Antibacterial Activity Directed Fractionation of *Woodfordia fruticosa* Kurz. Leaves

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Abstract

Background: ***Woodfordia fruticosa*** **is used traditionally as antidysenteric compound for fodder animals. Stem and flower extracts are previously flourished for their antibacterial activity. Leaves are also equally important, which are preliminary screened for their bioactive components in present study.**

Objective: Evaluation of antibacterial activity of leaves of *W. fruticosa* and detection of their active compound.

Methods: Different solvent i.e. petroleum ether, chloroform, diethyl ether and acetone extracts were studied against four bacterial strains using disc diffusion assay. Most potenz extract was chromatogram on preparative TLC. Further individual separated spots were back checked for their activity. Probable constituents of most active fraction were detected on GC-MS.

Results: All four extracts exhibited antibacterial activity. The acetone extract showed highest inhibition against *Bacillus subtilis* NCIM 2921. The 80μg and 120 μg of acetone extract exhibit comparable antimicrobial activity against the four different bacteria studied. Amongst the four different spots on TLC chromatogram three of them had activity against *B. subtilis* NCIM 2921. The most active fraction was collected by preparative TLC and used for GC-MS, which exhibited two peaks.

Conclusion: Extracts of petroleum ether, chloroform, diethyl ether and acetone were found to be effective against all the srains studied. The fraction of acetone extract, which is more active, constitutes two different compounds, in which both or one of them is responsible for antibacterial activity.

Keywords: ***Woodfordia fruticosa*****, Antibacterial activity, Solvent validation, Bioactive components**

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Introduction

Biologically active compounds are exploited vigorously in recent years due to growing cases of microbial resistance to the time honored antibiotics [8, 10]. In addition antibiotics produce much adverse effect on host, which includes immuno suppression and allergic reactions [1, 9]. One approach is to screen local medicinal plants for possible antimicrobial agents as they contain numerous biologically active compounds [7]. Contrary to the synthetic drugs, anti-microbial of plants origin is not associated with many side effects and has an enormous therapeutic potential to heal many infectious disease. Over the last 20 years, a large number of plant species have been evaluated for their antimicrobial activity. The trend of search is increased rapidly in recent years, which has been reported in several studies [4, 6, 11, 13, 14, 17]. One of the plants known for having many medicinal uses in traditional Indian system of medicine is *Woodfordia fruticosa* (Lythraceae). It is being used as a source of medicinal agents for anthelmentic, astringent, emetic, febrifuge, sedative, and stimulant. The decoction of the flower is used for biliousness, burns, diabetes, haemmorrhage, leprosy and skin diseases [16]. However it is not been proved scientifically for their bioactive components present in leaves. Present study is to give scientific basis for the therapeutic action of former and determine the antibacterial sensitivity for *Woodfordia fruticosa* Kurz. leaves.

Materials and Methods

Plant Material

Fresh and mature young leaves of *Woodfordia fruticosa* Kurz. were collected from Western Ghat, MS (INDIA) in November 2008. Authentication of collected plant material was carried out by Dr. Waman, Department of Botany, B.S.T. College Sangamner with specimen number NACASBC 049112008. The leaves were dried and homogenized to fine powder for further extraction process.

Solvent Extraction

A 10 g dried leaves powder of *W. fruticosa* was extracted separately in each different organic solvent. In present study non polar solvents (petroleum ether), semi polar solvent (chloroform and diethyl ether) and polar (acetone) were used separately as extraction solvents. The 10 g 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, centrifuged at 5000 g for 15 min and was dried in oven at 40 to 45 ºC. The extract was stored at 4ºC in airtight bottles.

Microorganisms Tested

The bacterial strains used to access the antibacterial properties of crude solvent extract of *Woodfordia fruticosa* are *Escherichia coli* NCIM 2065, *Bacillus subtilis* NCIM 2921, *Staphylococcus aureus* NCIM 5022 and *Pseudomonas aeruginosa* NCIM 5029. The investigated microbial strains were obtained from National Chemical Laboratory (NCL), Pune, India. The organisms were maintained on nutrient agar (Hi Media, India) slope at 4ºC and activated by sub culturing.

Antibacterial Assay

Antibacterial activity of the crude extracts in different solvents was tested by disc diffusion assay [3]. Mueller Hinton agar no. 2 (Hi Media, India) was used as the bacteriological medium. Medium was prepared and poured 20 ml each in sterilized

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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 is shown in the Table 1. The experiment was performed thrice and the mean values were presented with standard error.

Bioactive Compounds Isolation

After screening the antimicrobial activity of each solvent extract the maximum activity showing fraction was selected for further process. Bioactive components were isolated by thin Layer Chromatography (TLC) of acetone fraction (exhibited maximum activity). Aluminum TLC plates coated with silica gel G60 with dimension 200 × 200 mm of (length × width), Merck grade were used as stationery phase. The thickness of gel was 0.2 mm. The maximum activity showing sample of the active components (acetone extract) mixed in equal volume of same solvent was applied on stationary phase. The quantity of the sample was 4µl for each spots on plate. A 100ml of mobile phase used was Petroleum ether: Diethyl ether: Acetone in ratio of 50:40:10 v/v. All reagents and solvents used for TLC were of Merck, India. The antibacterial activity of each spot was rechecked by extracting with acetone.

GC-MS of the sample

The identification of metabolites in TC was carried using a QP2010 gas chromatography coupled with mass spectroscopy (Shimadzu). The ionization voltage was 70 eV. Gas chromatography was conducted in the temperature programming mode with a Restek column (0.25 mm, 60 m; XTI-5). The initial column temperature was 80 ^{0}C for 1 min, then increased linearly at 7 ^{0}C min^{-1} to 220 ^{0}C, hold for 3 min followed by linear increased temperature 10 ^{0}C min^{-1} upto 290 ^{0}C hold for 10 min. The temperature of the injection port was 290 ^{0}C and the GC/MS interface was maintained at 290 ^{0}C. The samples were introduced via an all-glass injector working in the split mode, with helium carrier gas flow rate was 1.2 ml min^{-1}. The identification of components was accomplished by comparison of retention time and fragmentation pattern, as well as with mass spectra in the NIST spectral library stored in the computer software (version 1.10 beta, Shimadzu) of the GCMS.

Results and Discussion

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 Tables 1a & 1b. Table 1a showed antibacterial activity of different extracts of *W. fruticosa* against four different bacterial strains at 4 µl (40 µl). The 80 µg and 120 µg of acetone extract showed proportional antimicrobial activity against all bacterial strains undertaken for study. The acetone fraction was more active and hence it was further tested with comparison of known antibiotic erythromycin (Table 1b). The traditional healers use primarily water as the

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Table 1a- Antibacterial activity of different solvent extracts of *W. fruticosa*. Data representing n=3 ± SE

Sr. No.	Organism	Zone of Inhibitions in cm (4µL)			
		Petroleum Ether	Chloroform	Diethyl Ether	Acetone
1	*Escherichia coli* NCIM 2065	0.7±0.002	0.8±0.003	0.7±0.004	1.2±0.02
2	*Bacillus subtilis* NCIM 2921	0.8±0.009	0.9±0.011	1.1±0.012	2.1±0.013
3	*Staphylococcus aureus* NCIM 5022	0.6±0.006	0.8±0.002	0.7±0.013	1.0±0.001
4	*Pseudomonas aeruginosa* NCIM 5029	1.0±0.010	0.9±0.007	0.6±0.004	1.0±0.01

Table 1b - Antibacterial activity of acetone extract of *W. fruticosa* in comparison with erythromycin. Data representing n=3 ± SE

Sr. No.	Organism	Zone of Inhibition in cm		
		Leaf Extract (8 µL)	Leaf Extract (12 µL)	Erythromycin (20µL)
1	*Escherichia coli* NCIM 2065	2.2±0.01	2.4±0.08	2.0±0.02
2	*Bacillus subtilis* NCIM 2921	2.2±0.03	2.5±0.07	2.1±0.04
3	*Staphylococcus aureus* NCIM 5022	1.8±0.007	1.9±0.012	2.1±0.09
4	*Pseudomonas aeruginosa* NCIM 5029	1.5±0.01	1.6±0.008	2.0±0.06

Plant extract concentration 10 mg / ml and erythromycin concentration 1mg/ml

solvent, but in present study we found acetone extract exhibits maximum inhibition compare to other semi polar (chloroform and diethyl ether) and non polar solvents (petroleum ether) Table -1. The work is in support with earlier report [2, 15]. The minimum zone of inhibition for *E. coli, B. subtilis, S. aureus* was observed with petroleum ether. The results revealed variability in the bactericidal concentrations of each extract for given bacteria [5]. Further, this might have resulted from the lack of solubility of the active constituents in non polar solutions [18]. Amongst the undertaken bacteria *B. subtilis* showed maximum inhibitory zone. Result is in contrary to Parekh and Chanda, (2008), who got no inhibition zone with *B. subtilis* in methanol extract. This could be due to use of different tissue, used in present study. Alternatively, active compound (s) may varies quantatively in the crude extracts to shows activity with the dose levels employed [19]. The results obtained indicate the existence of antimicrobial compounds in the crude acetone extracts and showed a good correlation between the reported uses of the plant in Medicine against different diseases and the experimental data of such extracts toward the most common pathogens.

Acetone fraction showed larger zone of inhibition and hence selected for TLC (Table 1a). The TLC chromatogram of acetone extract exhibited four different spots. These spots were crosschecked for antibacterial

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activity against *B. subtilis* to validate their role. Out of four, the three spots exhibited positive result against *B. subtilis*. Among these three spots, one was more active than the other two spots. Hence it was selected for GC-MS (Gas Chromatography – Mass Spectroscopy) analysis. Isolated single spot from preparative TLC revealed two different peaks in GCMS spectra. The Fig 1 shows MS spectra of these peaks having RT 22.625 and 28.733 min respectively. The GC spectra proved that only two compounds are present in active spot and both or one of them is responsible for the antibacterial activity.

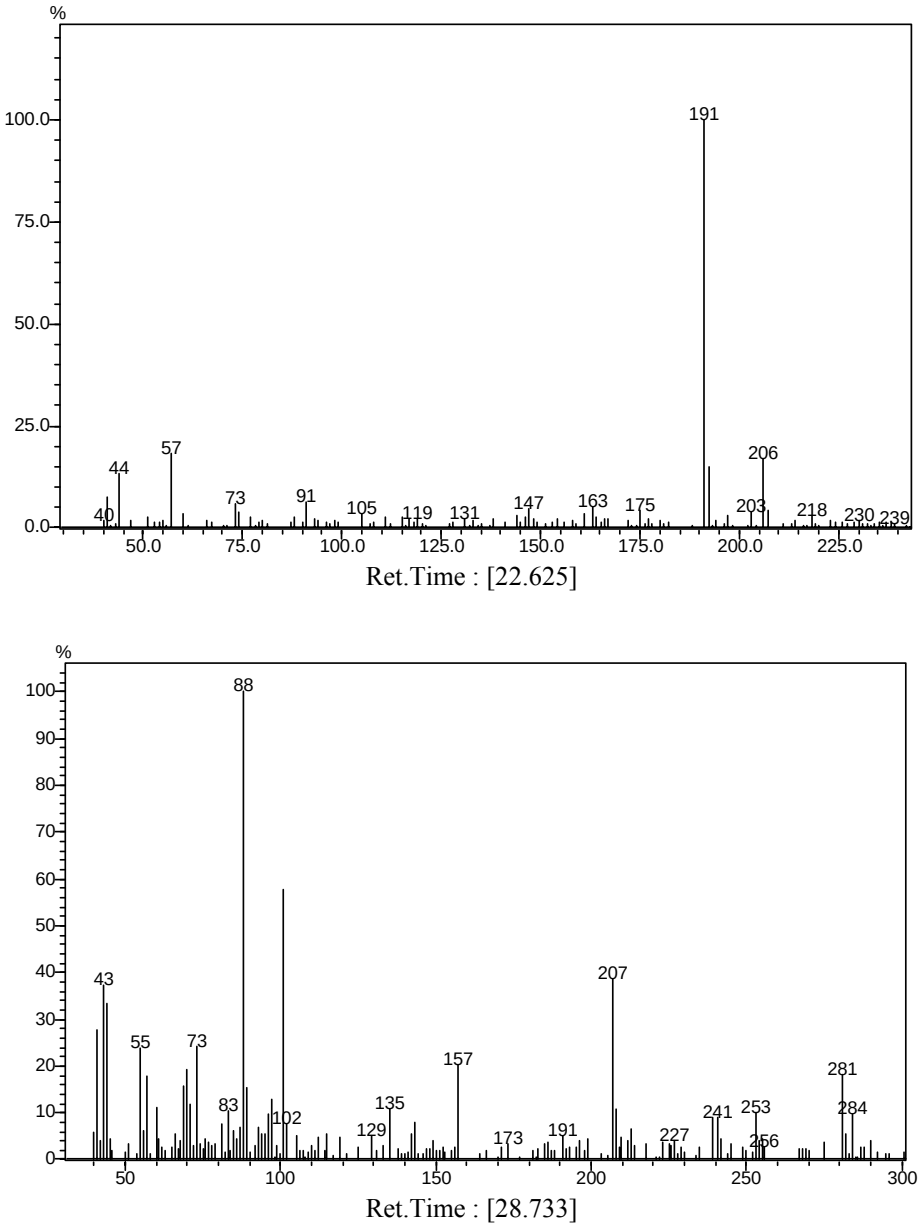


Fig. 1- Mass spectrum of two compounds found in active spot of *W. fruticosa* leaves

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From our investigation, the results confirm the presence of therapeutically potent compound in leaf extract of *Woodfordia fruticosa*. Presence of tannins, flavonoids, anthraquinone, glycosides and polyphenols in *W. fruticosa* have been reported is a supportive evidence [12]. In addition, these results form a good basis for selection of the plant for further phytochemical and pharmacological investigation. The results of the present study supports the folkloric usage of the studied plant and suggests that the plant extract possess certain constituents with antibacterial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. Therefore, results of the present study provide a scientific validation for the popular use of the medicinal plant studied and serve as a guide. This may help in selection of plants with antibacterial activities for further phytochemical work.

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