotary shaker for 24 hrs at room temperature. The extract was filtered through 42 mm Whatmann filter paper and was dried on water bath. The extract was stored at 4ºC in airtight bottles until further uses.

Micro-organisms tested: Total 8 strains including gram positive and gram negative bacteria were selected to assess the susceptibility test against the different drug extract. These are *Escherichia Coli* (ATCC-25922), *Pseudomonas aeroginosa* (ATCC-27853), *Salmonella paratyphi* (ATCC-9150), *Salmonella typhimorium* (ATCC-25241), *Shigella sonnei* (ATCC-25931), *Klebseilla pneumonia* (ATCC-27736), *Staphylococcus aureus* (ATCC-25923) and *Proteus vulgaris* (ATCC-6380). The investigated microbial strains were obtained from National Chemical Laboratory (NCL), Pune, India. The organisms were maintained on nutrient agar slope at 4ºC and activated by sub culturing [12]. The antibacterial activity was performed *in vitro* using Agar well diffusion assay and diameter of zone of inhibition was measured (**Fig. 1**).

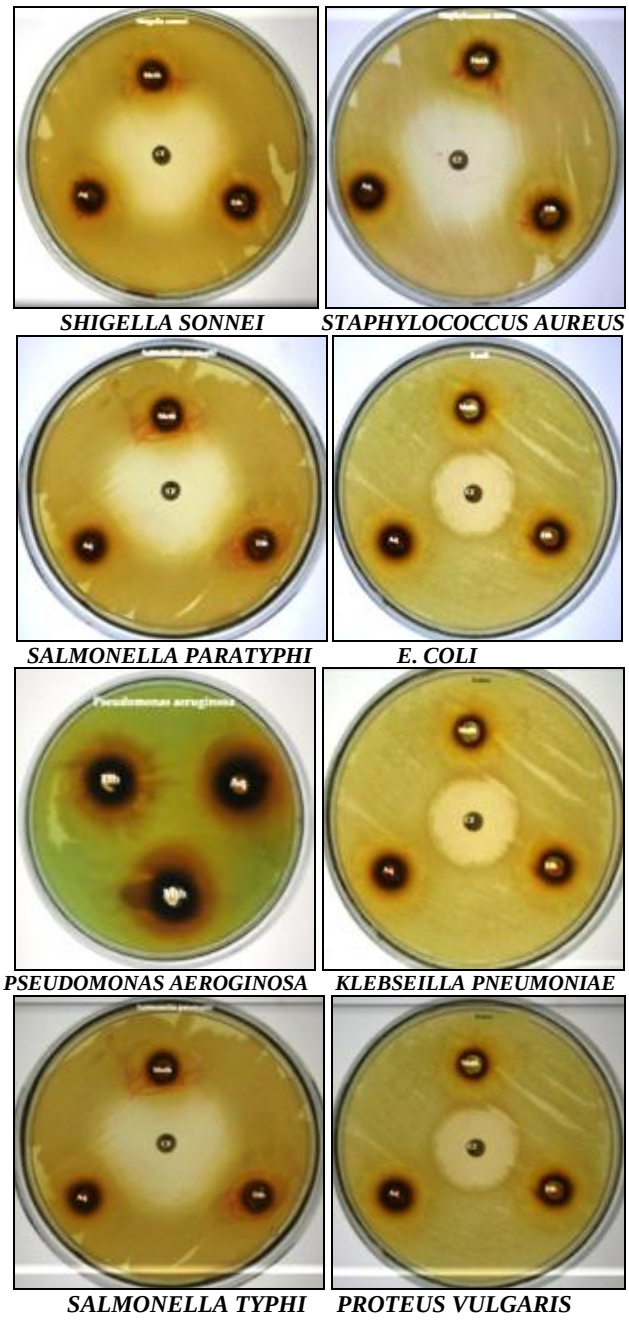

FIG. 1: ANTIBACTERIAL ACTIVITY (ZONE OF INHIBITION) OF DIFFERENT SOLVENT EXTRACTS OF *W. FRUTICOSA* SHOWING IN DIFFERENT PETRI PLATES

Antibacterial Assay: Antibacterial activity of the crude extracts in different solvents was tested by disc diffusion assay [13], Mueller Hinton agar no. 2 (Hi Media, India) was used as the bacteriological medium. Medium was prepared and poured 20 ml each in sterilized Petri plates of 9 cm diameter and allowed to solidify. Bacterial cultures grown in nutrient broth and on agar slants were used. Bacterial suspension was prepared aseptically from 10 ml of saline (0.085 g NaCl in 10 ml Distilled water) under laminar.

The plates, cultured with microbial suspension (100-150 µl) by spread plate technique. The zone of inhibition was measured after 24 hrs using disc diffusion assay. The concentration of extract was 10 mg/100 µl and 4 µl of each extract was used for antibacterial assay. For each bacterial strain controls were maintained where extract free pure solvents were used. The control zones were subtracted from the test zones and the resulting zone diameter [14] is shown in the **Table 1**.

Test Drug: Different extracts of *Woodfordia fruticosa* were used in this Study i.e. Ethanol, Methanol, Aqueous with the Concentration of 10 mg/100µl.

RESULTS: Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The data pertaining to the antibacterial potential of the plant extracts are presented in Table 1.

Results obtained in the present study relieved that tested medicinal plant extracts possess potential antibacterial activity against all selected bacteria. Among all the extracts Ethanolic and Methanolic extracts showed maximum antibacterial activity against all the bacterial strain used with a zone of inhibition ranges from 7.4-23.0 mm and the least activity was observed in Aqueous extract with zone of inhibition ranges from 6.0-15.5 mm.

The standard antibiotic Ciprofloxacin (1mg/ml.) shows highest zone of inhibition against *Salmonella paratyphi* i.e. 35 mm and the test drug was against *Shigella sonnei* i.e. 23mm of zone of inhibition. The plant extracts were also screened for qualitative analysis to know the presence of Phytochemical which may be responsible for the potent antibacterial activity [15] which is shown in **table 2**.

TABLE 1: ANTIBACTERIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF *W. FRUTICOSA*. DATA REPRESENTING N=3 ± SE

Sl. No.	Organism	Zone of Inhibition (mm.)			
		Ethanol	Methanol	Aqueous	Standard antibiotic: Ciprofloxacin
1.	*Staphylococcus aureus*	15.2±0.3	12.2±1.0	15.5±0.1	25±1.0
2.	*Salmonella typhimorium*	13.3±1.4	16.5±0.77	6±0.87	30±0.98
3.	*Salmonella paratyphi*	11.4±0.23	14.2±1.23	11.5±0.12	35±0.54
4.	*Shigella sonnei*	22±0.67	23±0.98	18.5±1.0	27±0.23
5.	*Proteus vulgaris*	7.5±0.12	8±0.41	11.5±0.35	30±0.13
6.	*Echerichia coli*	17.5±1.18	11.5±1.0	14±2.09	27±0.54
7.	*Pseudomonas aeruginosa*	14.5±0.56	13±0.97	13.5±0.49	30±1.12
8.	*Klebsiella pneumonia*	8±1.24	7.4±2.65	-	26±0.67

TABLE 2: PHYTOCHEMICAL ANALYSIS OF THE EXTRACTS OF THE FLOWERS OF *WOODFORDIA FRUTICOSA* SHOWED PRESENCE OF FOLLOWING PHYTOCHEMICALS

Phytochemicals	Name of Test	Solvents		
		Methanol	Ethanol	Aqueous
Sterol	Salkowski	+	+	+
Anthraquinone	Bromine test	+	+	-
Alkaloid	Dragendroff's	-	-	-
Tannin	Ferric chloride	+++	++	+
Flavonoid	Shinoda, NaOH	+	+	-
Fats and oil	Manual method	-	-	+
C. Glycoside	Conc.H_2SO_4, Aq. NaOH	+	+	+

DISCUSSION: The study shows that the crude Methanolic extract of *Woodfordia fruticosa* showed more pronounced antibacterial activity as compared to Aqueous extract and Ethanolic extract which is shown in fig. 1. At the molecular level *Woodfordia fruticosa* contains some chemical constituent like Tannin & Flavonoid [16]. Flavonoids, are known to be synthesized by plants in response to microbial infection, it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls, *Woodfordin fruticosin* C is the tannins which shows inhibitory activity towards DNA topoisomerase enzyme II [17].

CONCLUSION: The results of the present study Methanolic extract of *Woodfordia fruticosa* showed more antibacterial activity. This study provides a scientific validation for the popular use of the medicinal plant studied and serves as a guide. This may help in selection of plants with antibacterial activities for further Phytochemical work and mode of action.

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