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Antibacterial potential of plants traditionally used for curing diarrhea in Khyber Pakhtunkhwa, Pakistan

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Thirty-three plant species belonging to 26 families are traditionally used for curing diarrheal diseases in Khyber Pukhtunkhwa, Pakistan. The ethanolic extracts of these medicinal plants were screened for their antibacterial potential against five bacterial species. These tests were carried out using agar well diffusion method at three elevating concentrations (10, 20 and 30 mg/ml) of the crude extracts. The results indicated that all medicinal plants showed anti-bacterial activity on at least one bacterial species. The maximum anti-bacterial capacity was exhibited by *Ajuga bracteosa*, *Cedrella serrata*, *Juglans regia* and *Mentha viridis* against both gram-positive and gram-negative bacteria. The Minimum Inhibitory Concentrations (MICs) of the crude extracts of these plants determined by agar dilution method indicated that *M. viridis* was the most potent species inhibiting the growth of all tested bacterial species. The ethno-pharmacological knowledge of these 33 plant species was also documented. It was concluded that medicinal plants used for the treatment of various diarrheal diseases possess anti-bacterial elements and justify their use in traditional medicine.

Key words: Antibacterial activities, medicinal plants, diarrheal diseases, Khyber Pukhtunkhwa.

INTRODUCTION

Rescuing human race from the clutches of diseases has remained a sacred duty of man from the advent of civilization. The herbal medicine co-evolved with mankind within their societies and large proportions of rural and urban populations (about 80%) throughout the world depend upon herbal medicine for symbolic and medicinal value (Ahmad et al., 1998). The majority (1.5 billion) of the population of developing countries uses traditional medicine either because the people cannot afford to buy synthetic medicine or because traditional medicine is more acceptable (Singh and Khan, 1990).

Among the various diseases prevalent in the rural areas of Pakistan, the diarrheal disease is one of the

principal causes of human death, particularly in the malnourished infants (Havagiray et al., 2004). Majority of people exclusively uses traditional herbal medicine to treat diarrhea of both infectious and non-infectious nature. The major causative agents of diarrhea in humans include: *Shigella flexneri*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* (Anne and Geboes, 2002). *Candida albicans* has also been known to cause diarrhea in humans (Robert et al., 2001). It is well documented that herbal medicine provide a promising source of anti-diarrheal drugs and potentially useful antimicrobial plant compounds or their extracts ideally display activity against a wide range of microorganisms (Gram et al., 2002). The anti-microbial activity of plant extracts may reside in a variety of different components, including aldehyde and phenolic compounds (Lai and Roy, 2004). Naturally occurring combinations of these compounds can be synergistic and

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often result in crude extracts having greater antimicrobial activity than the purified individual constituents (Delaquis et al., 2002). Approximately 119 pure chemical compounds of plant origin are used in medicine throughout the world. Screening of plants and their products for antimicrobial activity has shown that plants hosts a potential source of new anti-microbial agents (Press, 1996), and possess diverse combinations of chemicals that can produce different results on pathogenic organisms.

Medicinal plants with antidiarrheal and antimicrobial properties have been widely used by traditional healers. However, the therapeutic potentials of some of these medicinal plants have not been scientifically evaluated (Havagiray et al., 2004). Due to the side-effects and the resistance developed by pathogenic microorganism against antibiotics, many scientists have recently paid attention to plant extracts used in herbal medicine (Essawi and Srour, 2000). The work of various scientists on different medicinal plants with anti-microbial potential from different parts of the world have helped in processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Sasidharan et al., 2007; Nalina and Rehman, 2007). Current study reports the antibacterial activity and traditional knowledge of various medicinal plants, which are used in the treatment of diarrheal diseases in Khyber Pukhtunkhwa, Pakistan.

MATERIALS AND METHODS

Collection of medicinal plants

We collected 33 medicinal plants traditionally used for curing diarrheal diseases in Khyber Pukhtunkhwa, Pakistan. The plants were identified with the help of available literature (Ali, 1967, 1977, 1983; Danief, 1979; Hedge, 1990; Jafri, 1973) and voucher specimens were deposited in the herbaria of Quaid-i-Azam University and Islamia College University, Pakistan. For preparing ethanolic extracts, the plants materials were shade dried for 14 days.

Tested bacterial species

We used gram-negative *E. coli*, *S. typhi*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and gram-positive *S. aureus* as test microorganism. Nutrient agar diffusion medium was used for the growth of bacteria following the protocol adopted by Mariam et al. (1993).

Preparation of ethanolic extracts

Different parts of medicinal plants were grinded to 60-mesh diameter powder in an electric grinder. Fifty grams of this powder per sample was soaked in 250 ml of 95% ethanol for 72 h. The ethanolic extract obtained was filtered thrice through Whatman filter paper, concentrated using rotary evaporator and stored at 4°C prior to use. The percentages of the crude extracts were calculated following the method of Miller (1980). For determining antibacterial activity, the ethanolic extracts and the standard drug (Streptomycin) were dissolved in dimethylsulphoxide (DMSO) at the rates of 10, 20

and 30 mg/ml respectively, following the protocol adopted by Mariam et al. (1993).

Determination of antibacterial activity

Antibacterial activity of the crude extracts was carried out using agar well diffusion method (Carron et al., 1987). Single bacterial colony was incubated in nutrient broth at 30°C, for 24 h. 10 ml of this culture was added to molten nutrient agar tube, mixed, and poured in petri plate. After solidification, 7 mm wells were made using sterilized borer. 100 µl of crude extract samples were added to each well, separately, and marked with specific code designated to each sample. The plates were incubated for 24 h at 30°C, after which zone of inhibition were measured for each sample. For control, 100 µl of reference antibacterial drug were added to wells, in the same plates.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations of the crude extracts of various plants were determined by agar dilution method (Mukherjee, 2002). The growth media, nutrient agar was first prepared in a usual fashion and sterilized by autoclaving. The sterilized media was allowed to cool to 50°C and 18 ml of the molten agar was added to test tubes which contained 2 ml of different concentration of the test drugs (crude extract) and the control (95% ethanol). The mixture of the media and the test drugs were thoroughly mixed and poured into pre-labeled sterile Petri-dishes on a level surface. Additional Petri-dishes containing only the growth media were prepared in the same way so as to serve for comparison of growth of the respective organisms. The concentrations of the extracts used in this test ranged from 12 to 0.75 mg/ml. The lowest concentration which inhibited the growth of the respective organisms was taken as MIC. All tests were carried out in triplicate.

RESULTS

The traditional medicinal knowledge of 33 medicinal plants used for diarrheal disease were collected and documented in the form of an inventory (Table 1). Maximum number (4 spp.) of plants used for curing diarrhea belong to family Lamiaceae followed by Apocynaceae (3 spp.) and Maliaceae represented by 2 plant species.

The antibacterial screening assays of the crude extracts of the selected medicinal plants is given in Table 2. Current study showed that the selected medicinal plants exhibited anti-bacterial activity on at least one of the selected bacterial species. The most efficient anti-bacterial plant species include *Juglans regia*, *Cedrella serrata*, *A. bracteosa* and *M. viridis*, which inhibited the growth of all tested bacterial species.

The highest antibacterial activity was shown by *Alstonia scholaris*, *Jastacia adhotoda* and *Lepidium sativum* against gram negative *P. aeruginosa*; whereas *A. scholaris* and *Eriobotrya japonica* inhibited the growth of *S. typhi*. The minimum antibacterial activity was shown by *Ailanthus altissima*, *Ficus carica* and *Sida cardifolia* that inhibited the growth of *S. aureus*, while the remaining plant species showed moderate activity against the

Table 1. Ethno-medicinal knowledge of 33 medicinal plants used for curing diarrhea in Khyber Pakhtunkhwa, Pakistan.

Plant material	Voucher number	Part used	Traditional uses
<i>Adiantum capillus veneris</i> L. (Adiantaceae)	SJ 15	Rhizome	The rhizome filtrate is used in case of diarrhea. The leaves juices mixed with sugar, is used for inflammation of stomach and fever, it provides coolness to the body.
<i>Ailanthus altissima</i> (Mill.) (Simaroubaceae)	SJ190	Bark	Bark is anthelmintic, in dysentery, Antispasmodic Root bark recommended for cardiac troubles, epilepsy and asthma.
<i>Ajuga bracteosa</i> Wall (Lamiaceae)	SJ85	Whole plant	The decoction of the plant is used in kidney pain stimulant and diuretic.
<i>Aloe barbadensis</i> Mill. (Liliaceae)	SJ94	Leaves juice	Fresh juices is refrigerant , cathartic and used in eye trouble.
<i>Alstonia scholaris</i> (Apocynaceae)	SJ25B	Bark	Bark is anthelmentic, used in chronic diarrhea asthma etc.
<i>Bergenia ciliata</i> (Haw). (Saxifragaceae)	SJ188	Rhizome	Rhizome is diuretic; demulcent and with honey given to children when teething. It dissolves gravel and stones in the bladder.
<i>Calotropis procera</i> (Willd) R. Br (Asclepidaceae)	SJ27	Latex of leaves, leaves and roots	Locally the bark is powdered and used in dysentery, the milky juice is used is various skin diseases.
<i>Cedrella serrata</i> Royle. (Meliaceae)	SJ99	Bark	Bark is antiperiodic, tonic, astringent; externally applied to ulcers; also used in chronic infantile dysentery.
<i>Eclipta alba</i> L. (Asteraceae)	SJ33A	Stem + leaves mix	Used in hepatic and spleen enlargement.
<i>Eriobotrya japonica</i> (Thumb.) Lindley. (Rosaceae)	SJ154	Leaves	Flower is expectorant. Fruit is sedative; used in reduce thirst and vomiting. Infusion of leaves is given in diarrhea.
<i>Eucalyptus camaldulensis</i> Dennhardt. (Myrtaceae)	SJ117	Leaves	The leaver are antiseptic, and smoked to relive asthma and repellant to mosquitoes.
<i>Euphorbia helioscopia</i> L. (Euphorbiaceae)	SJ62	Whole plant	The milky latex is poisonous and causes swelling on skin. Irritating the animals cause in animals purgation.
<i>Ficus carica</i> L. (Moraceae)	SJ108	Leaves latex	Locally fruits are used as a food. The milky juice of green fruits and leaves are used to destroy warts.
<i>Foeniculum vulgare</i> Hill (Apiaceae)	SJ22	Fruit	The fruit is used in cooling drink in fever and scalding of urine. Also used in confectionary; refrigeration used as anti-emetic and also used to improve eyesight.
<i>Justicia adhatoda</i> L. (Acanthaceae)	SJ13	Leaves	Leaves are powerful expectorant, antispasmodic; chiefly used in disease of respiratory tract, particularly in tuberculosis, all kinds of cough, chronic bronchitis and asthma.
<i>Juglans regia</i> L. (Rosaceae)	SJ83	Bark	Bark used for cleaning of teeth. Seed kernel sexual and mental tonic.

Table 1. Contd.

<i>Juniperus excelsa</i> (Cupressaceae)	M.B.	SJ03	Fruit	Fruit used in stomach cramps, asthma, diuretic, carminative, stimulant, used in dropsy, gonorrhea, gleet, leucorrhoea and some cutaneous diseases.
<i>Lantana camara</i> L. (Verbenaceae)		SJ206	Whole plant	The decoction of the plant is considered as diaphoretic, carminative, antiseptic and vulnerary, rheumatism, tetanus and malaria.
<i>Lepidium sativum</i> L. (Brassicaceae)		SJ42	Seed	Seeds are galactagogue, emmenagogue, and tonic, aphrodisiac, laxative and rubefacient. It allays irritation of the intestines in dysentery and diarrhea.
<i>Melia azedarach</i> L. (Meliaceae)		SJ100	Leaves	Leaf juices anthelmintic, diuretic and emmenagogue.
<i>Mallotus philippensis</i> (Euphorbiaceae)		SJ63	Whole plant	Carminative and smoked as cigarettes for relief of asthma.
<i>Mentha longifolia</i> L. (Lamiaceae)		SJ88	Leaves	Locally, the leaves are boiled in water along with cardamom seed and the extract is given to children as anti-emetic.
<i>Mentha piperata</i> Linn. (Lamiaceae)		SJ89	Leaves	Stimulant, stomachic and carminative.
<i>Mentha viridis</i> (Lamiaceae)		SJ90	Leaves	Leaves used locally for chutneys and flavoring agent. The infusion mixed with sugar is given to children during vomiting and dysentery.
<i>Paeonia emodi</i> (Paeoniaceae)		SJ124	Whole plant	Young fronds eaten and the decoction of whole plant are used in dysentery.
<i>Punica granatum</i> L. (Punicaceae)		SJ141	Bark	The bark is used for the expulsions of tapeworms and for snakebite.
<i>Quercus incana</i> , Rox. (Fagaceae)		SJ68	Bark	The bark is given as a diuretic and also as astringent in indigestion diarrhea.
<i>Salvadora oleoides</i> Decne (Salvadoraceae)		SJ183	Fruits	Used for enlarged spleen and rheumatism.
<i>Sesamum indicum</i> L. (Malvaceae)		SJ181	Seed	Seeds edible used in breads cookies cakes and confectionary.
<i>Sida cardifolia</i> L. (Apocynaceae)		SJ25	Leaves	Leaves are demulcent and diuretic. Boiled in oil applied to testicular swelling.
<i>Valeriana jatamansi</i> Jones (Valerianaceae)		SJ204	Rhizome	Rhizome is used in fever, diarrhea and cough.
<i>Verbascum thapsus</i> (Scrophulariaceae)	L.	SJ184	Leaves	Leaves are used for diarrhea and dysentery in cattle.

Table 2. Antibacterial activity of the plant ethanolic extracts against five bacterial species.

No	Plant species	Conc. (mg/ml)	Growth inhibition (mm) in Bacteria				
			<i>E. coli</i>	<i>S. typhi</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>P. vulgaris</i>
1	<i>A. capillus veneris</i>	30	11 ± 0.6	16 ± 0.8	11 ± 0.4	21 ± 0.2	10 ± 0.1
		20	5 ± 0.7	10 ± 0.9	10 ± 0.3	14 ± 0.4	-
		10	-	6.0 ± 0.3	-	11 ± 0.5	-
2	<i>Ailanthus altissima</i>	30	-	-	-	20 ± 0.2	4.0 ± 0.8
		20	-	-	-	13 ± 0.8	-
		10	-	-	-	5.0 ± 0.7	-
3	<i>Ajuga bracteosa</i>	30	23 ± 0.3	21 ± 0.2	14 ± 0.8	18 ± 0.1	21 ± 0.2
		20	17 ± 0.2	13 ± 1.0	9.0 ± 0.9	13 ± 0.8	15 ± 0.3
		10	11 ± 0.2	10 ± 0.1	5.0 ± 0.5	8.0 ± 0.2	10 ± 0.5
4	<i>Aloe barbidensis</i>	30	14 ± 0.8	15 ± 0.9	7 ± 0.9	20 ± 0.6	-
		20	-	10 ± 0.8	-	15 ± 0.4	-
		10	-	5.2 ± 0.5	-	6.0 ± 0.1	-
5	<i>Alstonia scholaris</i>	30	-	18 ± 0.2	24 ± 0.9	17 ± 0.2	14 ± 0.1
		20	-	13 ± 0.9	18 ± 1.0	14 ± 0.1	16 ± 0.5
		10	-	7.0 ± 0.3	9.0 ± 0.4	9 ± 0.2	19 ± 0.2
6	<i>Bergenia ciliata</i>	30	25 ± 0.3	24 ± 0.8	25 ± 0.4	24 ± 0.9	20 ± 0.7
		20	22 ± 0.2	23 ± 0.6	20 ± 0.9	18 ± 0.3	16 ± 0.5
		10	16 ± 0.4	20 ± 0.1	8.0 ± 0.7	10 ± 0.3	11 ± 0.2
7	<i>Calotropis procera</i>	30	-	15 ± 0.3	-	18 ± 0.3	16 ± 0.5
		20	-	10 ± 0.4	-	17 ± 0.8	5 ± 0.8
		10	-	-	-	9 ± 0.8	-
8	<i>Cedrela serrata</i>	30	17 ± 0.1	-	19 ± 1.0	18 ± 0.3	18 ± 0.5
		20	12 ± 0.4	-	10 ± 0.5	16 ± 0.1	13 ± 0.8
		10	-	-	6 ± 0.7	12 ± 0.6	-
9	<i>Eclipta alba</i>	30	-	15 ± 0.3	-	11 ± 0.8	5 ± 0.1
		20	-	7 ± 0.9	-	8 ± 0.4	-
		10	-	-	-	-	-
10	<i>Eriobotrya japonica</i>	30	-	18 ± 0.2	-	10 ± 0.2	22 ± 0.1
		20	-	12 ± 0.6	-	4 ± 0.1	18 ± 0.8
		10	-	8 ± 0.3	-	-	12 ± 0.2
11	<i>Eucalyptus camaldulensis</i>	30	22 ± 0.7	18 ± 0.8	-	20 ± 0.2	12 ± 0.2
		20	18 ± 0.5	14 ± 0.4	-	11 ± 0.4	8 ± 0.9
		10	13 ± 0.2	-	-	9 ± 0.3	-
12	<i>Euphorbia helioscopia</i>	30	-	16 ± 0.1	7 ± 0.9	-	14 ± 0.7
		20	-	7.0 ± 0.7	-	-	6 ± 0.4
		10	-	-	-	-	3 ± 0.1

Table 2. Contd.

13	<i>Ficus carica</i>	30	-			18 ± 0.2	
		20	-			13 ± 0.7	-
		10	-	-	-	8.0 ± 0.3	-
14	<i>Foeniculum vulgare</i>	30	15 ± 0.2	17 ± 0.3	10 ± 0.4	13 ± 0.7	18 ± 0.5
		20	11 ± 1.0	9.0 ± 0.1	6 ± 0.5	9 ± 0.6	6 ± 0.7
		10	4 ± 0.1	-	-	-	-
15	<i>Justicia adhatoda</i>	30	-	11 ± 0.3	22 ± 0.5	17 ± 0.9	-
		20	-	5.0 ± 0.9	16 ± 0.6	12 ± 0.4	-
		10	-	-	7 ± 0.1	10 ± 0.3	-
16	<i>Juglans regia</i>	30	22 ± 0.1	23 ± 0.4	20 ± 0.4	23 ± 0.5	18 ± 0.2
		20	12 ± 0.5	11 ± 0.2	16 ± 0.3	9 ± 0.2	12 ± 0.9
		10	9 ± 0.1	6 ± 0.3	9 ± 0.1	5 ± 0.1	10 ± 0.7
17	<i>Juniperus excelsa</i>	30	14 ± 0.5	15 ± 0.8	8 ± 0.4	15 ± 0.1	13 ± 0.7
		20	-	6 ± 0.2	-	10 ± 0.5	-
		10	-	-	-	-	-
18	<i>Lantana camara</i>	30	10 ± 0.6	16 ± 0.3	11 ± 0.5	-	-
		20	-	12 ± 0.4	-	-	-
		10	-	-	-	-	-
19	<i>Lepidium sativum</i>	30	16 ± 0.8	10 ± 0.2	24 ± 0.4	19 ± 0.4	14 ± 0.1
		20	7 ± 0.7	-	11 ± 0.9	15 ± 0.5	7.0 ± 0.9
		10	-	-	-	6 ± 0.3	-
20	<i>Melia azedarach</i>	30	15 ± 0.2	18 ± 0.1	17 ± 0.2	12 ± 0.2	-
		20	10 ± 0.1	6 ± 0.3	-	-	-
		10	-	-	-	-	-
21	<i>Mallotus philippensis</i>	30	-	4 ± 0.8	15 ± 0.8	7 ± 0.2	12 ± 0.7
		20	-	-	10 ± 0.9	-	5 ± 0.6
		10	-	-	-	-	-
22	<i>Mentha longifolia</i>	30	10 ± 0.2	13 ± 0.4	14 ± 0.7	17 ± 0.8	-
		20	-	8 ± 0.5	-	12 ± 0.9	-
		10	-	-	-	-	-
23	<i>Mentha piperata</i>	30	15 ± 0.8	17 ± 0.8	-	12 ± 0.8	-
		20	6 ± 0.9	9 ± 0.2	-	6 ± 0.4	-
		10	-	-	-	-	-
24	<i>Mentha viridis</i>	30	19 ± 1.0	17 ± 0.4	16 ± 0.5	19 ± 0.1	15 ± 0.7
		20	15 ± 0.7	14 ± 0.2	11 ± 0.1	12 ± 0.5	12 ± 0.1
		10	11 ± 0.5	6 ± 0.3	8 ± 0.9	9 ± 0.7	7 ± 0.2
25	<i>Paeonia emodi</i>	30	-	-	18 ± 0.7	16 ± 0.3	-
		20	-	-	12 ± 0.9	12 ± 0.1	-
		10	-	-	-	-	19 ± 0.3

Table 2. Contd.

26	<i>Punica granatum</i>	30	15 ± 0.2	11 ± 0.1	16 ± 0.2	18 ± 0.8	-
		20	11 ± 0.6	9 ± 0.3	7 ± 0.4	13 ± 0.6	-
		10	-	-	-	10 ± 0.8	-
27	<i>Quercus incana</i>	30	16 ± 0.7	16 ± 0.1	15 ± 0.6	13 ± 0.2	15 ± 0.8
		20	9 ± 0.4	10 ± 0.5	-	8 ± 0.9	11 ± 0.2
		10	-	-	-	-	-
28	<i>Salvadora oleoides</i>	30	-	8 ± 0.2	-	-	6 ± 0.2
		20	-	-	-	-	-
		10	-	-	-	-	-
29	<i>Sesamum indicum</i>	30	-	18 ± 0.3	15 ± 0.4	14 ± 0.8	-
		20	-	10 ± 0.2	-	6 ± 0.3	-
		10	-	-	-	-	-
30	<i>Sida cardifolia</i>	30	-	-	-	17 ± 0.9	-
		20	-	-	-	8 ± 0.2	-
		10	-	-	-	-	-
31	<i>Thevetia peruviana</i>	30	16 ± 0.5	8 ± 0.5	12 ± 0.6	14 ± 0.3	6 ± 0.9
		20	-	-	5 ± 0.3	6 ± 0.3	-
		10	-	-	-	-	-
32	<i>Valeriana jatamansi</i>	30	-	-	10 ± 0.2	15 ± 0.8	15 ± 0.3
		20	-	-	-	10 ± 0.3	8 ± 0.7
		10	-	-	-	-	-
33	<i>Verbascum thapsus</i>	30	-	13 ± 0.4	17 ± 0.5	11 ± 0.4	5 ± 0.1
		20	-	8 ± 0.6	11 ± 0.3	6 ± 0.7	-
		10	-	-	6.2 ± 0.2	-	-
34	Control (Streptomycin)	0.1	30	30	35	30	30

tested bacterial species as compared to control (Streptomycin). Based on the initial antibacterial screening test, *J. regia*, *C. cirrata*, *M. viridis* and *A. bracteosa* were further studied for the determination of MIC, as they were found to be most active against all tested bacterial species. The MIC study showed that *M. viridis* was the most potent anti-bacterial plant species, while the least effective plant species was *A. bracteosa* (Table 3).

DISCUSSION

Sketchy substantiation and the conventional use of plants as medicines put forward the basis for investigation of which plant product and extract may be useful for definite medical condition. It is imperative to study scientifically

those plants, which have been used in traditional medicines as potential source of novel antimicrobial compound (Mitscher et al., 1987). Also, the reappearance of concentration in natural therapies and increasing demand for effective, safe, natural products means that quantitative data on plant product and extract are required.

As revealed from the results presented in Table 3, the antibacterial activities of the tested plant species were more pronounced on the gram-positive bacterium (*S. aureus*) than the gram-negative bacteria (*E. coli*, *S. typhi*, *P. aeruginosa* and *Protoeas vulgaris*). This may be due to the fact that gram-negative bacteria have an outer phospholipidic membrane carrying the structural lipopolysaccharide components, which makes their cell wall impermeable to antibacterial chemical substances. The gram-positive bacteria on the other hand are more

Table 3. Minimum inhibitory concentration (MIC) values of the 95% ethanol extracts of the four selected plant species on the tested bacterial species.

No.	Plant species	Conc.	Presence/absence of bacterial growth				
			<i>E. coli</i>	<i>S. typhi</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>P. vulgare</i>
1	<i>Ajuga bracteosa</i>	12.0	-	-	-	-	-
		6.0	-	-	-	-	-
		3.0	+	+	+	-	-
		1.5	+	+	-	+	+
		0.75	+	+	+	+	+
2	<i>Cedrella serrata</i>	12.0	-	-	-	-	-
		6.0	-	-	-	-	-
		3.0	+	+	-	+	+
		1.5	+	+	+	+	+
		0.75	+	+	+	+	+
3	<i>Juglans regia</i>	12.0	-	-	-	-	-
		6.0	-	-	-	-	-
		3.0	-	+	-	+	-
		1.5	+	+	+	+	+
		0.75	+	+	+	+	+
4	<i>Mentha viridis</i>	12	-	-	-	-	-
		6.0	-	-	-	-	-
		3.0	+	+	-	-	-
		1.5	+	+	+	+	-
		0.75	+	+	+	+	+

susceptible having an outer peptidoglycan layer which is not an effective permeability barrier. Therefore, the cell walls of gram-negative organisms are more complex than the gram-positive ones and act as a diffusional barrier thus making them less susceptible to the antibacterial agents than the gram-positive bacteria (Nostro et al., 2000; Hodges, 2002).

The most resistant bacterial species used in this study was *E. coli*. In fact, gram-negative bacteria are frequently reported to have developed multi-drug resistance to many of the antibiotics currently available in the market (Alonso et al., 2000; Sadar et al., 2002; Uddin et al., 2005). These kinds of differences in susceptibility among the microorganisms against antimicrobial substances may be explained by the difference in inheritance of genes on plasmids that can easily be transferred among bacterial strains (Helander et al., 1998). However, some species of plants such as *A. bracteosa*, *C. serrata*, *J. regia*, and *M. viridis* are still of particular significance for advance investigations in this regard as they showed remarkably stronger activity against *E. coli* than the other gram-positive bacteria, a trend not observed for other plant species. According to Trease and Evans (1992), these plants contain steroids, tannins and volatile oil. The antimicrobial activities of these compounds are very well

established (Scalbert, 1991; Hussein et al., 1997; Cowan, 1999; Djipa et al., 2000).

The antimicrobial activity of plant extracts may reside in a variety of different components, including aldehyde and phenolic compounds (Lai and Roy, 2004). Literature review on the phytochemical constituents of different plants revealed that *Adiantum capillus-veneris*, *Eucalyptus camaldulensis*, *Foeniculum vulgare*, *J. regia*, *Mentha sp.* contain the essential oil (Trease and Evans, 1992; Hammer et al., 1999). The mechanism of the action of essential oil is probably related to the outer membrane disintegrating properties of Thymol and Carvicol (Helander et al., 1998). Some investigations suggest that these compounds penetrate inside the cell, where they interfere with cellular metabolism. Other studies (Farag et al., 1989; Ultee et al., 2002) indicated that these compounds disturb the structure of the cellular membrane and react with active sites of enzyme or act as H carrier depleting adenosine triphosphate pool. These results are further supported by the investigation of the antibacterial activity of the essential oil (at 10, 20, 30 and 40 ml) from the spices (Nanasombat and Pana, 2005) and leaves of *A. capillus-veneris* against various bacterial strains. The essential oil exhibited maximum inhibition zone in *S. typhi* and mild activity was detected

for *Pseudomonas* sp., *K. pneumoniae* and *Streptococcus pyogenes* (Vector et al., 2002). Promising anti-bacterial results could be obtained from extracts of *Aloe perryi*, *Indigofera oblongifolia*, *Meriandri benghalensis* and *Ziziphus spina Christi* (Ali et al., 2001; Ahmad et al., 1998) further supported the present investigations.

Traditional herbal medicine is just one of the many different approaches in practice pertinent to plant use as remedies. It aims to treat the whole person and not just the symptoms and to encourage the body to heal itself. The fairly good degrees of correlation of traditional medicine claims with biological activities as observed in the present study warrant further investigation.

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