

ORIGINAL RESEARCH ARTICLE

Antimicrobial Activity of Useful Parts of *Woodfordia fruticosa* (Linn.) Kurz. of Nepal

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ABSTRACT

In continuation of our screening of Nepalese medicinal plants, six extracts (two each of methanol, chloroform and hexane) prepared from leaf and flower samples of *Woodfordia fruticosa* were screened for antimicrobial activity against 14 microorganisms by disk diffusion method. The tested plant parts are used to treat ailments thought to be caused by bacterial infection, including boils, diarrhea, dysentery, fever, cough, menstrual disorders, and others. Among six extracts examined, 66% extracts showed antimicrobial property against *Bacillus subtilis*; 50% extracts against *Staphylococcus aureus*, *Salmonella Typhi*, *Salmonella paratyphi*, *Citrobacter freundii* each; 33% extracts against *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Shigella dysenteriae* each, and 16% extracts against *Enterobacter* spp., *Acenitobacter* spp., each. Extracts were more likely to inhibit Gram-positive bacteria with respect to Gram-negative bacteria. The importance of *Woodfordia fruticosa* in Nepal can perhaps be understood by the broad spectrum of its antibacterial activity. We hope that this study will encourage the researchers in Nepal to explore alternative, affordable and easily available medicines of plant origin.

Key Words: Antimicrobial activity, Medicinal plant, Nepal, *Woodfordia fruticosa***INTRODUCTION**

The diversity of medicinal plants is very high in Nepal but research on pharmacological properties is restricted to only few species. In a very real sense, only about 20% of the medicinal plants, so far documented from Nepal Himalaya, are studied, to some extent, for their biochemical property [1]. Several studies have suggested that medicinal plants offer great potential for discovery of novel molecules and new sources of active compounds, mainly because of the environmental stress to which they are subjected [2].

Plants have been a rich source of medicine because they produce many biologically active molecules, most of which likely evolved as chemical defenses against predation or interaction. Most plant species possess one or more medicinal property *viz.*, antibacterial, antifungal, antiviral, anticancer, and others. Many studies have investigated the uses of medicinal plants in Nepal [3, 4, 5, 6], but only a few species have been screened for biological activity [7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21].

Considering the negative effects of synthetic drugs people are looking for natural remedies, which are safe and effective. In this respect, medicinal plants used in the traditional therapy could be the alternative source for the development of new therapeutic agents to combat with the resistant organisms. At present a considerable number of plants derived drugs from medicinal plants have been shown to have diverse biological activities which are in the various clinical stages [22]. Therefore plants with medicinal values should be investigated by modern scientific techniques in order to establish their safety and efficacy and to determine their potential as a source of new drugs.

Now-a-days, numerous scientific investigations are going on in isolation of potent phytochemicals as lead compounds for antimicrobial therapy. Many useful plant derived drugs were discovered as a result of scientific follow up of well known plants used in traditional medicine. Even today's major diseases like cancer are being treated with an array of potential products derived from biodiversity.

Traditional medicine is the basis of health care in Nepal to treat various kinds of infectious diseases. Although some infectious diseases have been cured by modern medicines, new diseases are constantly emerging while others re-emerge in resistant forms. Therefore one of the most fruitful approaches to overcome the resistant microbes involves the search for new anti-infective agents of plant origin on the basis of an ethno-pharmacological approach. Nepal has an enormous wealth of information on ethno-pharmacology based remedies which are not only cheap and abundant but are culturally accepted.

In Nepal, the flower and leaf of *Woodfordia fruticosa* have exceptionally wide diversity of traditional uses to treat various illnesses. It is used to treat ailments that may be of bacterial origin, for example boils, diarrhea, dysentery, fever, cough, menstrual disorders, urinary disorders, wounds, swellings, cuts, skin diseases. Such wide uses of the plant in the management of various illnesses may be due to the presence of numerous bioactive compounds. Knowing the ethno botanical and pharmacological applications of the plant, the main objective of this research is to assess *in vitro* antimicrobial activity of flower and leaf samples of *Woodfordia fruticosa* against human pathogens. To screen for biological activity, the crude methanol, chloroform and hexane extracts were prepared and tested against fourteen different microorganisms. The aim of screening was to correlate and confirm the antimicrobial activity to the traditional uses of plants. This can be seen as the first step in the search for primary health care products that are socially acceptable and scientifically valuable. In Nepal, this is seen as an opportunity for sustainable income generation as well as strong means of conservation and primary health care [11, 12]

MATERIALS AND METHODS

Collection of plant samples for antimicrobial testing:

The samples for antimicrobial testing were collected from the Makawanpur and Nawalparasi districts of Nepal. The leaf and flower of the plant used in traditional medicine were collected in the cotton bags, cut into small pieces and air dried in the shade. Antimicrobial studies were carried out at Nepal Academy of Science and Technology (NAST) laboratory.

Preparation of culture media: Three media used in the study were Potato dextrose agar, nutrient agar (Becton Dickinson and Company) and

nutrient broth (DIFCO, Bacto: Dehydrated nutrient broth).

Preparation of plant extracts: Plant material was air dried and grounded in an electric grinder. Powder plant materials (each 30 g) were subjected to sequential Soxhlet extraction, using methanol, chloroform, and hexane respectively. Organic solvents were removed under reduced pressure and was evaporated *in vacuo* at 40 °C using a rotary evaporator. The crude evaporated plant extracts were dried at room temperature for 5-30 days. Then 50 mg of each crude plant extract was dissolved in 1 mL (1,000 microlitres) of the respective solvents (methanol, chloroform and hexane) to give a final concentration of crude extract in solvent of 50 mg/mL.

Preparation of test disks: From the 50mg/mL crude extract stock solution, 6 mm paper discs were impregnated with 20 µL extracts (20 µL/disc from a stock solution of 50 mg/mL) which is equivalent to 1 mg/disc. The stock solution was then subjected to serial dilution to find out the Minimum Inhibitory Concentration (MIC) using sterile solvent as the dilution medium.

Preparation of positive and negative control discs: Negative control discs were prepared by dipping the discs into methanol solution or impregnating the discs with 20 µL of the methanol. Similarly, standard positive control discs (Ciprofloxacin, Erythromycine, Gentamycine) were used in the study.

Microorganisms used: Fourteen different strains of microorganisms were used in the screening process including Gram-positive *Staphylococcus aureus*, *Bacillus subtilis* and Gram-negative *Salmonella* Typhi, *Salmonella paratyphi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Shigella dysenteriae*, *Citrobacter freundii*, *Enterococcus faecalis*, *Acinetobacter spp.* Two fungi, *Candida albicans* and *Aspergillus spp.*, were used in the study. These microorganisms were supplied by the Department of Clinical Microbiology, Teaching Hospital and the Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu.

Preparation of inoculum culture: The inoculum for most of the microorganisms was prepared by transferring a large number of microorganisms from the agar slant to a tube containing 5 mL of liquid media (nutrient broth) and incubating for 24 h at 37 °C. The tubes were shaken occasionally to aerate and promote growth. The overnight cultures

were diluted 1/5 to 1/10 with nutrient broth before use.

Inoculating the agar plates: This overnight culture (and diluted 1/5 to 1/10 with nutrient broth) was used to inoculate the nutrient agar test plates. Petri dishes is poured with 100 microliter of this diluted microorganisms solution in broth which had been growing overnight for 24 h at 37 °C and swabbed with a sterile cotton swab by rotating the Petri dishes.

Placing extracts discs and incubation:

Once inoculated, dried discs of plant extracts and controls (negative and positive) were added. The discs containing extracts of plant material and control discs were placed on inoculated agar plates aseptically. Plates were incubated upside down for 18-24 h at 37 °C.

Antimicrobial assay and Determination of Minimum Inhibitory Concentration (MIC):

The bioassay used was the standard disc diffusion assay, adapted from [17, 18, 21]. Results were recorded as presence or absence of zone of inhibition and testing was repeated three times to ensure reliability of results. The Minimum Inhibitory Concentration (MIC) method was applied on extracts that proved their high efficacy against microorganisms by the disk diffusion method. The active extract at 50 mg/mL concentration was subjected to sequential or serial dilution to find out the minimum inhibitory concentration using sterile solvent medium as a dilutant. The highest dilution of a plant extract that still retains an inhibitory effect against the growth of microorganisms is known as MIC.

RESULTS AND DISCUSSION

In this research, *in vitro* antimicrobial assays of 6 extracts (two each of methanol, chloroform and hexane) prepared from flower and leaf samples of *Woodfordia fruticosa* were examined against 14 microorganisms by disc diffusion method. Among six extracts examined, four (66%) extracts showed antimicrobial property against *Bacillus subtilis*, three (50%) extracts against *Staphylococcus aureus*, *Salmonella* Typhi, *Salmonella paratyphi*, *Citrobacter freundii* each; two (33%) extracts against *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Shigella dysenteriae* each, one extracts (16%) against *Enterobacter* spp., *Acenitobacter* spp., each. Rest of the two human pathogenic fungi, *Candida albicans* and *Aspergillus* spp., did not show any zone of inhibition against any extracts tested. It is possible that the extracts contain

antifungal compounds against pathogenic fungi other than those tested in this study.

Extracts were more likely to inhibit Gram-positive bacteria. For example, most extracts were active against *Bacillus subtilis*, and then *Staphylococcus aureus*. With respect to Gram-negative bacteria, it was more common for extracts to inhibit *Salmonella* Typhi, *Salmonella paratyphi*, *Citrobacter freundii* than *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Shigella dysenteriae*, *Enterobacter* spp., and *Acenitobacter* spp. The reason of inhibition of more extracts against Gram-positive bacteria is due to low number (2 species) of Gram-positive bacteria compared to Gram-negative bacteria (10 species) tested in this study.

The MIC method was used to further investigate extracts that showed broad spectrum activity against microorganisms. The active extract at 1 mg/disc concentration was subjected to serial dilution to find out the minimum inhibitory concentration. The highest dilution of a plant extract that still retained an inhibitory effect against the growth of microorganisms (absence of zone of inhibition) was reported as the MIC. In this study, a total of two extracts in methanol (from flower sample) and hexane (from leaf sample) showed lowest promising MIC of 0.12 mg/disc (**Table 1**). Similarly, six extracts of flower in methanol showed lowest MIC of 0.25 mg/disc. Full results can be seen in (**Table 1**).

The importance of *Woodfordia fruticosa* in the community can perhaps be understood by the broad spectrum of its antibacterial activity. Detailed phyto-chemical analysis research in the future may help to find alternative medicines from this species. The extraction with methanol, chloroform and hexane crudely separated the chemical components into groups of varying polarity. The activity of these extracts gives insight into the chemical nature of the biologically active constituents.

Bajracharya *et al.* [23] obtained the antimicrobial activity of the ethanol extracts of leaves of *Woodfordia fruticosa* against *Escherichia coli*, *Salmonella* Typhi, *Salmonella paratyphi*, *Proteus vulgaris*, *Proteus mirabilis*, *Klebsiella* spp., *Citrobacter* spp., *Enterobacter* spp., *Shigella* spp., and *Pseudomonas* spp. Similarly, Timsina [24] obtained the antimicrobial effect of methanol extracts of leaves against *Bacillus subtilis*, *Candida albicans*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella* Typhi, *Salmonella*

paratyphi, *Shigella dysenteriae*, *Staphylococcus aureus*, and *Vibrio cholera* but showed inactivity against *Escherichia coli*.

Overall, the activity of more plants against Gram-positive bacteria compared to Gram-negative was not a surprise finding because previous studies showed that greater number of extracts were active against Gram-positive bacteria than Gram-negative bacteria [17, 18, 25]. This is likely explained by the more complex cell wall/membrane structure of Gram-negative bacteria. It is also proved that *Woodfordia fruticosa* contain certain constituents like tannins with significant antibacterial property which enables the extract to

overcome the barrier in Gram-negative cell wall [26, 27].

The present research justified the traditional usage of plant in Nepal and link to scientifically correlate its promising usage in laboratory. Therefore, we recommend further investigation of new natural products from the Nepalese *Woodfordia fruticosa*. We hope that the results of antimicrobial activities may play a significant role in the conservation of traditional medicine knowledge of Nepalese *Woodfordia fruticosa* and encourage the scientific community for further investigations for the antibacterial effect observed.

Table 1. Minimum Inhibitory Concentrations (MIC) of *Woodfordia fruticosa* with methanol, chloroform and hexane extracts against variety of microorganisms

Part tested	Solvents used	Minimum Inhibitory Concentrations (mg/disc)													
		Microorganisms used													
		<i>Pa</i>	<i>Ec</i>	<i>Sa</i>	<i>Bs</i>	<i>Pm</i>	<i>Kp</i>	<i>Sd</i>	<i>Sp</i>	<i>St</i>	<i>Cf</i>	<i>Es</i>	<i>As</i>	<i>Ca</i>	<i>Asp</i>
Flowers	Methanol	0.5	0.25	0.5	0.12	0.25	0.25	0.25	0.25	0.25	-	-	-	-	-
Flowers	Chloroform	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Flowers	Hexane	-	-	1.0	0.12	-	-	-	2.0	2.0	2.0	-	-	-	-
Leaves	Methanol	1.0	0.5	1.0	2.0	1.0	0.5	0.5	1.0	2.0	2.0	2.0	-	-	
Leaves	Chloroform	-	-	-	-	-	-	-	-	-	-	-	-	-	
Leaves	Hexane	-	-	-	2.0	-	-	-	-	-	2.0	-	-	-	

Key: MIC: Minimum Inhibitory Concentrations in microgram per disc; '-' indicated no zone of inhibition; Bacteria tested: *Sa*: *Staphylococcus aureus*; *Bs*: *Bacillus subtilis*; *Ec*: *Escherichia coli*; *Pa*: *Pseudomonas aeruginosa*; *Pm*: *Proteus mirabilis*; *Kp*: *Klebsiella pneumoniae*; *Sd*: *Shigella dysenteriae*; *Sp*: *Salmonella paratyphi*; *St*: *Salmonella Typhi*; *Cf*: *Citrobacter freundii*; *Es*: *Enterococcus species*; *As*: *Acenitobacter species*; *Ca*: *Candida albicans*; *Asp*: *Aspergillus species*; All solvent controls (MeOH, Chloroform and Hexane) were negative, producing no zone of inhibition. Positive controls were Erythromycin, Ciprofloxacin and Gentamycine.

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