International Journal of Phytomedicine 6 (2014) 115-121
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Evaluation of anti-microbial potential of some medicinal plants

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Abstract

The ethanolic extracts of the eight medicinal plants were tested to determine antibacterial activities against fourteen gram positive and twenty two gram negative bacteria. Five out of eight extracts revealed prominent antibacterial activity. Ampicillin was used as a standard for anti-bacterial activity. The significant zone of inhibition was exhibited by Digitalis purpurae (23±2) against Corynebacterium hofmanii. Sambucus nigra and Urtica urens exhibited minimum inhibitory concentration (12 mg/ml) against Staphylococcus epidermidis and Streptococcus fecalis. Saprophytes, dermatophytes and yeasts were used to screen antifungal activities of these selected medicinal plants. Griseofulvin was used as a standard anti-fungal drug. Four out of eight of the tested plant extracts had significant antifungal activity. Urtica uren produced the most significant zone of inhibition (32±1) against Rhizopus specie. Whereas the lowest minimum inhibitory concentration was exhibited by Urtica urens (20mg/ml) against Aspergillus flavus. The above results justify the use of medicinal plants and its extracts in the formulation of anti-microbial medicaments.

Keywords: Medicinal plants, zone of inhibition, minimum inhibitory concentration, Ampicillin, Griseofulvin.

Introduction

Infectious diseases are caused by micro-organisms like bacteria, fungi, viruses and parasites. These micro-organisms are normally present in and on our bodies and are harmless but they may cause disease under certain conditions (microbes that cause illness are known as pathogens) by either disrupting normal body processes or by stimulating the immune system to mount a defensive mechanism[1]. Any immune response against a pathogen may include high fever, inflammation etc. Antibiotics have been used widely for the treatment of these infectious conditions but unfortunately, the development of resistance against the indiscriminate use of antibiotics have made it necessary to explore other ways of curing these infections [2]. Plants have been used to treat various pathologies and alleviate symptoms associated with the chronic inflammatory diseases, since the beginning of mankind. Plants have been known to possess anti-microbial and immunity enhancing constituents, such as, tannins, terpenoids, essential oils, alkaloids and flavonoids[3-4].The plant and their extracts that can kill or inhibit pathogens; as well as, have minimum or not any toxic effect to host are considered appropriate for developing new antimicrobial drugs. Researches are being carried out to explore the plants and their extract which have target sites other than that of conventional antibiotics, in order to ensure their effectiveness against antibiotic resistant pathogens [5-6]. In the present study different medicinal plant extracts were selected on the basis of their active constituents and evaluated for antimicrobial activity.

Materials and Methods

The medicinal plants; *Uva ursi*, *Urtica urens*, *Arnica montana*, *Cicuta virosa*, *Digitalis purpurae* and *Sambucus nigra, Thuja occidentalis* and *Apis mellifica*were were collected from different places. After identifion voucher specimen (FSMP-08-09) was deposited in the herbarium of Research institute of Pharmaceutical Sciences, University of Karachi, Pakistan. These plants were washed, shade dried and pulverized. All the drugs were extracted with ethanol at room temperature, filtered and evaporated under vacuum to obtain thick mass. These extract were further use for antimicrobial evaluation.

Chemicals and test organisms

All the chemicals and reagents were procured from the authorized dealers. The pathogenic bacteria (14 gram-positive and 22 gram-negative bacteria) and fungal isolates (6 saprophytic, 5 dermatophytic and 6 yeasts) were obtained from the Department of

Microbiology, Federal Urdu University of Arts, Science and Technology, Karachi-Pakistan.

Screening of anti-bacterial activity

The anti-bacterial activity of different medicinal plants against fourteen gram positive and twenty two gram negative bacteria were explored in this study. All the bacterial isolates were checked and identified on the basis of conventional methods for purity and maintained on nutrient agar at 4°C in the refrigerator for further work. Antibacterial activity of crude extract against the test organisms were determined by using agar-well method. Autoclaved Muller Hinton broth was used to keep the bacterial culture in log phase for 2 hours with constant agitation and subsequently wells were dug onto Muller Hinton Agar. Later, 10 microliters of culture were poured into the wells [7]. All plates were incubated at 28 ± 2 C for 24-48 hours and after incubation diameter of zone of inhibition was measured [8].

Determination of Minimum inhibitory concentration (MIC)

Minimum inhibitory Concentration (MIC) of was found out by Micro broth dilution method using 96-well micro-titre plate [9]. Stock solution of 100 mg/ml of crude extract was prepared in distilled water. Two fold serial dilutions of extracts was made in 100 µl broth and subsequently 10 µl of two hours old culture perfectly matched the innocula of each with 0.5 Mac Farland index later was added in all wells. One well served as antibiotic control while other served as culture control. Micro-titre plate was incubated for 24 hours at 37 ºC. The MIC was read as the well showing no visible growth. Ampicillin was used as a standard drug.

Screening of antifungal activity

The test organisms for this study were members of the 6 saprophytic fungi Penicillium sp, Aspergilus flavus, Aspergillus niger, Fusarium sp, Rhizopus and Helminthosporum, 5 dermatophytic Microsporum canis, Microsporum gypseum, Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton tonsurans and 6 yeast including Candida albicans, Candida albicans ATCC 0383,Saccharomyces cerevisiae, Candida galbrata, Candida tropicalis, Candida kruzei.All the fungal isolates were checked for purity and maintained on Sabourd Dextrose agar (SDA) at 4°C in the refrigerator until required for use. Anti-fungal activities of medicinal plants were tested using agar-well method. Autoclaved distilled water was used for the preparation of fungal spore suspension and transferred aseptically into each SDA plates. All plates were incubated at 28±2 C for 24-48 hours and after incubation diameter of zone of inhibition was measured [10].

Determination of Minimum inhibitory concentration (MIC)

Minimum inhibitory Concentration (MIC) of medicinal plants were determined by Micro broth dilution method using 96-well micro-titre plate .Stock solution of 100 mg/ml of medicinal plants were prepared in distilled water. Two fold serial dilutions of extracts was made in 100 µL broth and subsequently 10 µL of two hour refreshed culture matched with 0.5 Mac Farland index was added to each well. One well served as anti-fungal agent control while other served as culture control. Micro-titre plate was incubated for 24 hours at 37 ºC. The MIC was read as the well showing no visible growth. Griseofulvin was used as a standard drug.

Results

Anti-bacterial activity

Zone of inhibition of *A. montana* was found to be (20±2 mm) against *S. pyogenes* (gram-positive bacteria). Zone of inhibition by *D. purpurae* was (23±2 mm) against *C. hofmanii* (gram-positive bacteria). *S. nigra* had (20±2 mm) zone of inhibition against *A. hydrophila* (gram-negative bacteria). *U.urens* revealed (18±2 mm) zone of inhibition against gram-negative bacteria, *K. pneumonia*. Zone of inhibition (20±2 mm) was found in case of *U. ursi* against *C. diptheriae* (gram-positive bacteria).*A. mellifica*, *T. occidentalis* and *C. virosa* did not exhibited zone of inhibition against bacterial pathogens (table 1).

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration of *A. montana* was 22 mg/ml against the *S. pyogenes* gram-positive bacteria. Gram negative bacteria, *E. coli* were inhibited at the MIC of 24 mg/ml of *A.montana*. MIC against *D. purpurae* had found to be 32 mg/ml against the *S. fecalis* (gram-positive bacteria), while the gram-negative bacteria, *S. typhi* were inhibited at 20 mg/ml. MIC of *S.nigra* against *S. epidermidis* and *S. pyogenes* (gram-positive bacteria) were observed at 12 mg/ml and 22mg/ml, respectively. *U. urens* exhibited minimum inhibitory concentration at 12 mg/ml and 22 mg/ml against *S. fecalis* and *S. epidermidis* (gram-positive bacteria) respectively. Whereas, *U. ursi* demonstrated minimum inhibitory concentration (34 mg/ml) against *K. pneumonia* (gram-negative bacteria). From these results it was observed that *A. montana* inhibited *E.coli* at a lower dose than then standard ampicillin. No minimum inhibitory concentrations against bacterial pathogen were observed in case of *A. mellifica*, *T. occidentalis* and *C. virosa* (table 2).

Table 1: Zone of inhibition of some medicinal plants against different bacteria

Test Organisms	Zone of inhibition in mm (mean±S.D)							
	A.montana	*D.purpurae*	*S.nigra*	*U.urens*	*U.ursi*	*A.mellifica*	*T.occidentalis*	*C.virosa*
Gram positive bacteria								
Bacillus cereus	17±1	-	10±0	10±0	-	-	-	-
Bacillus subtilis	18±3	-	15±0	15±0	-	-	-	-
Bacillus thruingiensis	16±2	-	14±2	14±2	-	-	-	-
Corynebacterium diptheriae	-	21±1	-	-	20±0	-	-	-
Corynebacterium hofmanii	-	23±2	-	-	18±1	-	-	-
Corynebacterium xerosis	-	18±0	-	-	14±3	-	-	-
Staphylococcus epidermidis	-	-	13±2	13±2	-	-	-	-
Streptococcus saprophyticus	-	-	-	12±1	-	-	-	-
M. smegmatis	-	-	-	-	-	-	-	-
Streptococcus fecalis	-	22±1	-	16±2	-	-	-	-
Streptococcus pyogenes	20±2	-	13±1	13±1	-	-	-	-
Gram negative bacteria								
Enterobacter aerogenes	12±2	-	-	-	-	-	-	-
Escherichia coli ATCC 8739	12±1	-	07±1	-	-	-	-	-
Escherichia coli	10±2	-	-	-	-	-	-	-
E. coli multi drug resistance	14±2	-	-	-	-	-	-	-
Klebsiella pneumoniae	-	15±2	-	18±2	19±2	-	-	-
Salmonella typhi	-	10±2	-	-	-	-	-	-
Salmonella paratyphi A	-	-	-	-	-	-	-	-
Salmonella paratyphi B-	-	-	-	-	-	-	-	-
Shigella dysenteriae	-	-	-	-	-	-	-	
Serratia marcesens	-	13±1	-	-	12±1	-	-	
Acinetobacter baumanii	10±1	16±0	10±1	13±0	15±2	-	-	
Campylobacter jejuni	-	-	-	-	-	-	-	
Campylobacter coli	-	-	-	-	-	-	-	
Helicobacter pylori	-	-	-	-	-	-	-	
Hemophilus influenzae	-	-	-	-	-	-	-	
Vibrio cholerae	-	-	-	-	-	-	-	
Aeromonas hydrophila	-	-	20±2	-	-	-	-	

Table 2: Minimum Inhibitory Concentration (MIC) of some medicinal plants against different micro-organism

Test Organisms	MIC mg/ml								
	Ampicillin	*A.montana*	*D.purpurae*	*S.nigra*	*U.urens*	*U.ursi*	*A.mellifica*	*T.occidentalis*	*C.virosa*
Gram positive bacteria									
Bacillus cereus	-	80	-	88	80	-	-	-	-
Bacillus subtilis	0.39	80	-	74	44	-	-	-	-
Bacillus thruingiensis	0.048	74	-	80	60	-	-	-	-
Streptococcus pyogenes	-	22	-	22	34	-	-	-	-
Corynebacterium diptheriae	0.97	-	70	-	-	70	-	-	-
Corynebacterium hofmanii	0.024	-	40	-	-	40	-	-	-
Corynebacterium xerosis	0.097	-	54	-	-	54	-	-	-
Streptococcus fecalis	-	-	32	-	12	-	-	-	-
Staphylococcus epidermidis	1.56	-	-	12	22	-	-	-	-
Staphylococcus aureus ATCC 6538	0.781	-	-	-	-	-	-	-	-
Streptococcus saprophyticus	0.39	-	-	-	10	-	-	-	-
Gram negative bacteria									
Enterobacter aerogenes	12.5	38	-	-	-	-	-	-	-
Escherichia coli ATCC 8739	-	44	-	80	-	-	-	-	-
Escherichia coli	>100	24	-	-	-	-	-	-	-
Escherichia coli(MDR)	>100	-	-	-	-	-	-	-	-
Acinetobacter baumanii	-	84	34	94	74	74	-	-	-
E. coli multi drug resistance	-	92	-	-	-	-	-	-	-
Klebsiella pneumoniae	-	-	40	-	34	34	-	-	-
Serratia marcesens	-	-	78	-	-	98	-	-	-
Salmonella typhi	0.048	-	20	-	-	-	-	-	-
Salmonella typhi ATCC-14028	0.097	-	-	-	-	-	-	-	-
Salmonella paratyphi A	>100	-	-	-	-	-	-	-	-
Salmonella paratyphi B	0.097	-	-	-	-	-	-	-	-
Aeromonas hydrophila	-	-	-	82	-	-	-	-	-
Shigella dysenteriae	6.25	-	-	-	-	-	-	-	-
Shigella flexeneri	>100	-	-	-	-	-	-	-	-
S. boydii	6.25	-	-	-	-	-	-	-	-
Proteus mirabilis	0.19	-	-	-	-	-	-	-	-

Table 3: Zone of inhibition of some medicinal plants against different fungus

Test Organisms	Zone of inhibition (mm)							
	C.virosa	*D.purpurae*	*U.urens*	*U.ursi*	*T.occidentalis*	*S.nigra*	*A.montana*	*A.mellifica*
Yeasts								
Candida albicans	16±3	16±3	10±2	19±3	-	-	-	-
Candida albicans ATCC 0383	18±1	18±1	12±1	19±1	-	-	-	-
Saccharomyces cerevisiae	-	-	15±2	25±2	-	-	-	-
Candida galbrata	20±1	20±1	17±1	12±1	-	-	-	-
Candida tropicalis	-	19±1	13±0	12±2	-	-	-	-
Candida kruzei	-	20±1	11±2	21±1	-	-	-	-
Dermatophytes								
Microsporum canis	12±2	12±2	-	-	-	-	-	-
Microsporum gypseum	-	-	-	-	-	-	-	-
Trichophyton rubrum	-	-	-	-	-	-	-	-
Trichophyton mentagrophytes	-	-	-	-	-	-	-	-
Trichophyton tonsurans	16±2	-	-	-	-	-	-	-
Saprophytes								
Aspergillus flavus	22±2	22±2	10±2	20±0	-	-	-	-
Aspergillus niger	16±0	-	-	-	-	-	-	-
Fusarium specie	-	-	12±2	-	-	-	-	-
Penicillium sp	15±2	-	15±2	21±2	-	-	-	-
Rhizopus	-	-	32±1	-	-	-	-	-
Helminthosporum	-	-	-	-	-	-	-	-

Table 4: Minimum inhibitory concentration of some medicinal plants against different fungus

Test Organisms	MIC mg/ml								
	Griseofulvin	*C.virosa*	*D.purpurae*	*U.urens*	*U.ursi*	*T.occidentalis*	*S.nigra*	*A.montana*	*A.mellifica*
Yeasts									
Candida albicans	250	80	40	82	42	-	-	-	-
Candida albicans ATCC 0383	-	94	44	94	44	-	-	-	-
Saccharomyces cerevisiae	>2000	-	-	22	32	-	-	-	-
Candida galbrata	-	88	80	70	30	-	-	-	-
Candida tropicalis	-	-	76	76	36	-	-	-	-
Candida kruzei	-	-	72	82	42	-	-	-	-
Dermatophytes									
Microsporum canis	1.563	32	98	-	-	-	-	-	-
Microsporum gypseum	0.39	-	-	-	-	-	-	-	-
Trichophyton rubrum	0.39	-	-	-	-	-	-	-	-
Trichophyton mentagrophytes	1.563	-	-	-	-	-	-	-	-
Trichophyton tonsurans	1.563	98	-	-	-	-	-	-	-

.....Continue Table 4

Saprophytes									
	Griseofulvin	*C.virosa*	*D.purpurae*	*U.urens*	*U.ursi*	*T.occidentalis*	*S.nigra*	*A.montana*	*A.mellifica*
Aspergillus flavus	-	60	62	20	22	-	-	-	-
Aspergillus niger	-	64	-	-	-	-	-	-	-
Fusarium specie	-	-	-	78	-	-	-	-	-
Penicillium sp	-	76	-	82	-	-	-	-	-
Rhizopus	-	-	-	66	-	-	-	-	-
Helminthosporum	-	-	-	-	-	-	-	-	-
Neurospora	-	-	-		-	-	-	-	-

Anti-fungal activity

C. virosa and *D. purpurae* showed significant results against *A. flavus* (Saprophytes) (Zone of inhibition 22±2 mm). *U. urens* had 32±1 mm zone of inhibition against *Rhizopus* (Saprophytes). *U.ursi* exhibited 25±2 mm zone of inhibition against *S. cerevisiae* (yeasts).Whereas, *T. occidentalis*, *S. nigra*, *A. montana* and *A. mellifca* did not exhibited any zone of inhibition against fungus pathogens (table 3).

Minimum Inhibitory Concentration (MIC)

C. virosa significantly inhibited *M. canis* (Dermatophytes) at the dose of 32 mg/ml. MIC of *D. purpurae* was 40 mg/ml against *C. albicans* (yeasts). MIC of *U. urens* is 20 mg/ml against *A. flavus* (Saprophytes). Whereas, the minimum inhibitory concentration of *U. ursi* is 22mg/ml against *A. flavus* (Saprophytes).*D. purpurae* and *U. urens* exhibited more pronounced inhibition of *C. albicans* and *S. cerevisiae* respectively in comparison to the standard, griseofulvin. On the other hand no inhibitory concentration was observed against fungus pathogens in case of *T. occidentalis*, *S. nigra*, *A. montana* and *A. mellifica* (table 4).

Discussion

Infectious diseases are the major cause of death in developing countries. In Pakistan the major infectious diseases are bacterial diarrhea, hepatitis A and B, typhoid and respiratory tract infections. The other contributing factor is an increase in antibiotic resistance to the community acquired infectious diseases [11]. Antimicrobial drugs derived from plant source has vast therapeutic potential. They are valuable in the treatment of infectious diseases and also concurrently alleviating many of the adverse effects commonly accompanied with synthetic antimicrobials [12].

The results of our studies revealed the strong potential of the medicinal plants to be used in the formulation of anti-microbial and anti-infective drugs. The rich chemical constitution of the plants forms the basis of biological action including anti-bacterial and anti-fungal activities [13,14]. In our present study, the tested medicinal plants revealed antimicrobial activity in terms of zone of inhibition in following sequence *D. purpurae* > *A.montana* > *U.ursi* > *U.urens* > *S.nigra*. While in case of minimum inhibitory concentration against various bacteria pathogens; the medicinal plants exhibited minimum inhibitory concentration in the following series *U. ursi* > *A. montana* > *D.purpurae* > *S.nigra* > *U.urens*. Ampicillin was used as a standard anti-bacterial drug.

Anti-fungal activity was carried out on different types of yeasts, dermatophytes and saprophytes. Anti-fungal activity in terms of zone of inhibition was observed in following order *U. urens* > *U. ursi* > *D.purpurae* > *C.virosa*. Whereas, the minimum inhibitory concentration of the tested medical plants was perceived as follows: *D.purpurae* > *C.virosa* > *U. ursi* > *U.urens*. Griseofulvin was used as a standard anti-fungal drug.

D.purpurae showed anti-microbial activity might be due to the presence of volatile oils in it [15]. Anti-microbial action of *U. ursi* is owing to the flavonoids, triterpenes and volatile oils in it [16]. *U. urens* possesses the very potent anti-microbial effects due to the presence of flavonoids, acetophenone, acetylcholine, amines, agglutinins, alkaloids, astragalin, butyric acid, caffeic acids, carbonic acid and chlorogenic acid [17]. *C. virosa* presented anti-fungal activity due to presence of cicutoxin, sesquiterpene and monoterpene compounds in it [18]. No anti-fungal activity was observed in *T. occidentalis*, *S. nigra*, *A. montana* and *A. mellifica*.

Conclusion

Plants in past, present and future are a great source in the derivation and formulation of medicaments of great therapeutic efficacy and yet being mild. Many anti-microbial constituents have been extracted; isolated and preliminary studies have been carried on them. Further researches are required for authentication of the structures of those isolated anti-microbial constituents and to carry out clinical studies for the righteous marketing of the plant-based anti-microbial drugs by the population to protect them against the lethal side-effects associated with the use of antibiotics or to give better choice to the patients who have already developed resistance against antibiotics.

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