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Screening Of Six Medicinal Plants Used In North Indian Unorthodox Medicine For Pathogenic Microorganisms

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ABSTRACT

Six Indian medicinal plants *Enicostema axillare* Blume, *Melia azedarach, Citrus maxima*, *Hibuscus rosa-sinensis, Adhatoda zeylanica* and *Nyctanthes arbor-tristis* used by local peoples for the treatment of several ailments of microbial and non-microbial origins were investigated for *in vitro* antimicrobial potential. Fresh plant materials were collected from local areas of northern region of India. Water and ethanol extracts of the shredded plants were obtained by standard methods. The Bacterial cultures used were *E.coli, Bacillus cereus, Staphylococcus aureus, Salmonella typhi, Shigella dysentareae, Klebseilla pneumoneae and Enterococcus fecalis*. Susceptibility testing and Phytochemical screening of the plant extracts were performed by standard procedures. Controls were maintained for each test batch. Both water and ethanol extracts of all the selected plants were effective on most of the organisms. The Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) of the ethanol and water extracts of these plants were obtained between the range from 0.242 to 24.832 and 6.20 to 50 mg/ml respectively.

KEYWORDS

Ethanol extracts;
Indian medicinal plants;
Minimum inhibition concentration;
Unorthodox medicine.

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INTRODUCTION

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants[1]. Therefore, such plants should be investigated to better understand their properties, safety and efficacy. The use of plant extracts with known antimicrobial properties can be of great significance in therapeutic treatments. Herbal medicine has been widely used and formed an integral part of primary health care in India. Traditional medical practitioners in India use a variety of herbal preparations to treat different kinds of microbial diseases. Also over the years, the World Health Organization advocated that countries should interact with traditional medicine with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and non-microbial origins[2]. In view of this, the present study has been designed to investigate the antimicrobial potential of six medicinal plants commonly found in north India. The plants selected for the present investigation are following:

Enicostema axillare blume is commonly found as a weed. The crushed plant water taken as restorative, also as carminative and in diabetes. Plant decoction used in fever and snakebites. *Melia azedarach*, a moderate sized deciduous tree. Bark dark grey, flowers lilac and fruit an ellipsoidglobuse drupe with 4-5 seeds. Leaves, fruits and seeds are useful in skin diseases as well as in rheumatic pains, also as antiseptic and pulp is regarded as an abortifacient. *Citrus maxima* is a low shrub, fruit is nutritive, cardio tonic and refrigerant. Leaves are useful in epilepsy, cholera and convulsive cough. It is used in the treatment of hemorrhagic diseases, cures bronchial troubles and vertigo. *Nyctanthes arbor-tristis* is a hardy large shrub or small tree, up to ten meter high with a grey and greenish white rough bark. This plant is cholagogue, antihelmentic and laxative. It is used in dysentery, menorrhagia, sores and ulcers. *Hibuscus rosa-sinensis* is an evergreen woody shrub five to eight feet high, mainly used as demulcent and used for cough, gonorrhoea and stomachic troubles. *Adhatoda* zeylanica is an evergreen, gregarious, stiff perennial shrub, 1.2-6.0 meter in height. Leaf and roots are used in cough, asthma, bronchitis and rheumatism[3].

MATERIALS AND METHODS

Fresh plant materials were collected from users of these plants in Garhwal region of India. Their botanical identities were determined and authenticated by Dr. H.C.Pandey taxonomist, Botanical survey of India, Dehradun, India. Samples were deposited in the department of pharmaceutical sciences.

2.1 Preparation of extract

The extraction method used in this study was a modification of Akinside & Olukoya,[4] and Akinyemi *et al*[5]. In line with the traditional methods of preparation, shredded plant materials were put in sterile bottles containing either distilled water or 40% ethanol. Plant materials of different plants were air dried in shade at room temperature for 6 days. They were subsequently grounded into fine powder. 100 g of each of the powdered plant materials were put in a soxhlet extractor and extracted with ethanol and water. The resulting ethanol and aqueous extracts were subsequently weighed to produce 1.5 and 0.80, 2.0 and 0.5, 1.5 and 0.5, 1.0 and 0.35, 3.5 and 1.0 and 2.5 and 0.5 % w/w respectively for each plant. They were further labeled as ethanol and aqueous extracts and designated as (A, A-1) for *Enicostema axillare* Blume, (B, B-1), *Melia azedarach* (C, C-1), *Citrus maxima,* (D, D-1) *Nyctanthes arbor-tristis,* (E, E-1) *Adhatoda zeylanica* and (F, F-1) *Hibuscus rosa-sinensis* respectively.

2.2 Bacterial cultures

The bacterial cultures *E.coli, Bacillus cereus, Staphylococcus aureus, Salmonella typhi, Shigella dysentereae, Klebseilla pneumoneae and Enterococcus fecalis* were procured from department of microbiology, Sardar

Bhagwan Singh post graduate Institute of biomedical sciences and research Balawala Dehradun. The organisms were maintained on agar slants at 4°C and sub-cultured for 24 h before use.

2.3 Bacterial susceptibility testing

A standardized inoculum (1–2 × 10^7 cfu/ml 0.5 McFarland standards) was introduced onto the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculum. A sterile paper disc previously soaked in a known concentration of extract (20 mcg/ml per disc) was carefully placed at the center of the labeled seeded plate. The same procedure was used for all the strains used. The plates were incubated aerobically at 37°C and examined for zones of inhibition after 24 h. The inhibition zones were measured with a ruler and compared with the control disc (solvent)[6].

2.4 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC of the extracts was determined by dilution of all the extracts to various concentrations 0.0060-50 mg/ml respectively. Equal volume of each extract and nutrient broth were mixed in a test tube. Specifically 0.1 ml of standardized inoculum (1–2 × 10^7 cfu/ml) was added to each tube. The tubes were incubated aerobically at 37°C for 18–24 h. Two control tubes were maintained for each test batch. These included antibiotic control (tube containing extract and the growth medium without inoculum) and organism control (the tube containing the growth medium, physiological saline and the inoculum). The lowest concentration (highest dilution) of the extract that produced no visible bacterial growth (no turbidity) when compared with the control tubes was regarded as MIC. However, the MBC was determined by sub culturing the test dilution on to a fresh drug-free solid medium and incubated further for 18–24 h. The highest dilution that yielded no single bacterial colony on a solid medium was taken as MBC.

Phytochemical screening methods

All plant parts were extracted on the day of collection. The screening procedures were adapted from Wall *et al.*,[7] and Sofowora[8]. An extraction of each plant part was prepared by macerating a known weight of the fresh plant material in a blender with redistilled ethylated spirit. Each extract was suction-filtered and the process repeated until all soluble compounds had been extracted, as judged by loss of color of the filtrate. Extract from each plant part was evaporated to dryness in vacuo at about 45°C and further dried to a constant weight at the same temperature in a hot-air oven. A portion of the residue was used to test for plant constituents.

RESULTS AND DISCUSSION

The profile of six medicinal plants used in this study is shown in TABLE 1. The results of antibacterial activity of the crude extracts of these plants revealed that all the six plants: *Enicostema axillare* Blume, *Melia azedarach, Citrus maxima*, *Hibuscus rosa-sinensis, Adhatoda zeylanica* and *Nyctanthes arbor-tristis* showed good antibacterial activity against pathogenic cultures used. Both water and ethanol extracts of these plants were effective on pathogenic strains. On the other hand, the crude extracts (water & ethanol) of. *Nyctanthes arbor-tristis* and *Hibuscus rosa-sinensis* were most effective against the *Enterococcus fecalis* and *Shi-*

TABLE 1: Profile of the six medicinal plants used

Botanical Name	Family	Local name	Plant part used	Voucher Number
Enicostema axillare Blume	Gentianaceae	Chhota	Aerial parts	EA-26
Melia azedarach,	Meliaceae	Neem	Leaves	MA-29
Citrus maxima	Rutaceae	Chakotra	Leaves	CM-10
Nyctanthes arbor-tristis	Oleaceae	Harsinghar	Leaves	NT-69
Hibuscus rosa-sinensis	Malvaceae	Gurhal	Leaves	HR-09
Adhatoda zeylanica	Acanthacea	Arusa	Roots	AZ-22

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TABLE 2: Antibacterial activity of the crude plant extracts on pathogenic microorganisms

Plants used→ Organisms ↓	*Enicostema Axillare B*lume		*Melia azedarach*		*Citrus maxima*		*Nyctanthes Arbor-tristis*		*Adhatoda zeylanica*		*Hibuscus rosa-sinensis*	
	A	**A-1**	**B**	**B-1**	**C**	**C-1**	**D**	**D-1**	**E**	**E-1**	**F**	**F-1**
B.cereus	15	12	20	16	10	10	16	11	18	12	20	15
S.dysentereae	18	14	18	14	12	9	14	13	16	10	22	16
K.pneumoneae	12	10	20	13	15	8	11	8	19	15	16	10
S.typhi	15	12	21	17	12	8	15	10	20	18	11	9
E. fecalis	20	18	21	14	11	10	10	8	16	11	16	12
S. aureus	16	14	20	19	13	10	19	12	14	8	14	12
E.coli	19	13	19	12	14	9	13	11	19	12	20	17

A, B, C, D, E, F = Ethanol extract
A-1, B-1, C-1, C-1, D-1, E-1, F-1= aqueous extract

TABLE 3: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of crude extracts of six medicinal plants of northern region of India

Organism→ Extract↓	*Enterococcus. fecalis*		*Shigella dysenterae*		*Bacillus cereus*	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
A	0.388	24.83	3.104	>50	0.776	24.83
A-1	1.552	24.83	12.416	>50	6.20	>50
B	0.0485	6.20	0.097	6.20	0.194	12.416
B-1	0.194	12.416	1.552	24.83	1.552	24.83
C	0.388	24.83	0.194	12.416	0.776	24.83
C-1	1.552	24.83	3.104	>50	0.776	24.83
D	0.194	12.416	0.194	12.416	3.104	>50
D-1	6.20	>50	24.832	>50	12.416	>50
E	0.097	6.20	0.194	12.416	3.104	>50
E-1	3.104	>50	1.552	24.83	12.416	>50
F	0.097	6.20	0.0485	6.20	0.194	12.416
F-1	0.242	24.83	0.194	12.416	0.388	24.83

TABLE 4: Phytochemical analysis of six crude plant extracts

Components	*Enicostema axillare B*lume (aerial parts)	*Melia azedarach* (leaf)	*Citrus maxima* (leaf)	*Nyctanthes arbor-tristis* (leaf)	*Adhatoda zeylanica* (root)	*Hibuscus rosa-sinensis* (leaf)
Alkaloids	++	++	++	+++	++	++
Tannins	++	+++	++	++	+++	++
Saponins	++	+++	++	++	+	++
Glycosides	-	-	-	-	+	-
Flavonoides	+	+	+	+	+	+

+++ = Appreciable amount, ++ = Moderate amount, + = Trace amount, - = Completely absence

gella dysentereae as judged by the zones of inhibition (TABLE 2). The MIC and MBC values obtained for the extracts against *Enterococcus fecalis, Shigella dysentereae* and *Bacillus cereus*. The Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) of the ethanol and water extracts of these plants were obtained between the range from 0.242 to 24.832 and 6.20 to 50 mg/ml respectively (TABLE 3). The result of photochemical screening showed that all the six tested plants exhibited positive reactions to alkaloids, tannins and saponins. However, flavonoides was only found in

the four active plants in this study (TABLE 4).

CONCLUSION

Medicinal plants constitute an effective source of both traditional and modern medicines, herbal medicine has been shown to have genuine utility and about 80% of rural population depends on it as primary health care. World Health Organization advocated that countries should interact with traditional medicine with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and non-microbial origins. The results of the study indicated that all the six medicinal plants commonly used by traditional medical practitioners showed good antimicrobial activity. Our results therefore offer a scientific basis for the traditional use of both water and ethanol extracts of *Enicostema axillare* Blume, *Melia azedarach, Citrus maxima*, *Hibuscus rosa-sinensis, Adhatoda zeylanica* and *Nyctanthes arbor-tristis* separately. But *in vivo* studies on these medicinal plants are necessary and should seek to determine toxicity of the active constituents, their side effects, serum-attainable levels, pharmacokinetic properties and diffusion in different body sites. The antimicrobial activities could be enhanced if the active components are purified and adequate dosage determined for proper administration. This may go a long way in curbing administration of inappropriate concentration; a common practice among many traditional medical practitioners.

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