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Antibacterial Potential of Extracts of *Woodferdia fruticosa* Kurz on Human Pathogens

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Abstract: *Woodferdia fruticosa* Kurz was tested for antibacterial activity against fourteen human pathogenic bacteria. The dried flowers were extracted with deferent solvents viz., petroleum ether, chloroform, methanol, ethanol and water using soxhlet apparatus. All the solvent extracts were evaporated to dryness using rotary flash evaporator. Dry residue was dissolved in respective solvents (1:10 w/v) and tested for antibacterial activity. The result revealed that among five solvents tested, petroleum ether extracts showed significant antibacterial activity when compared with Gentamicin for human pathogens.

Key words: *Woodferdia fruticosa* Kurz · Antibacterial · Human pathogens

INTRODUCTION

Bacterial infection is one of the serious global health issues in 21st century [1]. There are several reports of antibiotic resistance of human pathogens to available antibiotics [2-6]. Antimicrobial resistance setting has failed to address these essential aspects of drug usage [7]. The multiple drug resistance and associated adverse effects of antibiotics on the host including hypersensitivity, immune –suppression and allergic reaction are growing and because of this outlook for the use of antimicrobial drugs in the future is still uncertain [8]. Natural products, either as pure compounds or as standardized plant extract, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity [9].

Thus green plants represent a reservoir of effective chemotherpeutants and provide valuable source of natural products [10,11]. In recent years, secondary metabolites, previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents [12]. Plants are rich in a wide variety of secondary metabolite, such as tannins, terpenoids, alkaloids and flavonoids, which have been found *in vitro* to have antimicrobial properties. Thousands of secondary plant products have been identified and it is estimated that thousands of these compounds still exist. Since secondary metabolites from natural resources have been elaborated within living systems, they are often perceived as showing more "drug-likeliness and biological friendliness than totally synthetic molecules" [13] making them good candidates for further drug development [14,15]. Biomolecules of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human pathogens and hence in the present investigation, the antimicrobial activity of Indian medicinal herb *Woodfordia fruticosa* Kurz. (family: Lythraceae) against fourteen human pathogens.

The selection of this plant for evaluation was based on its traditional usage. A Survey of the literature revealed that the plant has been recommended for use in various traditional systems of medicine for the treatment, among others, of bowel disorders [16]. The dried flowers of *Woodfordia fruticosa* Kurz have been also used as an astringent tonic in disorders of mucous membranes, haemorrhoids and in derangements of the liver [17]. The original Sanskrit name *Agnijwala* or *Tamra-pushpin* appears to be derived from the bright red color of the flower and the bark. In India, it is much- used medicinal plant in Ayurvedic and Unani systems of medicine [18,19]. The leaves of *Woodfordia fruticosa* are used as a folk medicine in India and Nepal. In case of fever, decoction of *Dawai* (a popular name of this plant in this region) leaves in combination with sugar and dried ginger is recommended [20]. The flowers of this plant possess high content of tannins and they have astringent, acrid,

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refrigerant, stimulant, styptic, uterine sedative, anthelmintic, constipating, antibacterial, vulnerary, alxeteric and febrifuge properties. The extracts of *Woodfordia fruticosa* Kurz. flowers showed the presence of carbohydrates, gums, flavonoids, sterols and phenolic compounds/tannins [21].

MATERIALS AND METHODS

Collection of Plant Materials: Fresh flowers of *Woodferdia fruticosa* Kurz. free from disease were collected, washed thoroughly 2-3 times with running tap water and once in sterile water, shade-dried, powdered and used for extraction.

Preparation of Extractions: Fifty gm of shade dried, powder of flowers of *Woodferdia fruticosa* Kurz were filled separately in the thimble and extracted successively with 200ml each of Petroleum ether, Chloroform, Methanol, Ethanol and Water using a Soxhlet extractor for 48 hrs. All the extracts were concentrated using rotary flash evaporator. After complete solvent evaporation, each of these solvent extract was weight and preserved at 5°C in an airtight bottle until further use. One gram of each solvent residue was dissolved in 10 ml of methanol which served as the test extracts for antibacterial activity assay and one gram of water extract was dissolved in 10ml of water which served as a the test extract for antibacterial activity assay.

Human Pathogenic Bacterial Cultures: *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 109), *Proteus mirabilis* (MTCC 1429), *Pseudomonas aeruginosa* (MTCC 1688), *Salmonella paratyphi* A (MTCC 735), *Salmonella typhi* (MTCC 733), *Salmonella typhimurium* (MTCC 98), *Shigella flexneri* (MTCC 1457), *Shigella sonnei* (MTCC 2957), *Staphylococcus aureus* (MTCC 737), *Streptococcus faecalis* (MTCC 459) and authentically identified clinical isolates of *Citrobacter* sp., *Salmonella paratyphi* B and *Shigella boydii* were obtained from the Department of Microbiology, Government Medical College, Mysore, India. All test strains were maintained on nutrient agar slopes (Hi-Media Laboratories Pvt. Limited, Mumbai, India) at room temperature and were sub-cultured, every two-weeks. These bacteria served as test pathogens for the assay.

Anti-bacterial Activity Assay: Antibacterial activity of aqueous extract and solvent extracts was determined by cup diffusion method on nutrient agar medium [22]. Cups were made in nutrient agar plate using sterile cork borer (5 mm) and inoculum containing 10^6 CFU/ml of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then 50 µl each of all aqueous and solvent were placed in the cups made in inoculated plates. The treatments also included 50 µl of sterilized distilled water and methanol separately which served as control. The plates were incubated for 24 hours at 37ºC and zone of inhibition if any around the wells were measured in mm (millimeter). For each treatment six replicates were maintained. The data was subjected to statistical analysis using SPSS for windows software. The aqueous and solvent extracts showed highest antibacterial activity, were further subjected to antibacterial activity assay at 50 µl concentrations along with synthetic antibiotic Gentamycin for comparison.

RESULTS

Widespread use of antibiotics is thought to have spurred evolutionarily adaptations that enable bacteria to survive these powerful drugs. The combat with the bacterial resistance demands a search for alternative newer molecules. For this reason, the five different solvent extracts of *Woodferdia fruticosa* in this study, were screened for their antibacterial activities. Their growth inhibitory activity was tested against fourteen human pathogens (Table 1). Petroleum ether extract was shown to be highly potent antibacterial activity. Their antibacterial activity was much stronger than that of gentamicin which is a powerful antibiotic used to overcome bacterial infections.

DISCUSSION

Plants have formed the basis of sophisticated traditional medicine system and natural products make excellent leads for new drug development. Approximately 80% of the world inhabitants rely on traditional medicine for their primary health care and plants also play an important role on the health care system of the remaining 20% of the population [23]. The rediscovery of the connection between plants and health is responsible for launching new generation of botanical therapeuticals, multicomponent botanical drugs, dietary supplements, functional foods and plant produced recombinant proteins [24]. Species of higher plants are much less surveyed for antibacterial activity [10, 25].

Table 1: Antibacterial activity of different solvent extracts of *Woodferdia fruticosa* Kurz on human pathogenic bacteria (zone of inhibition measured in mm)

	Zone of inhibition in mm(Mean±SD)					
Human pathogenic bacteria	PE	C	M	E	W	GEN
Citrobacter sp.	14.3±0.2	11.0±0.3	13.3±0.4	-	08.0±0.3	14.3±0.5
Escherichia coli MTCC 443	13.3±0.3	09.1±0.3	11.5±0.4	-	07.8±0.3	13.3±0.4
Klebsiella pneumoniae MTCC109	19.8±0.3	14.8±0.3	13.8±0.3	-	09.8±0.3	14.5±0.4
Pseudomonas aeruginosa MTCC1688	18.1±0.3	12.5±0.2	17.3±0.3	-	08.8±0.3	09.5±0.3
Proteus mirabilis MTCC1429	17.3±0.3	12.8±0.3	14.8±0.3	-	07.8±0.3	13.6±0.3
Salmonella typhi MTCC733	17.3±0.3	14.3±0.3	15.3±0.3	-	07.8±0.3	19.1±0.2
Salmonella paratyphi A MTCC735	18.6±0.3	18.0±0.3	12.3±0.4	-	09.1±0.3	17.5±0.2
Salmonella paratyphi B	21.0±0.3	13.8±0.3	12.8±0.4	-	10.1±0.3	20.5±0.3
Salmonella typhimurium MTCC98	16.1±0.3	11.5±0.4	12.1±0.3	-	07.8±0.3	15.5±0.3
Shigella boydii	21.1±0.4	14.5±0.2	14.8±0.3	-	08.8±0.3	20.5±0.6
Shigella flexneri MTCC1457	18.6±0.3	17.6±0.3	19.0±0.3	-	07.8±0.3	13.5±0.4
Shigella sonnei MTCC 2957	20.0±0.3	18.8±0.3	18.8±0.3	-	09.1±0.3	17.5±0.5
Staphylococcus aureus MTCC737	21.8±0.4	15.1±0.3	20.3±0.3	-	11.8±0.3	21.6±0.2
Streptococcus faecalis MTCC459	22.7±0.3	22.5±0.2	22.0±0.4	-	10.5±0.3	17.5±0.3

PE-Petroleum ether, C-Chloroform, M-Methanol, E-Ethanol, W-Water extract, GEN-Gentamicin, Gentamicin disc (10 µg) as a positive reference standard; Values are mean inhibition zone (mm)±S.D of six replicates, - no activity

Antibiotics provide the main basis for the therapy of bacterial infections. However, the high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. In recent years development of multidrug resistance in the pathogenic bacteria and parasites has created major clinical problems in the treatment of infectious diseases [26,27]. This and other problems such as toxicity of certain antimicrobial drugs on the host tissue [28,29] triggered interest in search of new antimicrobial substances/drugs of plant origin. Considering the rich diversity of plants, it is expected that screening and scientific evaluation of plant extracts for their anti-microbial activity may provide new anti-microbial substances. Hence the present investigation clearly reveals the antibacterial nature of this plant and suggests that this plant could be exploited in the management of diseases caused by these bacteria in human systems. From the results obtained it supports the folkloric usage of *Woodfordia fruticosa* Kurz as a therapeutic agent. In addition, this result form a good basis for selection of the plant for further phytochemical and pharmacological investigation and suggests that the petroleum ether extract contain certain constituents with antibacterial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. Since compounds of biological origin are known to posses minimal residual effect. The most active extracts can be further subjected for the isolation of therapeutic antimicrobials and carry out pharmacological evaluation.

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