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Full Length Research Paper

Antimicrobial activity of some plant extracts having hepatoprotective effects

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The antimicrobial activity of hot water and ethanolic extracts of six plant extracts, utilized in Pakistan for the cure of liver damage, were studied. The extracts of *Acacia arabica, Nymphaea lotus, Sphareranthus hirtus, Emblica officinalis, Cinchorium intybus* and *Cardus marianum* were tested *in vitro* against seven bacterial species and two fungal species by well-diffusion method and micro-dilution methods. The patterns of inhibition varied with the plant extracts, the solvent used for extraction and the organisms tested. *Escherichia coli, Salmonella typhi, Pseudomonas aeroginosa* were the most inhibited microorganisms. The extract of *Sphareranthu hirtus* was the most active against multi-drug resistant *Pseudomonas aeruginosa and* enterohemorrhagic *E. coli* 0157 EHEC. The ethanolic extract of *S. hirtus* exhibited a higher effect than the hot water extract. These plants extracts were analyzed for elemental composition.

Key words: Antimicrobial agents, plant extracts, Enterohemorrhagic *Escherichia coli* 0157 EHEC, multi-drug resistant *Pseudomonas aeruginosa, Salmonella typhi,* elemental composition.

INTRODUCTION

According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance (Diallo et al., 1999) . The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds (Edeoga et al., 2005).

Developing countries like Pakistan depend on plant resources mainly for herbal medicines, food, forage, construction of dwellings, making household implements, sleeping mats, and for fire and shade. The use of medicinal plants as traditional medicines is well known in rural areas of many developing countries (Sandhu and Heinrich, 2005; Gupta et al., 2005). Traditional healers claim that their medicine is cheaper, more effective and impart least side effects as compared to synthetic medi-cines. In developing countries, low-income people such as farmers, people of small isolate villages and native communities use folk medicine for the treatment of common infections (Rojas et al., 2006).

Six plant species used in folk medicine were selected to determine their antimicrobial and elementological activity. In general, these plants are used in folk medicine in the treatment of skin diseases, venereal diseases, respiratory problems and nervous disorders. There is lack of scien-tific studies on these selected plants especially antimi-crobial studies (Kloucek et al., 2005). The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants (Erdogrul, 2002). Screening of medicinal plants for antimicrobial and elementological activities are important for finding potential new compounds for therapeutic use. Total six elements (Sodium, Potassium, Zinc, Magne-sium, Copper, Calcium and Iron) are analyzed in these

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Table 1. Uses and properties of medicinal plants collected for antimicrobial screening.

Botanical name	Family	Local Name	Part of plant used
Acacia arabica	Mimosaceae	Kikar	Leaves
Nymphaea lotus	Nymphaeceae	Nilofar	Flowers
Sphaeranthus hirtus	Compositae	Mandibooti	Seeds
Emblica officinalis	Euphorbiaceae	Amla	Fruits
Cinchorium intybus	Compositae	Kasini	Pods
Silybum marianum		Milk thistle	Seeds

plant extracts. The biological functions of these plants are mentioned in this study. This paper reports the results of a bioassay and elemental analysis for different plant extracts.

METHODS

Plant materials and preparation of extracts

Fresh plant materials used in this study consisted of *Acacia arabica (*leaves*), Nymphaea lotus (*flower*), Sphaeranthus hirtus (seeds), Emblica officinalis* (fruit*), Cinchorium intybus (*pods) and *Silybum marianum* (seeds*)* which were collected from local cultivated areas of Lahore. Their botanical identities were determined and authenticated by Dr. Abdul Waheed Sabir PSO (Rtd) Botanist PCSIR Labs. Complex. The air-dried plant materials were grounded into fine powder and extracted with hot water and 80% ethanol. After filtration of total extracts, the extracts were evaporated to dryness in vacuo and weighted.

Bacterial cultures

Seven bacterial strains, Methicilin- resistant *Staphylcoccus aureus,* muti-drug resistant *Pseudomonas aeruginosa,* enterohemorrhagic *Escherichia coli* 0157 EHEC*, Salmonella typhi, Proteus vulgaris, Klebsiella pneumoniae, Bacillus subtilus (*reference strain) and two fungal strains (*Candida albican* and *Aspergillus niger*) were used for testing the activity.

Bacterial susceptibility testing

The antimicrobial activity was determined by the well diffusion method according to National Commmittee for Clinical Laboratory Standards (NCCLS) (National Committee for Clinical Laboratory Standards, 1993). Petri plates containing 20 ml of Muller Hinton Agar medium were seeded with 24 h cultures of bacterial inoculums (a standardized inoculums 1- 2 x 10^{7} cfu/ml 0.5 Mcfarland standard). Wells (6 mm diameter) were cut into agar and 50 µl of plant extracts were tested in a concentration of 100 mg/ml and incubation was performed at 37^{O}C for 24 h. The same procedure was performed for the determination of anti -fungal activity and incubated at 25^{O}C for 24 h. The assessment of antimicrobial activity was based on measurement of the diameter of the inhibition zone formed around the well. A standardized 30 µg Methicillin and Oxacillin were used as antibiotic control.

Determination of Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

MIC was determined by micro-dilution method using serially diluted (2 folds) plant extracts according to the National Committee for Clinical Laboratory Standards (NCCLS) (National Committee for Clinical Laboratory Standards, 2000). MIC of the extracts was determined by dilution of *A. arabica, Nymphaea lotus, S. hirtus, E. officinalis, C. intybus,* and *S. marianum* of various concentrations of 0.0-45, 0.0- 55, 0.0-42, 0.0- 55, 0.0-36, 0.0-42, 0.0- 45 mcg/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 $\cdot 10^{7}$cfu/ml) was added in each tube. The tubes were incubated aerobically at 37^{O}C for 18-24 h. Two control tubes were maintained for each test batch. These included antibiotic control (tube containing extract and growth media with out inoculum) and organism control (tube containing the growth medium, 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 were 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 signal bacterial colony on the solid medium was taken as MBC.

Elemental composition

Pre weighted samples of dried medicinal plants were subjected to dry ashing method in Muffle furnace 1200^{O}C. Metals were estimated at Atomic Absorption Spectrophotometer Perkin Elmer Analyst 800, with 0.5, 1.0 and 1.5 ppm standard solution for all metals (Sodium, Potassium, Zinc, Magnesium, Copper, Calcium and Iron) to obtain calibration curve.

RESULTS AND DISCUSSION

The profile of six medicinal plants used in this study is shown in Table 1. The result of antimicrobial activity of the crude extracts of these plants *A. arabica, S. hirtus, E. officinalis, C. intybus,* and *S. marianum* showed good antimicrobial activity against different bacteria but intermediate activity against fungus.

Both water and ethanolic extracts of these plants were effective on bacterial strains, on the other hand, the extracts (water extract and ethanolic extract) of *S. hirtus, Cardus marianum* and *C. intybus* were weakly effective against the fungus as judged by zones of inhibitions (Table 2). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values obtained for extracts against the bacterial strains varied from plant extract to the other. For instance, MIC values of 29.8, 29.2, 29.5, 34.4, 29.5, 27.3, and 18.9 mcg/ ml were obtained for ethanolic extracts of *E. officinalis,* while

Table 2. Anti-microbial activity of the crude plant extracts on different bacterial and fungal strains.

Plant name	Zone of inhibition (mm)																	
	Ethanol extraction									Hot water extraction								
	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
Acacia arabica	34	27	35	30	36	37	30	29	30	32	24	28	26	30	32	25	27	26
Nymphaea lotus	29	20	36	30	23	31	32	21	27	25	18	33	29	21	30	19	16	19
Sphaeranthus hirtus	28	30	47	44	40	29	29	24	24	25	22	33	31	37	22	24	18	-
Emblica officinalis	46	23	55	43	34	40	35	-	37	29	23	43	31	20	30	31	35	20
Cinchorium intybus	30	38	49	38	41	43	31	23	20	24	37	43	31	39	37	26	23	12
Cardus marianum	35	25	33	29	28	30	49	-	-	30	24	30	17	26	26	43	-	-

Activity key: Table indicates average zone of inhibition (in mm), (-)= no inhibition, Methicillin and Oxacillin = commercial antibiotics, 1= *S. aureus, 2= B. subtilus, 3= E.coli, 4= P. vulgaris, 5= P. aeruginosa, 6= S. typhi, 7= K. pneumoniae, 8= Candida albican,* and 9= *A. niger.*

Table 3. MIC and MBC of ethanolic plant extracts on different bacterial species (in mcg/ml).

Plant Name	*1*		*2*		*3*		*4*		*5*		*6*		*7*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
A.arabica	44.2	51.2	40.7	52.1	35.2	37.9	40.2	49.8	25.4	32.5	32.9	40.0	40.1	42.0
N. lotus	32.5	41.7	35.2	39.9	35.3	39.5	32.5	33.5	29.3	40.1	30.7	31.2	27.3	30.4
S. hirtus	28.9	32.2	27.3	29.8	41.2	45.4	49.3	55.0	18.4	25.5	28.4	30.3	20.4	25.6
E.officinalis	29.8	31.9	29.2	35.4	29.5	40.0	34.4	39.8	29.5	35.2	27.3	31.4	18.9	20.3
C. intybus	32.7	37.8	36.3	39.5	32.9	24.8	28.2	32.4	18.5	24.4	25.3	27.8	19.3	24.5
C.marianum	35.2	39.9	39.8	45.2	41.2	25.1	25.4	28.9	21.3	29.5	23.4	27.3	20.0	26.2

Activity key: mcg= microgram, 1= *S. aureus, 2= B. subtilus, 3= E.coli, 4= P. vulgaris, 5= P. aeruginosa, 6= S. typhi, 7= K. pneumoniae, 8= Candida albican,* and 9= *A. niger.*

the corresponding MBC values are 31.9, 35.4, 40.0, 39.8, 35.2, 31.4 and 20.3 mcg/ml Methicilin-resistant *S. aureus, B. subtilus,* enterohemorrhagic *E. coli* 0157 EHEC, *Proteus vulgaris, muti*-drug resistant *P. aeruginosa, S. typhi,* and *K. pneumoniae* (Table 3). The MIC and MBC values of 44.2 and 51.2; 40.7 and 52.1; 35.2 and 37.9; 40.2 and 49.8; 25.4 and 32.5; 32.9 and 40.0; 40.1 and 42.0 were recorded for ethanolic extract and 40.4 and 44.0; 30.7 and 52.1; 31.4 and 37.4; 36.2 and 38.6; 25.2 and 30.9; 22.5 and 30.0; 30.1 and 32.0 are values for water extract of *A. arabica* against Methicilin- resistant *S. aureus, B. subtilus,* enterohemorrhagic *E. coli* 0157 EHEC, *P. vulgaris,* multi-drug resistant *P. aeruginosa, S. typhi,* and *K. pneumoniae,* respectively (Tables 3 and 4). These plants were bacteriostatic at lower concentrations and bactericidal at higher concentrations as released by MIC and MBC values shown in Tables 3 and Table 4.

The medicinal plants constitute an effective source of both traditional and modern medicines. Herbal medicines have been shown to have genuine utility and about 80% of rural population depends on its primary health care. Over the years, the world health organizations advocated that countries should interact with traditional medicines with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and non-microbial origin (World Health Organization, 1978).

The results of present study indicated that six medicinal plants commonly used by traditional medical practitioners to cure liver damage were active against bacterial strains. The crude extracts of *Nymphaea lotus* and *S. hirtus* were weakly active against fungal strains with ethanolic extract of both plants excreting stronger anti-bacterial activity than water extracts.

The investigations further showed that both water and ethanolic extracts of *A. arabica, N. lotus, S. hirtus, E. officenalis, C. intybus* and *S. marianum* were active against bacterial species. The MIC values of these active plants extracts obtained in this study were lower than MBC values suggesting that plant extract were bacterio-static at lower concentration and bactericidal at higher concentrations. The ethanol extract of these plants exerted greater antibacterial activity than corresponding water extract at same concentration (Table 2). These observations may be attributed to two reasons; firstly, the nature of biologically active components (Alkoloids, anthraquinone, saponins and tannins) which could be enhanced in presence of ethanol. It has been documen-

Table 4. MIC and MBC of aqueous plant extracts on different bacterial species (in mcg/ml).

Plant Name	1		2		3		4		5		6		7	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
A.arabica	40.4	44.0	30.7	52.1	31.4	37.4	36.2	38.6	25.2	30.9	22.5	30.0	30.1	32.0
N. lotus	33.5	41.2	31.2	39.9	32.9	36.7	29.5	34.5	23.3	29.1	26.7	29.2	24.3	27.3
S. hirtus	22.9	30.5	23.3	29.8	39.4	43.4	39.3	45.1	14.4	22.8	26.4	27.3	19.4	22.6
E.officinalis	25.8	31.7	22.6	35.4	25.5	35.0	29.4	29.8	24.5	32.1	24.3	29.4	16.5	22.0
C. intybus	29.4	31.8	30.3	39.5	33.9	36.8	25.2	32.5	17.2	22.3	26.3	32.8	14.3	20.5
Silimarine	25.7	33.9	39.2	45.2	38.2	40.1	20.4	22.6	20.6	23.1	18.4	22.9	20.4	23.7

Activity key: mcg= microgram, 1= *S. aureus, 2= B. subtilus, 3= E.coli, 4= P. vulgaris, 5= P. aeruginosa, 6= S. typhi, 7= K. pneumoniae, 8= Candida albican,* and 9= *A. niger.*

ted that these components are well known for anti-microbial activity (Tshesche, 1970). Secondly, the stronger extraction capacity of ethanol could have produced greater active constituents responsible for anti-microbial activity.

Traditionally, these plants are soaked in ethanol or water (in case of patients forbidding alcoholic intake due to religious belief) for days, large quantities of these extract, which lack specific concentration are usually administrated to patients. Our results therefore tend to support the traditional claim that these medicinal plants are preferably extracted in ethanol.

The analysis of metals reveals that maximum concentration of zinc (6.052 mg/100 g of dried plant) was found in *S. birtus*, Magnesium was found (5.4780 mg/100 g) in *A. arabica,* Sodium was found (231.76 mg/100 g) in *N. lotus* and maximum amount of Potassium (881.772 mg/100 g) was found in *S. birtus.* The minimum amount of these metals (Sodium, Potassium, Zinc, Magnesium, Copper, Calcium and Iron) was found as 0.9205, 0.06175, and 0.4299, 29.455 mg/100 g of plant, in *E. officinalis, S. birtus, N. lotus,* and *C. intybus.*

Conclusion

The present results therefore offer a scientific basis for traditional use of both water and ethanol extracts of *A. arabica, N. lotus, S. hirtus, E. officinalis, C. intybus* and *S. marianum* separately against liver damage. But *in vivo* studies on these medicinal plants are necessary and should seek to determine toxicity of active constituents, their side effects, serum- attainable levels, pharmacokinetic properties and diffusion in different body sites. The anti-microbial activities could be enhanced if active components are purified and adequate dosage determined for proper administration. This way goes a long way in curbing administration of inappropriate concentration, a common practice among many traditional medicines practitioners in Pakistan. This represents the first preliminary report on the anti-microbial activity of these medicinal plants in Pakistan.

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