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The Antimicrobial activity of essential oil and plant extracts of *Woodfordia fruticosa*

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Abstract

The essential oil of *Woodfordia fruticosa* obtained by hydrodistillation was characterized. The main components present in the essential oil of leaves are sesquiterpenoids (β-caryophyllene, γ-curcumene, germacrene-D, β-selinene, elemol); and monoterpenoids (α-pinene, 2,6 dimethyl 1,3,5,7 octatetraene). The antibacterial activity of the essential oil was evaluated. The essential oil was most active against *Pseudomonas aerogenosa* and *Bacillus subtilis.* The plant extracts of *Woodfordia fruticosa* was evaluated for antimicrobial activity. The hexane extract was found to be most active against *Pseudomonas aerogenosa.*

Keywords: *Woodfordia fruticosa*; Antimicrobial activity; Hydrodistillation; Medicinal value; Agar disc diffusion method.

Introduction

Herbal medicines represent one of the most important fields of traditional medicine all over the world. To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants which have folklore reputation in a more intensified way [1]. Over the past 20 years, there has been an increased interest in the investigation of natural materials as a source of new antibacterial agents. Different extracts and essential oils from traditional medicinal plants have been tested to identify the source of therapeutic effects. As a result some natural products have been approved as new antibacterial drugs, but there is still an urgent need to identify novel substances that are active towards pathogens with high resistance [2,3]. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanism of action [4-7]. Contrary to synthetic drugs

antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases [8].

Woodfordia fruticosa Kurz belongs to the family *Lythraceae*. The English names that are most frequently used for the plant are Fire flame bush and Shiranjitea. The plant is abundantly present throughout India, ascending up to an altitude of about 1500 m, and also in the majority of the countries of South East and Far East Asia like Malaysia, Indonesia, Sri Lanka, China, Japan and Pakistan as well as Tropical Africa [9]. The local and traditional names are innumerable, especially in India, because of the widespread traditional use. In India, a few popularly known names are *Dhataki*, *Dhawi*, *Jargi*, *Dhai* etc. The full-grown leafy shrub is about 3.5 m high, having long and spreading branches with fluted stems. The bark, characteristically cinnamon brown colored and smooth. The leaves are opposite or sub-opposite in nature. Flowers are brilliant red, innumerable. The fruit are small capsules and membranous. The seeds are brown, numerous, minute, smooth, shining, angular and obovated [10,11].

According to the Indian systems of medicine, the flower is pungent, acrid, cooling, toxic, alexiteric, uterine sedative and anthelmintic, and is useful in thirst, dysentery, leprosy, erysipelas, blood diseases, leucorrhea, menorrhagia and toothache. Many marketed drugs comprise flowers, fruits, leaves and buds mixed with pedicals and thinner twigs of the plant[12-14]. The flowers are being used in the preparation of Ayurvedic fermented drugs called '*Aristhas*' and '*Asavas*' [15] and very popular in the Indian sub-continent and also in other South Asian countries [16]. A popular crude drug (called '*Sidowaya*' or '*Sidawayah*') of Indonesia and Malaysia chiefly contains dried flowers of *Woodfordia fruticosa* [17]. It has been used as an astringent to treat dysentery and sprue, and also for the treatment of bowel compliant, rheumatism, dysuria and hematuria in many South East Asian countries. It is also an ingredient of a preparation used to make barren women fertile [18]. Tribal people in Chhatisgarh district of central India randomly use fresh flowers to stop bleeding in emergency cuts, while they prefer to employ dried flower powder to heal wounds. Oral use of powdered bark in managing diarrhoea is well known. Successful treatment of otorrhoea by dried powdered flowers in tribal areas of Chhatisgarh is reported to be popular [19]. Management of female specific disorders like leucorrhea and dysmenorrhoea with flower based preparations is very popular among these tribes. An herbal composition containing *Woodfordia fruticosa* has been patented for the management of gynaecological disorders; it claims to prevent and treat anemia due to excessive bleeding associated with menstrual disorders [20].

The leaves of *Woodfordia fruticosa* are used as a folk medicine in India and Nepal. In case of fever, decoction of the *Dhawi* leaves (a popular name of the plant in this region) in combination with sugar and dried ginger is recommended.

The aim of this study is to find out the medicinal importance of the essential oil and plant extracts of *Woodfordia fruticosa* against a diverse range of organisms comprising gram-positive and gram-negative bacteria.

Materials and Methods

2.1. Plant Material:
Woodfordia fruticosa was collected from Satletta, Una region of Himachal Pradesh. The plant was taxonomically identified by Dr. Suman Arora, Taxonomist, and D.A.V Ayurvedic College Jalandhar.

2.2. Isolation of oil:
The essential oil of leaves of *Woodfordia fruticosa* was obtained by hydro distillation for 4 h in a Clevenger type apparatus [21]. The oil was taken by dissolving in HPLC grade n-hexane [22]. The oil was dried over anhydrous sodium sulphate and the corresponding oil was collected in the yield of 0.2 ml. The oil was stored in sealed vial at low temperature before analysis [23].

2.3.GCMS Analysis:
Sample dissolved in n-hexane was subjected to GCMS analysis. GC was carried out on Thermo System fitted with a PE-5 (5% phenyl, 95% dimethyl polysiloxane), capillary column (30 mm x 0.25 mm); film thickness 0.20 µm; carrier gas H_2 . Oven temperature 100º C for 2 min and then programmed from 100-280º C at 3º C/min. Injector and detector temperature 200º C and 300º C respectively. GC-MS analysis was carried out on a Thermo System coupled with GC- XL, MS at 70 eV; column and temperature programme same as above using carrier gas Helium. Inlet pressure 10 psi. The constituents were identified by comparing their retention indices with those of authentic samples or identified in essential oils of known compounds. The mass spectra were compared with those stored in spectrometer database and built in libraries.

2.4. Preparation of extracts:
For extraction; hexane, methanol and acetone were used as solvents. Hot extraction with Soxhlet apparatus was carried out. 35 g of leaves were extracted with hexane (300ml) for 6-8 h. The extract was concentrated under reduced pressure using rotavapour. The marc so obtained was subsequently extracted with acetone (300 ml) for 6-8 h. The extract so obtained was concentrated using rotavapour. The marc left behind was than extracted with methanol (300 ml) for 6-8 h. The extract was than concentrated.

2.5. Antimicrobial activity:
Bacterial Strains:
Two gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*; and two gram negative bacteria *Escherichia coli* and *Pseudomonas aerogenosa* were used in the study (Table I).

Table I: Pathogenic microorganisms used for antimicrobial activity tests

Group	Strain	Cultivation condition
Gram (+)	*Bacillus subtilis*	Nutrient agar/37ºC
Gram (+)	*Staphylococcus aureus*	MacConkey agar/37ºC
Gram (-)	*Escherichia coli*	Nutrient agar/37ºC
Gram (-)	*Pseudomonas aerogenosa*	MacConkey agar/37ºC

2.5.1. *Agar disc diffusion method:*
The essential oil and plant extracts of *Woodfordia fruticosa* was tested for antimicrobial activity using agar disc diffusion method on solid media [24]. Luria agar was used as basal medium for *E. coli* and *B. subtilis*; and nutrient agar was used as basal medium for *P. aeroginosa, S.aureus*. Luria agar and nutrient agar was poured in the sterile Petri plates. Mother culture of each organism was set up 24 h before the assays in order to reach stationary phase of growth [25]. The tests were assessed by inoculating Petri dishes from the mother cultures which had been surface spread with 0.1 ml of each bacteria, with the aim of obtaining microorganism concentration of 10^5 colony forming units (CFU/ml) [26]. 0.5 ml of hexane was added to the 0.2 ml of *Woodfordia* oil. 1 ml of DMSO was added to the extract in order to obtain 10 mg/ml concentration range. The stock solution of above concentration was absorbed on the sterile Whatmann filter paper No.1 discs (5mm disc diameter), which were subsequently placed in inoculated Petri plates. Discs with only hexane and DMSO were used as control. Therefore the Petri plates were than incubated at 37° C for 24 h. The antibacterial activity was determined by measuring the diameter of zone of inhibition surrounding bacterial growth [27].

2.5.2. *Minimum inhibitory concentration of plant extracts:*
The test bacteria used to determine MIC involves *E. coli, B. subtilis, P.aeroginosa* and *S. aureus*. The broth dilution method was used to measure MIC in order to determine the antibacterial effect of plant extracts. Two fold serial dilutions were prepared in broth media to obtain a concentration range of 0.142 mg/ml to 4.571 mg/ml using sterile screw bottles [28]. Bacterial colonies (mentioned above) were suspended in saline solution (0.85%) and turbidity of the saline solution was adjusted to 0.5 Mc Farland standards [29]. To each test tube 100 µl of standardized suspension of test bacteria were added and incubated at 37º C for 24 h. The end result of the test was the minimum concentration of the plant extract which gave clear solution i.e. no visual growth [30].

Results

The composition and identification of the main components present in the essential oil of *W. fruticosa* are shown in (Table: II). The presence of various constituents and their percentage were examined by GC and GC-MS. The fragmentation patterns of various components of oil were correlated with that reported earlier. The antimicrobial activity of essential oil against four human pathogenic bacteria (*P. aerogenosa, B. subtilis*, *S. aureus*, *E.coli)* was determined. The essential oil obtained from the leaves possessed activity against two bacteria (*P. aerogenosa, B. subtilis)* (Table: III). The antimicrobial activity of plant extracts was determined by agar disc diffusion method. The microorganisms that were used for the tests were sensitive to the all three plant extracts (Table: IV). It was found that the hexane extract of *W. fruticosa* showed maximum activity against *P. aurogenosa*. However the hexane and acetone extract of *W. fruticosa* showed minimum activity against *B.subtilis*. The minimum inhibitory concentration was also evaluated for these three plant extracts by broth dilution assay. The minimum inhibitory dilution for the plant obtained from the leaves varied between 0.142 mg/ml to 4.571 mg/ml for the entire microorganisms that were tested. The minimum inhibitory concentration for disappearance of visible microbial growth was observed against *P. aurogenosa* and *E.coli* by the hexane extract of *W.fruticosa* (Table: IV).

Table II: The chemical composition of essential oil of leaves of *W.fruticosa*

Sr.No	Components	Retention Index	Percentage
1.	α-pinene	935	23.53%
2.	2,6-Dimethyl-1,3,5,7- Octatetraene	1173	6.79%
3.	β-Caryophyllene	1398	36.37%
4.	γ-curcumene	1460	7.76%
5.	Germacrene D	1465	6.16%
6.	β-selinene	1471	4.59%
7.	Caryophylleneoxide	1575	6.95%
	Total Percentage		92.15%

Table III: Antimicrobial activity of Essential oil of leaves of *W.fruticosa*

Sr. No.	Bacterial strain	Z.O.I
1.	*Pseudomonas aerogenosa*	8 mm
2.	*Bacillus subtilis*	7 mm
3.	*Staphylococcus aureus*	-
4.	*Escherichia coli*	-

Z.O.I: Zone of inhibition in diameter mm.

Table IV: Antimicrobial activity of plant extracts

Sr.No.	Bacterial Strain	Plant Extract	Z.O.I in mm	M.I.C mg/ml
1.	*P.aurogenosa*	Hexane	15 mm	0.142
		Acetone	11 mm	0.857
		Methanol	13 mm	0.857
2.	*S.aureous*	Hexane	13 mm	0.857
		Acetone	13 mm	1.000
		Methanol	10 mm	1.000
3.	*E.coli*	Hexane	11 mm	1.142
		Acetone	13 mm	0.142
		Methanol	11 mm	1.142
4.	*B.subtilis*	Hexane	9 mm	1.000
		Acetone	9 mm	0.857
		Methanol	10 mm	1.000

M.I.C: Minimum inhibitory concentration in mg/ml

Discussion

The various constituents and their percentage were examined by GC and GC-MS studies. It was found that the main constituents of essential oil are β-caryophyllene 36.37%; α-pinene 23.53%; elemol 7.86%; γ-curcumene 7.76%; caryophylleneoxide 6.95%; 2,6 dimethyl 1,3,5,7 octatetraene 6.79%; Germacrene D 6.16% and β-selinene 4.59%. The essential oil of *W. fruticosa* exhibited significant activity against *P. aurogenosa* and *B.subtilis* with zone of inhibition 8mm and 7 mm respectively; while no activity was against *S. aureus* and *E.coli*. The plant extracts of *W.fruticosa* were tested for antimicrobial activity. The plant extracts showed significant activity against all the four test organisms.

The hexane extract showed maximum zone of inhibition of 15 mm against *P. aurogenosa*. Both the hexane and acetone extract showed minimum zone of inhibition of 9 mm against *E.coli* and *B.subtilis*.The minimum inhibitory concentration was also observed for the these three plant extracts. The hexane and acetone extract showed minimum inhibitory concentration of 0.142 mg/ml against *P. aurogenosa* and *E.coli*.

It is quite evident from the above results that the plant extracts are very active against most of the tested microorganisms and the minimum inhibitory concentration is quite low which indicated that very small volume of the extract is needed to kill microorganisms.

Conclusion

The objective of the present work was to find the medicinal importance of the plant *W. fruticosa*. The essential oil of *W. fruticosa* was studied as no work on its essential oil was done earlier. The antibacterial activity of essential oil was also carried out and essential oil was found to be potent against *Bacillus Subtilis* and *Pseudomonas aerogenosa*. Apart from this extraction of leaves of *W. fruticosa* with different solvents was also carried out. The antibacterial activity of plant extracts was evaluated. It was found that hexane extract of *W. fruticosa* was most active against *P.aerogenosa*.

Thus the results obtained confirm the therapeutic potency of *W.fruticosa* used in traditional medicine. This forms a good basis for the selection of plant for further phytochemical and pharmacological investigation. So present work gives a direction for future investigators to carry out research on the extracts and oil of the plant so that they could get some medicinally important drugs.

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