International Journal of Phytomedicine 5 (2013) 154-158
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Antimicrobial activity of medicinal plants against some pathogenic microbial strains

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Abstract

Methanol extracts of ten plant species have been screened for their antimicrobial potential against fungal pathogens namely *Aspergillus niger* and *Candida albicans* and bacterial strains *Escherichia coli* and *Bacillus subtilis*. Extracts of all the plant species taken for the present study except *Cucumis anguri* showed fungicidal activity against both the fungal pathogens used. Leaves extract of *Withania somnifera* exhibited maximum 80-95% inhibition against both of the fungal pathogens, while extract of *Azadirachta indica* showed 88% growth inhibition against *C. albicans*. *Acacia nilotica* and *Withania somnifera* have shown inhibition zones of 17 and 19mm respectively against *E. coli* and *B. subtilis*. All the plant species have shown significant antibacterial potential in the range of 1-18mm against *B. subtilis*. *Azadirachta indica, Cucumis anguri, Emblica officinalis* and *Solanum nigrum* did not exhibit any antibacterial activity against *E. coli*. The identification of these potential herbs as antimicrobial agents will be helpful in replacing some commercially synthesized antimicrobial drugs.

Keywords:*Aspergillus niger, Candida albicans, Escherichia coli, Bacillus subtilis* and medicinal plants.

Introduction

The medicinal plants are the plants whose parts (leaves, seeds, stem, roots, fruits, foliage etc.), extracts, infusions, decoctions, powders are used in the treatment of different diseases of humans, plants and animals [1]. The medicinal plants occupy a significant place in modern medicine as a raw material for some important drugs, although synthetic drugs and antibiotics brought about a revolution in controlling different diseases.

Extracts of many plants are highly efficient against parasitic as well as microbial infections. The Ayurvedic approach to the prevention and treatment of microbial infection recognizes the emergency use of modern drugs, but recommends traditional herbal combinations and extracts known to balance the individual and improve health, as well as herbs that help to combat or prevent microbial infections. The most important of bioactive constituents of plants are alkaloid, tannin, flavonoids, and phenolic compounds [2]. It is estimated that around 70,000 plant species, from lichens to tall trees, have been used at one time to other for medicinal purposes. The use of different parts of several medicinal plants to cure specific ailments has been in vogue from ancient times. The indigenous system of medicine namely Ayurvedic, Siddha and Unani have been in existence for several centuries [3].

In the present scenario, an emergence of multiple drug resistance in human pathogenic microbes and the small number of antimicrobial classes available stimulated research directed towards the discovery of novel antifungal and antibacterial agents from other sources, such as medicinal plants [4]. The plant botanicals are known to possess medicinal properties and biocidal activity against microbial and other pests and pathogens [5,6].

Keeping in view the importance of the subject some local potential medicinal plants were examined for their antimicrobial activity.

Materials and Methods

Collection of plants

The leaf sample of ten plant species were collected from Medicinal Garden, Banasthali University, Rajasthan, India and Botanical Garden, Patanjalai Yogpeeth, Haridwar, Uttrakhand, India (Table-1). Plant material was dried in shade at 35ºC for 15-20 days. The shade dried leaves of each plant spp. were grinded in mixer and stored in airtight containers after grinding. Dry powder of leaves was extracted four times with 5 ml methanol /g of plant material for 48 hrs at 40°C. All these extracts were combined and concentrated by flash evaporation at 40°C to a volume that would make 20 µl of extract equivalent to 100 mg of plant material.

Test organisms

Fungal strains

Two test organisms, *Aspergillus niger* (ATCC 9763), and *Candida albicans* (ATCC 7596) were collected from Plant Pathology Laboratory, University of Rajasthan, Jaipur, Rajasthan. The fungal cultures of *A. niger* and *C. albicans* were maintained on Saboraud dextrose agar (SDA), incubated at 25ºC. The inoculated medium was incubated at 25ºC for two days for the *C. albicans* and three days for *A. niger*.

Bacterial strains

Bacterial culture of *Escherichia coli* (MTCC 119) *Bacillus subtilis* (MTCC 619), were collected from Department of Biotechnology and Bioscience, Banasthali University, Rajasthan. They were cultured in Petri plates containing Nutrient Agar media and incubated at 28˚C for two days for both of the pathogens taken in present study.

Screening of plant extracts for fungicidal activity

Paper disc method was used for initial screening of anti-fungal potential of plant spp. chosen for present investigations. This method was based on diffusion capacity of test chemical(s) through agar medium [25]. Fungal plug were placed at the center of assay plate containing sterilized SDA (Hi-Media, Mumbai, India) and allowed to grow. After 2-3 cm growth of fungus, two sterilized paper discs of 0.5 cm diameter, one loaded with 20µl plant extract for test and one with same amount of pure solvent for control, were placed at equal distance from center. The plates were left for 30 min. at room temperature to allow the diffusion of the extract, and then they were incubated at 25˚C for two to three days. Inhibition zones were measured after one to three days of incubation depending upon the growth of pathogen. The zone of inhibition was measured with a ruler.

Food poisoning technique was used to find percent inhibition of methanolic plant extracts showing potential activity against certain test organisms. For this purpose 50µl of alcoholic extract equivalent to 0.25 gm initial plant material was spread to each petri-dish after pouring the sterilized medium, while in control treatment equal amount of pure solvent was added. The fungal plug were placed at the centre of petridish. Growth of fungus was recorded after one to three days depending upon the growth of pathogen. The percent inhibition was calculated using the formula of Vincent[26].

$$\text{Inhibition (\%)} = (C\text{-}T)/C \times 100$$

Where C is the growth in control in mm and T is growth in treatment in mm.

All the experiments were carried out in triplicate in randomized block design and average value was used for interpretation of results.

Screening of plant extracts for antibacterial evaluation

Agar Disc Diffusion

The antibacterial activity was assessed using the simple disc diffusion method 25 where plant extract impregnated filter paper disc are placed on nutrient agar (Hi-Media, Mumbai, India) media incubated with the test bacteria so as to get a lawn culture on incubation. The drug diffuses in to the medium and inhibits bacterial growth around the disc, if it is effective this indicates the antibacterial activity of the drug tested. Larger the inhibition zone higher will be the antibacterial activity. If the plant extract is not effective or is not having antibacterial activity then the zone of inhibition will not be observed.

It was performed using a 48 hrs culture at 28˚C in 20ml of nutrient broth (Hi-Media, Mumbai, India). 50µl of the test bacterial suspensions were spread over the plates containing Nutrient Agar using a sterile cotton swab in order to get a uniform bacterial growth on test plates two sterile whatman filter paper discs of 0.5 cm diameter were loaded in condensed (one loaded with 20µl plant extract for test and one with same amount of pure solvent for control) placed at equal distance from center. The plates were left for 30min. at room temperature to allow the diffusion of the extract, and then they were incubated at 28˚C for 48 hrs. After the incubation period, the zone of inhibition was measured with a ruler.

Results and Discussion

Antifungal and bacterial potential of plant extracts taken for present study has been reported in (Table 2& 3)

It is clear from the data reported in (Table-2) that Cassia alata, Acacia nilotica, Solanum nigrum, Tinospora cordifolia, Commiphora wightii, Emblica officinalis, Carica papaya and Withania somnifera possess 20-80% inhibitory effect against A. niger. Azadirachta indica has shown significant inhibition of 88% against C. albicans but it was not found effective against A. niger.

Tinospora cordifolia has shown 35% inhibition against C. albicans while Commiphora wightii, Cassia alata, Acacia nilotica and Carica papaya caused 40-65% inhibition in the growth of C. albicans. Solanum nigrum was found to be significant growth inhibiter against A. niger but it was not found to be ineffective against C. albicans. Extract of Cucumis anguri did not show activity against any of the pathogens taken in the present investigation.

Plant extracts of *Withania somnifera*, *Carica papaya*, *Emblica officinalis* and *Azadirachta indica* exhibited significant fungicidal potential against *A. niger* and *C. albicans* respectively.

From the results obtained in (Table-3) it seems that the antibacterial action of the extracts of *Commiphora wightii, Carica papaya, Tinospora cordifolia, Acacia nilotica* and *Azadirachta indica* were (10-18mm) effective against *B. subtilis*. *Withania somnifera* has shown maximum inhibition zone of 19 mm against *B. subtililis*. *Emblica officinalis, Cucumis anguri, Cassia alata* and *Solanum nigrum* have shown 1-9 mm inhibition zones against *B. subtilis*. *Acacia nilotica* exhibited maximum inhibition of 17mm against *E.coli*. Plant extract of *Withania somnifera, Tinospora cordifolia* have exhibited inhibition zones in the range of 14-16mm against in *E. coli*. *Commiphora wighti, Cassia alata* and *Carica papaya* were found to possess minimum activity (2-7mm) against *E. coli*. *Solanum nigrum, Emblica officinalis, Cucumis anguri* and

Azadirachta indica did not exhibit antibacterial potential against *E. coli.*

Therefore extracts of Azadirachta indica, Acacia nilotica, Tinospora cordifolia and Withania somnifera could be subjected to further isolation and purification of active compound to discover novel lead antibacterial agents.

The result lends credence to the folkloric use of these medicinal plants in treating microbial infection and shows that extract of *Azadirachta indica*, *Acacia nilotica, Cassia papaya*, *Emblica officinalis* and *Withania somnifera*, could be exploited for new potent antimicrobial agents. The effective plant extracts are expected to contain wide spectrum antimicrobial constituents.

These plant species may contain chemical compounds having selective/ specific antimicrobial properties. Therefore, the results described above may be helpful in developing/ synthesizing the plant based natural fungicides and antibacterial agents that may be used for preventing the incidence of many diseases caused by the fungi and bacteria involved in the present investigations.

Table-1 Plant species collected for screening of antimicrobial potential.

Name of plant species	Family	Plant part used	Location	Altitude (ft)
Acacia nilotica [7,8]	Mimosaceae	Leaves	Botanical Garden, Patanjali Yogpeeth, Haridwar, Uttrakhand, India	865
Azadirachta indica [9]	Meliaceae	Leaves	Medicinal Garden, Banasthali University, Rajasthan, India	1297
Cassia alata [10,11]	Caesalpiniaceae	Leaves	Botanical Garden, Patanjali Yogpeeth, Haridwar, Uttrakhand, India	864
Carica papaya [12,13]	Caricaceae	Leaves	Medicinal Garden, Banasthali University, Rajasthan, India	1297
Commiphora wightii [14,15]	Burseraceae	Leaves	Botanical Garden, Patanjali Yogpeeth, Haridwar, Uttrakhand, India	864
Cucumis anguri [16]	Cucurbitaceae	Leaves	Botanical Garden, Patanjali Yogpeeth, Haridwar, Uttrakhand, India	864
Emblica officinalis [17,18]	Euphorbiaceae	Leaves	Medicinal Garden, Banasthali University, Rajasthan, India	1297
Solanum nigrum [19,20]	Solanaceae	Leaves	Medicinal Garden, Banasthali University, Rajasthan, India	1297
Tinospora cordifolia [21,22]	Menispermaceae	Leaves	Botanical Garden, Patanjalai Yogpeeth, Haridwar, Uttrakhand, India	864
Withania somnifera [23,24]	Solanaceae	Leaves	Botanical Garden, Patanjalai Yogpeeth, Haridwar, Uttrakhand, India	864

Latitude range = 27˚06'17.65" N to 29˚54'11.26" N
Longitude range = 75˚32'23.01" E to 77˚59'45.86"

Table-2 Antifungal activity of methanol extract of medicinal plant species

Name of plant species	Name of fungal pathogens			
	A. niger		C. albicans	
	Inhibition zone (mm)	% inhibition	Inhibition zone (mm)	% inhibition
Acacia nilotica	9	49	10	45
Azadirachta indica	0	0	17	88
Cassia alata	5	22	8	42
Carica papaya	14	72	12	65
Commiphora wightii	11	55	8	40
Cucumis anguri	0	0	0	0
Emblica officinalis	11	52	17	85
Solanum nigrum	10	49	0	0
Tinospora cordifolia	9	50	8	35
Withania somnifera	15	80	17	95

Table-3 Antibacterial activity of methanol extract of medicinal plant species

Name of plant species	Antibacterial activity[(inhibition zone size(mm)]	
	E. coli	B. subtilis
Acacia nilotica	17	15
Azadirachta indica	-	18
Cassia alata	5	7
Carica papaya	2	11
Commiphora wightii	7	10
Cucumis anguri	-	8
Emblica officinalis	-	9
Solanum nigrum	-	1
Tinospora cordifolia	14	15
Withania somnifera	16	19

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