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Full Length Research Paper

Antimicrobial activity of selected medicinal plants of Margalla Hills, Islamabad, Pakistan

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The present studies focus on the antimicrobial activities of the crude methanolic extracts of different plant parts of thirteen selected medicinal plants namely: *Woodfordia fruiticosa*, *Adhatoda vasica*, *Chenopodium ambrosoides*, *Viburnum cotinifolium*, *Euphorbia hirta*, *Vitex negundo*, *Peganum harmala*, *Broussonetia papyrifera*, *Taraxacum officinale*, *Urtica dioica*, *Verbascum thapsus*, *Caryopteris grata* and *Mimosa rubicaulis* collected from Margalla Hills Islamabad, Pakistan against four Gram positive namely: *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus* and *Enterococcus faecalis* and five Gram negative namely: *Escherichia coli*, *Pseudomonas aeruginosa*, *Klibsella pneumonia*, *Vibrio cholera* and *Enterobacter coccus* bacterial strains. The fungal strains used were *Aspergilus niger*, *Aspergilus flavus*, *Aspergilus fumigates* and *Rhyzoctonia solani*. *Woodfordia fruiticosa*, *Chenopodium ambrosoides*, *Viburnum cotinifolium*, *Euphorbia hirta*, *Vitex negundo* and *M. rubicaulis* exhibited higher antibacterial activity showing minimum inhibitory concentration (MIC) values as 1 mg/ml against *E. faecalis* and *E. coccus*. *E. hirta* was most affective against *E. coli* by inhibiting the growth showing MIC value of 1.0 mg/ml. Maximum antifungal activity against *A. niger* was exhibited by *C. grata* (87.77%) followed by *E. hirta* (79.72%) and *V. cotinifolium* (72.39%), respectively. *A. vasica* showed greater inhibitory properties (89.50%) against *A. fumigates*. Maximum inhibitory activity against *R. solani* was shown by *V. negundo* (100%). *V. cotinifolium* exhibited maximum activity against *A. flavus* (88.93%) and *A. fumigates* (82.6%).

Key words: Medicinal plants, Margalla Hills, antibacterial, antifungal.

INTRODUCTION

Many medicinal plants around the world contain many compounds with antibacterial activity (Marjorie, 1999). Chemotherapy is the main approach in the treatment of bacterial infections but in clinical treatment antibiotics encounter a major problem of resistance and thus results in treatment failure (Mckeegan et al., 2002). Secondly various antibiotics are toxic, owing high cost but low efficacy. Because of the resistance that pathogenic build against antibiotics, there is a great interest in the search of new antimicrobial natural drugs from medicinal plants (Bisignano et al., 1996). Medicinal plants contribute to a significant proportion of pharmaceutical products in current used medicines derived from plants (Cowan 1999; Raksin et al., 2002). The antimicrobial properties of the plant extracts and natural products have been intensively investigated as the demand for safe drugs due to the misuse of the commercially available antibiotics and an increase in immuno-deficiency (Grayer and Harborne, 1994). A large number of phytochemicals belonging to several chemical classes have shown inhibitory effects on all types of microorganisms *in vitro* (Cowan, 1999) and some plant extracts have shown activity on both Gram positive and Gram negative bacteria (Nascimento et al., 2000). Presently there is no single plant derived antibacterial chemical entity used clinically (Gibbons, 2004). Different compounds have been used for this purpose mostly isolated from medicinal plants (Carballo et al., 2002). Fungal related diseases may not be as common as other microbial infections but, when present, they are difficult to treat especially in immunosupressed persons. According to the W.H.O important progress has been made in controlling major

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infectious diseases. However, about 43% of total deaths occurred in developing countries due to the infectious diseases in recent years. The search for new antimicrobial agents is necessary due to the appearance of microbial resistance and occurrence of fatal opportunistic infections (Kivack et al., 2001). The discovery and development of new antimicrobial agent is therefore, an on going process. Remarkable diversity of chemicals present in biological samples has tremendous potential in search of new antimicrobial agents. Therefore, the aim of the current investigation was to determine the antimicrobial potential of selected medicinal plants of Margalla Hills (Table 1) Islamabad, Pakistan.

MATERIALS AND METHODS

Medicinal plants used

The fresh plant materials collected were rinsed with water and kept under shade at room temperature for drying. After complete dryness, these plant parts were ground into powder and were kept in sealed plastic bags duly labeled. Extraction of these plant parts were carried out one by one by simple maceration process. Fifty grams of each powder was soaked in 500 ml of methanol and was blended for vigorous shaking and mixing. The poorly homogenized mixture was kept at room temperature (25±2)°C for 3 weeks. Then maximum solvent was separated from the mixture. Filtrate was filtered twice initially using the ordinary filter paper and then Whatman filter paper # 41. Solvent was completely evaporated by rotary evaporator (Heidolph model, Germany) and the gummy extract was put in separate bottles and labeled. The residues were again soaked in their respective recovered methanolic solvent for another two weeks and the process was repeated with three washings for each plant extract. After dryness the extracts were weighed. *Woodfordia fruiticosa* leaves, *Adhatoda vasica* leaves and twigs, *Chenopodium ambrosioides* aerial parts, *Viburnum cotinifolium* leaves, *Euphorbia hirta* aerial parts, *Vitex negundo* leaves, *Peganum harmala* aerial parts, *Broussonetia papyrifera* leaves, *Taraxacum officinale* flowers, *Urtica dioica* aerial parts, *Verbascum thapsus* aerial parts, *Caryopteris grata* leaves and *Mimosa rubicaulis* stem yielded 1.465, 1.01, 0.890, 1.371, 0.825, 1.785, 0.976, 0.682, 0.700, 0.854, 0.787, 0.811 and 0.900 g of the methanolic extracts respectively.

Antibacterial assay

Agar diffusion method (well diffusion method) or (cylinder plate method) was used for antibacterial activity as reported by Kavanagh (1963) and Leven et al., (1979). Wells were made in seeded agar and the test sample was then introduced directly into these wells. After incubation, the diameter of the clear zones around each well was measured and compared against zone of inhibition of the known concentrations of the standard antibiotics.

Preparation of stock solution

150 mg of the methanolic extract of each plant was dissolved in 10 ml of DMSO to prepare the stock solution (15 mg/ml).

Microorganisms used

4 gram-positive and 5 gram-negative bacterial strains were used for this assay. Gram positive strains: *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Micrococcus luteus* (ATCC 10240), *Enterococcus faecalis* (ATCC 19433). Gram negative strains: *Escherichia coli* (ATCC 15224), *Pseudomonas aeruginosa* (ATCC 7221), *Klibsella pneumonia* (ATCC UC57), *Vibrio cholera* (ATCC 11623) and *Entrobacter coccus* (ATCC 13048). All the microorganisms were maintained at nutrient agar medium at 4°C.

Preparation of inocula

Centrifuged pellets of bacteria from 24 h old culture in broth medium of selected strains were mixed with physiological saline and turbidity was corrected by adding sterile physiological saline until it matched with McFarland 0.5 $BaSO_4$ turbidity standard. Now this inoculum was ready for seeding.

Preparation of seed agar plates

Nutrient agar media already prepared by dissolving 20 g of the medium in 1 L of distilled water with pH 7 and autoclaved, was allowed to cool down to 45°C and were seeded with 10 ml of prepared inocula in order to have 106 CFU per ml. Petriplates (14 cm) were prepared by pouring 75 ml of the seeded nutrient agar in each plate and were solidified. 11 wells/holes per plate were made with sterile cork borer (8 mm).

Pouring of test solutions, incubation and measurement of zones of inhibition and determination of MIC

100 µl of the test solutions (8 conc. of each extract), 2 solutions for + control (one for each antibiotic that is, erythromycin and cefixime) and DMSO as negative control were poured in the respective wells/holes. All the plates were incubated in paraffin oven at 37°C. Diameter of the clear zones showing no bacterial growth around each well/ hole were measured in mm after 24 h (Collins et al., 1989). Plates were prepared in triplicate for each plant sample and bacterium tested.

Antifungal assay

The agar tube dilution method was used for antifungal activity as reported by Choudhary et al., (1995). Four fungal strains were used which are:

(1) *Aspergilus niger* (0198)
(2) *Aspergilus flavus* (0064)
(3) *Aspergilus fumigates* (66)
(4) *Rhizoctonia solani* (18619)

Each fungal strain was maintained on sabouraud dextrose agar mediumat 4°C. 12 mg of the methanolic extract of each plant was dissolved in 1 ml of DMSO to prepare the initial stock solution which was further diluted to get final concentration of 200 µg/ml. Solution of antibiotic terbinafine 12 mg/ml in DMSO was prepared for positive control.

Pure DMSO was used as negative control. The test tubes were incubated at 28°C for 7 days and the inhibition of growth was calculated with reference to negative control. % inhibition of fungal growth was determined by the following formula:

% inhibition of fungal growth = 100 - linear growth in test sample/ linear growth in control (mm) × 100

Table 1. List of selected medicinal plants collected from Margalla Hills for the study.

S/N	Family	Botanical name	Vernacular name	Part of the plant used
1.	Lytheraceae	*Woodfordia fruticosa* (L.) S. Kurz	Dhawi	Leaves
2.	Acanthaceae	*Adhatoda* Nees in wall *vasica*	Bhekkar	Leaves and twigs
3.	Chenopodiaceae	*Chenopodium ambrosio-ides* Linn.	Chandan bathwa	Aerial parts
4.	Caprifoliaceae	*Viburnum cotinifolium* D. Don	Taliana	Leaves
5.	Euphorbiaceae	*Euphorbia hirta* Linn.	Dudhi	Aerial parts
6.	Verbenaceae	*Vitex negundo* Linn.	Banna	Leaves and twigs
7.	Zygophyllaceae	*Peganum harmala* Linn.	Harmal	Aerial parts
8.	Moraceae	*Broussonetia papyrifera* Vent.	Jangli Shahtoot / toot	Leaves
9.	Asteraceae	*Taraxacum officinale* Weber	Dudal	Flowers
10.	Urticaceae	*Urtica dioica* Linn.	Bichu booti	Aerial parts
11.	Crophulariaceae	*Verbascum thapsus* Linn.	Gidar tambaku	Aerial parts
12.	Verbenaceae	*Caryopteris grata*	Benth and Hook. f.	Leaves
13.	Mimosaceae	*Mimosa rubicaulis* Lam	Ral	Stem

RESULTS AND DISCUSSION

Plants produce a wide range of biologically active molecules making them a rich source of different medicines which play a dominant role in the maintenance of human health since time immemorial (Farombi, 2003). During present investigation, the methanolic extracts of selected medicinal plants were tested against 9 bacterial strains (both Gram +ve and –ve) and minimum inhibition concentration was determined (MIC). The MIC for erythromycin and cefixime (antibiotics as control) was 1 mg/ml against all strains but cefixime was not effective against 3 strains.

The results presented in Table 2 revealed that against *Micrococcus luteus*, only *T. officinale* and *M. rubicaulis* were effective showing 1 and 5 mg/ml MIC respectively. Against *Enterococcus faecalis*, the *A. vasica*, *P. harmala, T. officinale, U. dioica, V. thapsus* and *C. grata* did not show antibacterial activity. However, the plant species *W. fruticosa, C. ambrosoides, V. cotinifolium, E. hirta, V. negundo, B. papyrifera and M. rubicaulis* were effective in inhibiting the growth of *E. faecalis* showing MIC values as 1 mg/ml. The growth of *Enterococcus bacter* was inhibited only by *W. fruticosa, C. ambrosoides, V. cotinifolium*, *E. hirta*, *V. negundo* and *M. rubicaulis* as indicated by MIC values of 1 mg/ml. All the selected plant species exhibited MIC values against *Vibrio cholera* as 1 mg/ml except *T. officinale* (12.5 mg/ml), *V. thapsus* (10 mg/ml) and *C. grata* (5 mg/ml). Against *S. aureus*, the *V. negundo*, *B. papyrifera*, *T. officinale, U. dioica,* and *C. grata* did not show any activity. *P. harmala* showed higher MIC value (12.5 mg/ml) as compared to remaining plants having MIC values as 1 mg/ml and *E. hirta* as 2 mg/ml. The *T. officinale* did not show any activity against *B. subtilis* at all but rest of the plants showed lower MIC values of 1 mg/ml except *A. vasica, U. dioica* and *C. grata* which showed higher MIC values of 10 mg/ml and 7.5 mg/ml respectively. The *T. officinale* was ineffective against *P. aeruginosa* and *B. papyrifera* and *C. grata* showed higher MIC values (7.5 mg/ml) as compared to other plant species showing MIC as 1mg/ml except for *A.vasica* showing MIC 2 mg/ml. Against *Klibesella pneumonia*, the plant species *P. harmala, T. officinale, C. grata* did not show any activity. The higher MIC values were recorded as 12.5 mg/ml for *V. thapsus* and 7.5 mg/ml for *B. papyrifera* and *U. dioica* while remaining plant species showed MIC value of 1 mg/ml except *A. vasica* (2 mg/ml). Nearly half of the plants showed no activity against *Escherichia coli*. Higher MIC values were recorded for *V. thapsus* (15 mg/ml), *U. dioica and C. grata* (7.5 mg/ml), *V. cotinifolium* (5 mg/ml) and *W. fruticosa* (3 mg/ml). *E. hirta* showed lowest MIC (1 mg/ml).

Dabur et al. (2007) screened out 77 different plant extracts from 24 plants against eight bacterial strains using microbroth dilution assay. They found that aqueous extracts of *Acacia nilotica*, *Justicia zylanica*, *Lantana* camara and *Saraca asoca* exhibited good activity against all the tested bacterial strains and the MIC was recorded in the range of 9.375 to 37.5 µg/ml. They also reported the antimicrobial activity of *W. fruticosa* with MIC range of 75 to 1200 µg/ml. Similarly, Bajracharya et al. (2008) determined the antibacterial properties of plant extracts against different species of entero pathogenic bacteria like *E. coli*, *Klebsiella* sp*, Citrobacter* sp*, Enterobacter sp, Salmonella typhi, Salmonella paratyphi* etc. They also reported that *W. fruticosa* was found effective against all the enteric bacteria. During the present investigation it was also found that *W. fruticosa* was effective against all tested bacterial strains except *M. luteus*. The methanolic extract of *E. hirta* exhibited higher antibacterial activity against *E. coli*. Similar results were found by Abubakar (2009) that methanolic extract of *E. hirta* could be beneficial in treating enteric infections caused by *E. coli*. The methanolic extracts of *W. fruticosa*, *E. hirta* and *V. cotinifolium* were effective against Gram –ve bacterial

Table 2. Minimal inhibitory concentration (mg/ml) of selected medicinal plants collected from Margalla Hills against different bacterial strains. The data represents mean of three replicates.

Plant species	*M. luteus*	*E. faecalis*	*E. bacter*	*V. cholerae*	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*K. pneumonia*	*E. coli*
W. fruticosa	0	1	1	1	1	1	1	1	3
A. vasica	0	0	0	1	1	10	2	2	0
C. ambrosoides	0	1	1	1	1	1	1	1	0
V. cotinifolium	0	1	1	1	1	1	1	1	5
E. hirta	0	1	1	1	2	1	1	1	1
V. negundo	0	1	1	1	0	1	1	1	0
P. harmala	0	0	0	1	12.5	1	1	0	0
B. papyrifera	0	1	0	1	0	1	7.5	7.5	0
T. officinale	1	0	0	12.5	0	0	0	0	0
U. dioica	0	0	0	1	0	10	1	7.5	7.5
V. thapsus	0	0	0	10	1	1	1	12.5	15
C. grata	0	0	0	5	0	7.5	7.5	0	7.5
M. rubicaulis	5	1	1	1	1	1	1	1	0
Erythromycin	1	1	1	1	1	1	1	1	1
Cefixime	0	1	0	1	1	0	1	1	1

strains. Gram –ve bacteria are usually resistant to plant extracts as described by Kambezi and Afolayan (2008) because of the outer phospholipids membrane with the structural lipopolysaccharide components, which prevent the antimicrobial agents to pass through the cell wall (Nikaido and Vaara, 1985). However, during the present study it was found that some of the selected medicinal plants collected from Margalla Hills were highly effective in inhibiting the growth of Gram –ve and Gram +ve bacteria. From the results it can be inferred that methanolic extracts of *W. fruticosa*, *C. ambrosoides*, *V. negundo*, *E. hirta*, *V. cotinifolium* and *M. rubicaulis* could be utilized in the formulation of bioactive antimicrobial drugs. Fungi are major destroyers of foodstuffs and grains during storage, making them ailing for human utilization by retarding their nutritive value and often by producing mycotoxins (Marin et al., 1999). Keeping in view the aforementioned contexts, during present study, the methanolic extracts of selected medicinal plants were tested against 4 fungal strains.

The results presented in Table 3 revealed that maximum (87.77%) antifungal activity against *Aspergilus niger* was shown by *Caryopteris grata* followed by *E. hirta* (79.72%) and *V. cotinifolium* (72.39%) respectively. The ranking for antifungal activity of metahanolic extracts of different plant species against *A. niger* was as follows: *C. grata* > *E. hirta*> *V. cotinifolium*> *M. rubicaulis* = *T. officinale*> *Chenopodium ambrosoides*> *U. dioica* >*Broussonetia papyrifera*> *Verbascum thapsus*> *Vitex negundo*> *A. vasica* = *Woodfordia fruticosa*> *P. harmala.* The results showed that *Aspergilus flavus* showed greater susceptibility to methanolic extracts of *Euphorbia hirta*, *Viburnum cotinifolium*, *Urtica dioica, T. officinale*, *Vitex negundo*, *Verbascum thapsus, C. grata* and *Chenopodium ambrosoides* respectively. It was found that methanolic extracts of *Broussonetia papyrifera, M. rubicaulis*, *P. harmala*, *A. vasica and W. fruticosa* were not highly effective in inhibiting linear growth of *Aspergilus flavus* (Table 3). The methanolic extract of *A. vasica* was ineffective against *Aspergilus niger* and *Aspergilus flavus* but exhibited greater inhibitory properties (89.50%) against *Aspergilus fumigatus* as compared to methanolic extracts of other plant species. However, the inhibitory effect of methanolic extract prepared from *Woodfordia fruticosa* (89.50%) was comparable to that of *Adhatoda vasica*. According to their inhibitory activity against *Aspergilus fumigatus*, the different plant species were ranked as follow:

Adhatoda vasica = *Woodfordia fruticosa*>

Table 3. Antifungal activity of selected medicinal plants collected from Margalla Hills. The data represents mean of three replicates. The fungicide terbinafine was used as positive control.

Plant species	Linear growth of inhibition (%)			
	A. niger	***A. flavus***	***A. fumigatus***	***R. solani***
W. fruticosa	2.8 ±0.34	7.05±0.57	89.50 ±0.25	77.48±0.514684
A. vasica	2.8±0.36	7.05±0.35	89.50±0.36	77.48±0.47
C. ambrosoides	36.71±0.49	53.01± 0.99	56.88 ±0.28	42.25 ±0.23
V. cotinifolium	72.39±0.83	88.93±0.58	82.62±0.41	15.14±0.26
E. hirta	79.72±0.67	98.67±0.88	28.99±0.18	20.78±0.18
V. negundo	13.29±0.72	61.06±1.10	31.9±0.53	100±0.00
P. harmala	0.70±0.70	19.12±0.47	12.68±0.24	44.20±1.57
B. papyrifera	29.03±0.75	39.26±0.37	0.73±0.72	0.7±0.35
T. officinale	37.41±0.91	70.81±0.42	84.8±0.43	77.47±0.51
U. dioica	31.82±0.41	88.59±0.30	2.9±0.33	0.70±0.35
V. thapsus	25.53±0.65	58.39±0.30	23.18±0.50	64.09±0.28
C. grata	87.77±0.89	53.69±0.65	6.16±0.30	22.17±0.86
M. rubicaulis	37.07±0.43	24.48±1.33	26.81±0.45	20.43±0.24
Terbinafine	100	100	100	100

Taraxacum officinale> *Viburnum cotinifolium*> *Chenopodium ambrosoides*> *Vitex negundo*> *Euphorbia hirta*> *Mimosa rubicaulis*> *Verbascum thapsus*> *P. harmala*> *Caryopteris grata*> *Urtica dioica*> *Broussonetia papyrifera*. The results further revealed that methanolic extract of *C. grata* was highly effective against *Aspergilus niger* but exhibited non-significant effects against *Aspergilus fumigatus* and *Rhyzoctonia solani*. Maximum inhibitory activity against *Rhyzoctonia solani* was exhibited by methanolic extracts of *Vitex negundo*, *Woodfordia fruticosa, Adhatoda vasica, T. officinale* and *V. thapsus* respectively (Table 3). The results further revealed that methanolic extract of *V. cotinifolium* was highly effective against *Aspergilus niger*, *Aspergilus flavus* and *Aspergilus fumigatus* but ineffective against *Rhyzoctonia solani*. The *Chenopodium ambrosoides* exhibited moderate antifungal activity against selected fungal strains. Sathiamoorthy et al. (2006) isolated a new flavone glycoside along with five known compound from the ethanolic extract of *V. negundo* the new flavones glycoside that is vitegnoside and negundoside showed significant antifungal activity against *Trichophyton mentagrophytes* and *Cryptococcus neoformans.* Son et al. (2001) isolated Papyriflavonol A from *B. papyrifera* root bark showed strong antifungal activity. Plant metabolites and pesticides derived from plants are better alternatives as they are environmental friendly and biodegradable as compared to synthetic pesticides (Verma and Dubey, 1999).

Conclusion

It is inferred that methanolic extracts of *W. fruticosa*, *C. ambrosoides*, *V. negundo*, *E. hirta*, *V. cotinifolium* and *M. rubicaulis* are highly effective against Gram +ve and –ve bacterial strains as well as against the tested fungal strains and can be utilized as sources of natural antimicrobial agents. However, further researches are needed to explore the bioactive compounds in these selected medicinal plants.

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