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Full Length Research Paper

Biological screening of seventeen medicinal plants used in the traditional systems of medicine in Pakistan for antimicrobial activities

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Antibacterial properties of 51 aqueous, ethanolic and n-hexane extracts of seventeen medicinal plants from fourteen families used in the traditional system of medicines in Pakistan were tested against ten commonly prevalent gram negative and gram positive bacteria. In particular we examined the enteric pathogens and yeast using agar well diffusion method. The extracts of *Eucalyptus globulus, Emblica officinalis* and *Sphaeranthus* were found to have high levels of activity against all ten of the microorganisms. Ethanolic extracts of *Azadirecta indica, Cedrella toona, Punicia granatum, Berginia ciliata and Lawsonia alba* have shown considerable activity. Aqueous extracts of *B. ciliata, L. alba and P. granatum* have also shown low to moderate antimicrobial activity. The hexane extracts of the other fourteen medicinal plants and extracts from *Calotropics procera, Melia azdirecta, Myrtus communis, Berberis aristata, Aspholedus tinuipholius* had either no activity against most of the organisms or moderate activity against few organisms. Antibacterial activity of some of these plant extracts was comparable with commercially available antibiotics. Pakistan has a rich flora with broad potential for new antimicrobial agents which can be used as a substitute for current antibiotics against which wide spread resistance has developed.

Key words: Antimicrobial properties, crude extracts, Pakistani medicinal plants.

INTRODUCTION

In the Asian sub-continent, the uses of different parts of several medicinal plants have been used from ancient times to cure specific ailments (Bhatachargee, 1998). Traditionally higher and aromatics plants have been used in greco-roman or unani medicine to extend the shelf life of foods because they showed inhibition against bacteria, fungi and yeasts (Hulin et al., 1998). Currently, the number of drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are increasing and becoming more widespread. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, intravenous catheters, organ transplantation and other infections (Dean et al., 1996; Gonzalez et al., 1996). This situation has provided the impetus to the search for new antimicrobial substances from various sources such as medicinal plants (Cordell, 2000). Plant extracts have been used for centuries as a popular method for treating several health disorders. Studies have been carried out on various natural products screening their antimicrobial activity (Yoshikawa et al., 2008; Parekh and Chanda, 2007; Pesewu et al., 2008). Systematic screening of these plants may result in the discovery of new effective compounds and can reduce the specter of untreatable bacterial infections (Tomoko et al., 2002). It has been reported that only approximately 20% of the plants found in the world have been subjected to pharmacological or biological testing.

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Additionally, a substantial number of new antibiotics introduced on the market are obtained from natural or semi-synthetic resources (Mothana and Lindequist, 2005). In our study, we chose seventeen promising medicinal plants used traditionally in Pakistan and like to be associated with the micro-organism and evaluated them for potential antimicrobial activity.

METHODS AND MATERIALS

Plant material and extract preparation

All of the plants for this study were collected from various parts of Pakistan that is, Islamabad, Punjab, Sarghodha Division, Kalar Kahar, and Hazara Division and dried (GACP and FCP guidelines, 2004). Plant identification was confirmed in the Herbarium of National Agricultural Council, Islamabad, Pakistan. The plants were prepared using a modified extraction method (Alade and Irobi, 1993). Three 100 g portions of the each powdered plants were separated for (a) aqueous extraction in soxhlet apparatus (b) ethanolic extraction by maceration of the powdered material in ethanol from 7 - 10 days (c) n-hexane extraction with maceration. The extracts were then passed through whatman filter 1. The filtrates obtained were evaporated under vacuum at 40°C using rotary vacuum evaporator to obtain the dry extracts. The last traces of water or solvent were evaporated using water bath. The stock solutions of the dried materials of aqueous, ethanolic, and n-hexane extracts were prepared in their respective solvents, in an appropriate concentration of 100 mg/ml. The WHO reference standards of antibiotics that is, tetracycline, streptomycin, and Chloramphenicol were used for comparison and a positive control 100 µg/ml dilution were prepared in their respective solvents/buffers.

Microorganisms

The following strains of bacteria were used in this study: *Bacillus subtilis*, ATCC #6633, *Escherichia coli,* ATCC, #10536, *Klebsiella pneumoniae,* ATCC #10031, *Micrococcus pyogens*, ATCC #6538, *Salmonella typhi,* ATCC# 19430, *Shigella dysentriae,* ATCC# 11835, *Staphylococcus aureus*, ATCC #6538, *Staphylococcus epidermidis*, ATCC # 12228, *Sacchromyces cerevisiae*, ATCC #9763, *Vibrio cholerae*, ATCC # 25870. The medium used for the sub culturing of the microorganisms was nutrient agar and sabouraud dextrose agar and was autoclaved at 121°C for 15 min. The final pH was 7.0 ± 0.2. All of the culture media were prepared and treated according to the manufacturer's guidelines (DIFCO). The inoculums were prepared in Na/saline or broth.

Antibacterial activity

The agar well plate diffusion method for aqueous, ethanolic, n-hexane extracts and solvent extract (Perez et al., 1990) was employed for the determination of antimicrobial activity, in which the wells were used as a reservoir of the sample dilutions and the standard dilutions. 25 ml of the media was poured into 90 mm sterilized Petri dishes to have a mean depth of 4.0 ± 0.5 mm and the reservoir containing the sample dilutions were brought into contact with this media and were incubated for 18 - 24 h. The diameter of the clear zones of inhibition around the reservoirs was measured with Vernier Caliper (Rios et al., 1988). In cases where the samples showed no activity, no zone of inhibition was developed (recorded as 0). The concentration of each extract tested was 100 mg/ml. The test was carried out in triplicate.

RESULTS

A total of 51 aqueous, ethanolic and n-hexane extract of seventeen medicinal plants from fourteen families used in the unani system of medicines were screened for their antimicrobial activity against ten commonly prevalent bacteria particularly the enteric pathogens and yeast that is, *E. coli*, *S. typhi, S. dysentriae*, *V. cholerae*, *K. pneumoniae*, *S. aureus, S. epidermidis, B. subtilis, M. pyogens*, *S. cerevisiae*.

Table 1 shows the common uses of seventeen medicinal plants in the unani system of medicines in Pakistan. The aqueous, ethanolic and n-hexane extracts of *Eucalyptus globulus*, *Emblica officinalis* and *sphaeranthus* were found to have very good activity against all the ten microorganisms. Hexane extracts of other fourteen medicinal plants showed no activity against any of microorganisms. The ethanolic extracts of *Calotropics procera* had no activity only against *K. pneumoniae*, while it is active against all other test organisms. The aqueous and n-hexane extracts had no activity against all the test organisms. The ethanolic and aqueous extracts of *Melia azdirecta* had no activity against *S. dysentriae, E. coli, S. typhi, V. cholerae, K. pneumonia, S. epidermidis, B. subtilis, M. pyogens, S. cerevisiae*. These extracts are active against *S. aureus, B. subtilus and S. typhi,* the ethanolic and aqueous extracts of *Myrtus communis* had no activity against *K. pneumonia, S. epidermidis, Bacillus subtilis, M. pyogens and Saccharomyces cerevisiae*, while ethanolic and aqueous extracts of *M. communis* had moderate activity against *S. aureus*, *S. typhi*, *S. dysentriae*, *E. coli*, *S. typhi*, *V. cholerae*, The n-hexane extract had no activity against all the test organisims. *Berberis aristata, Aspholedus tinuipholius* had no activity against most of the organisms. The ethanolic extracts of *Azadirecta indica*, *Cedrella toona*, *Punicia granatum*, *Berginia ciliata* and *Lawsonia alba* has shown considerable activity while aqueous extract of *Berginia ciliata*, *Lawsonia alba* and *P. granatum* has also shown moderate activity (Table 2).

DISCUSSION AND CONCLUSION

In our study, aqueous, ethanolic, and hexane extracts of 17 commonly used medicinal plants from 14 different families were tested for the presence of antimicrobial activity against 1 yeast strain and 10 gram positive and gram negative bacteria. These plants are frequently used in Pakistan and have been previously described as antiseptics, astringents, diuretics, and for the relief of stomach aches (Said, 1969). In studies conducted in Turkey, Cambodia, Yemen, Peru, Brazil, Mexico and Cameron, screening of antibacterial activity were conducted but

Table 1. Common uses of medicinal plants used in traditional system of medicines in Pakistan.

S/No.	Botanical name (family)	Voucher	Local name	Part used	Therapeutic uses
01	*E. globulus* (Myrataceae)	101-L	Safeda	L	Expectorant, Diarrhea, Skin diseases
02	*E. oficinalis* (Euphorbiacea)	102-F	Amla	F	Diuretic, typhoid, fever
03	*C. procera* (Asclepdiaceae)	103-L	AK	L	Anthelmentics, Liver diseases.
04	*A. maritime* (Compositae)	104-Ap	Tarakh	Ap	Whooping cough, Fever
05	*N. chinesis* (Lauraceae)	105-B	Maida Sak	B	Diarrhea, Dysentery
06	*A. Indica* (Metiaceae)	106-L	Neem	L	Blood purifier, Astringent
07	*C. toona* (Meliaceae)	107-L	Tun	L	Ulcer, Rheumatism, dysentery
08	*M. azadirecta* (Meliaceae)	108-L	Dhrek	L	Anthelmentics, Diuretic
09	*P. granatum* (Punicae)	109-Rf	Anar	Rf	Diarrhea, Dysentery
10	*N. sativa* (Renunculaceae)	110-S	Kalongi	S	Urinary tract infections
11	*B. Ciliata* (Saxifragaceae)	111-Ri	Bud piah	Ri	Fever, Ulcer
12	*M. Communis* (Myrataceae)	112-F	Habul Ass	F	Diarrhea, dysentery
13	*B. aristata* (Berberideae)	113-F	Rasaut	F	Scabies
14	*A. Tinuipholius* (Liliaceae)	114-S	Piazi	S	Diuretic
15	*T. Cordifolia* (Merispermaceae)	115-Sm	Gilo	Sm	Antipyretic, Anthelmentics
16	*L. alba* (Lythraceae)	116-L/Sm	Mehndi	L, Sm	Astringent, Ulcer
17	*S. indicus* (Compositae)	117-L/Sm	Gorak Mundi	L, Sm	Skin diseases, Blood purifier

L (Leaves), F (Fruit), Ap (Aerial parts), B (Bark), Rf (Rind of fruit), Ri (Rhizome), S (Seeds), Sm (Stem).

Table 2. Antimicrobial activity of medicinal plants/zone of inhibition mm.

S/ No.	Botanical name and family	Material	A	B	C	D	E.	F.	G	H	I	J
			Staphylococcus	***S. typhi***	***S. dysentriae***	***V. cholerae***	***E. coli***	***K. pneumoniae***	***S. epidermidis***	***M. pyogens***	***B. subtilus***	***S. cerevisiae***
01	*E. globulus* (Myrataceae)	Aq.	24	19	24	24	21	30	24	24	23	30
		Etoh	30	24	30	23	21	29	23	23	29	29
		n-hex	15	14	16	16	15	16	14	16	14	15
02	*E. oficinalis* (Euphorbiacea)	Aq.	24	30	24	24	30	28	19	23	24	20
		Etoh	24	30	29	24	30	29	19	23	24	19
		n-hex	0	0	0	0	0	0	0	0	0	0
03	*C. procera* (Asclepdiaceae)	Aq.	0	0	0	0	0	0	0	0	0	0
		Etoh	16	15	16	14	15	0	13	16	12	14
		n-hex	0	0	0	0	0	0	0	0	0	0

Table 2. Contd.

04	*A. maritime* (Compositae)	Aq.	0	0	0	0	0	0	0	0	0	0
		Etoh	14	15	12	16	16	16	12	14	14	15
		n-hex	0	0		0	0	0	0	0	0	0
05	*N. chinesis* (Lauraceae)	Aq.	0	0	0	0	0	0	0	0	0	0
		Etoh	0	19	0	0	0	0	0	0	20	15
		n-hex	18	20	20	0	19	0	0	0	18	18
06	*A. Indica* (Metiaceae)	Aq.	0	0	0	0	0	0	0	0	0	0
		Etoh	17	22	19	0	24	0	23	19	24	23
		n-hex	0	0	0	0	0	0	0	0	0	0
07	*C. toona* (Meliaceae)	Aq.	0	0	0	0	0	0	0	0	0	0
		Etoh	16	0	0	0	14	0	0	0	13	16
		n-hex	0	0	0	0	0	0	0	0	0	0
08	*M. Azadirecta* (Meliaceae)	Aq.	19	16	0	0	0	0	0	0	18	0
		Etoh	20	15	0	0	0	0	0	0	20	0
		n-hex	0	0	0	0	0	0	0	0	0	0
09	*P. granatum* (Punicae)	Aq.	20	16	14	14	12	19	15	16	16	16
		Etoh	24	23	24	16	22	23	19	20	24	21
		n-hex	0	0	0	0	0	0	0	0	0	0
10	*N. sativa* (Renunculaceae)	Aq.	0	0	0	0	0	0	0	0	0	0
		Etoh	18	20	13	12	18	12	0	0	17	0
		n-hex	0	0	0	0	0	0	0	0	0	0
11	*B. Ciliata* (Saxifragaceae)	Aq.	19	20	29	14	22	18	15	16	17	23
		Etoh	18	21	26	14	23	20	20	19	22	24
		n-hex	0	0	0	0	0	0	0	0	0	0
12	*M. Communis* (Myrataceae)	Aq.	12	14	16	14	14	0	0	0	0	0
		Etoh	15	16	16	13	14	0	0	0	0	0
		n-hex	0	0	0	0	0	0	0	0	0	0

Table 2. Contd.

13	*B. aristata* (Berberideae)	Aq.	12	14	16	16	15	0	0	0	0	0
		Etoh	14	14	13	15	12	0	0	0	0	0
		n-hex	0	0	0	0	0	0	0	0	0	0
14	Aspholedus (*Tinuipholius Liliaceae*)	Aq.	14	16	16	12	16	0	0	0	12	0
		Etoh	15	16	16	15	15	0	0	0	15	0
		n-hex	0	0	0	0	0	0	0	0	0	0
15	Tinospora (*Cordifolia erispermaceae*)	Aq.	14	14	15	16	14	0	0	0	13	0
		Etoh	15	16	15	14	16	0	0	0	12	0
		n-hex	0	0	0	0	0	0	0	0	0	0
16	*Lawsonia alba* (Lythraceae)	Aq.	23	24	19	18	20	12	19	16	23	14
		Etoh	24	22	18	19	24	14	23	14	24	15
		n-hex	0	0	0	0	0	0	0	0	0	0
17	*Sphaeranthus indicus* (Compositae)	Aq.	24	22	23	24	24	16	14	15	23	14
		Etoh	24	29	22	22	24	20	16	15	22	18
		n-hex	22	28	22	21	22	18	12	13	22	18

Key: - Inhibition zone diameter = mm Aq (Aqueous), Etoh (Ethanolic), n-hex (n-hexane), Zone of inhibition in mm. *(A) S. aureus (B) S. typhi (C) S. dysentriae (D) V. cholerae (E) Escherichia coli (F) K. pneumoniae (G) S. epidermidis (H) M. pyogens (I) B. subtilus (J) S. cerevisiae*.

but were less extensive than the study described here (Turker and Usta, 2008; Kong, 2007; Al-Fatimi et al., 2007; Duarte, 2006; Kloucek et al., 2006).

Many of the plants described in this study showed antimicrobial activity which was comparable to antibiotics like tetracycline, streptomycin and Chloramphenicol. These plants can offer alternative treatments for drug resistant enteropathogens. Medicinal plants have a great economic value and professional importance as 80% of the population in developing countries which is the 65% of the total global population relies on traditional medicines for their primary health care needs (Fabricant and Farnsworth, 2001). Pakistan is rich in medicinal and aromatic plants and there is need for the investigation of unexplored flora to screen for new antimicrobial agents and determine the compounds within which are responsible for their activity.

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