

Studies on Antimicrobial Activity of Plant Extracts on Phytopathogenic Fungi and Bacteria

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Many angiosperm plants have been reported to have an antimicrobial compounds which capable of inhibiting the growth of many pathogenic microorganisms cause diseases on plant as well as animals including human. The effect of various plant parts like root, stem, leaves etc. have been reported earlier for their effect on inhibition of growth of various plant pathogens including bacteria and fungi. Hence, the present study is undertaken to screen few plant extracts from leaves against the phytopathogenic bacteria and fungi. Therefore the Plant extracts from *Abutilon indicum*, *Aristolochia bracteata*, *Cleome viscosa*, *Morinda tinctoria*, *Pongamia glabra*, *Solanum trilobatum* and *Thespesia populanea* were obtained from leaves by simple solvent extraction method at ambient conditions. The crude extracts were screened for antifungal and antibacterial activity by bioassay method against *Alternaria solani*, *Fusarium graminearum*, *Helminthosporium solani*, *Pseudomonas fluorescens*, *Xanthomonas campestris* and *Erwinia caratovora*. Subsequently the inhibitory effect of plant extracts at various concentration was determined by micro-dilution method. The solvents used in the present study were water, ethanol, Petroleum ether and Butanol individually and in combination with water. Of the six plant species screened, 3 showed antimicrobial activity against one or more species of bacteria and fungi extracted from ethanol and butanol solvents. The most effective plants were *Cleome viscosa* and *Pongamia glabra*. The effect of biocompounds extracted from various solvent mixtures on different pathogenic microorganisms was discussed in the present study. The aim of this article is to explore the possibility of using antimicrobial compounds extracted from plants in control of plant diseases.

Key words: Plants extracts, Antimicrobial compounds, Fungi, Bacteria, Minimal inhibitory test, Bioassay.

Antimicrobial properties of many angiosperm plants are being and increasingly reported from various parts of the world (Mahesh and Satish, 2008; Dabur *et al.*, 2007; El Astal *et al.*,

2005; Adiguzel *et al.*, 2005; Martin and Earst, 2003; and Cutler, 2000; Gislene *et al.*, 2000 and Ashebir and Ashenafi, 1999). The various parts of plants as root, stem, bark, leaves, flowers and fruits containing active compounds capable of curing microbial diseases in animals and plants, which are having scope and economically important. Therefore, the plant kingdom is a virtual goldmine of potential bioactive compounds including bactericide, fungicide and virucide which are

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waiting to be discovered completely. However, the use of plant compounds in medicinal, pharmaceutical, agricultural, food and cosmetics is increasing and it requires systematic study.

The use of plant parts and its extracted compounds in various agricultural practices to prevent the pathogens which are responsible to cause losses in standing crops as well as in stored commodities. The effect of plant antimicrobial compounds from herbs, shrubs, trees and climbers on various microorganisms like bacteria, Fungi and viruses which cause various diseases have been reported by various authors in vitro laboratory conditions. (Sathish *et al.*, 1999; Shiraishi *et al.*, 2003; Shitut *et al.*, 1999; Karamanoli *et al.*, 2005; Satya *et al.*, 2005; Saha *et al.*, 2005 and Modak *et al.*, 2004). Based on the above experimental proof, many plant compounds are currently used to control the plant pathogens in the field condition. The effect of plant compounds including essential oils and aromatic products on microorganisms were reported in *neem* and *pongamia* species.

The objective of the present investigation is to make a small survey of preliminary screening of wild plant species available commonly near cultivating agricultural areas around Chennai, Tamil Nadu. Since the fungi and bacteria are commonly occurring causative pathogenic organisms on crop plants, the antimicrobial properties of the above plant species have been focused to isolate those compounds for bio-control method.

MATERIAL AND METHODS

Standard methods were followed for sample collection, processing, extraction and assay of compounds on microorganisms throughout the present study (Sathish *et al.*, 1999; Saha *et al.*, 2005).

Collection of Plant samples and processing

The leaves of different plants given below were collected from cultivating land area located in and around Chennai, Tamil Nadu for the present investigation during January to March 2009. The details of plant species taken for present study are *Abutilon indicum* Linn., *Aristolochia bracteata* Retz., *Cleome viscosa*, Linn, *Morinda tinctoria* Roxb., *Pongamia glabra* Vent., *Solanum trilobatum* Linn. and *Thespeasea populanea* (L.) Sol. ex Correa.

The leaves were freshly collected and washed with tap water and distilled water to remove all the external contaminants. Then the samples were air dried at ambient condition and made into fine powder after sieving through nylon mesh. Finally the powdered leaf samples were filled in sterile glass bottles and labeled for further work.

Preparation of Microbial organisms:

Selective microbial organisms from fungi and bacteria in the form of agar slants were obtained from the culture collection centre of Institute of Microbial Technology (IMTECH), Chandigarh, India. The details of species are the bacteria species *Pseudomonas fluorescens*, *Xanthomonas campestris*, *Erwinia carotovora* and the fungi as *Alternaria solani*, *Fusarium graminearum* and *Helminthosporium solani*. The microbial organisms were sub cultured in the laboratory in Nutrient agar for bacteria and Potato Dextrose agar for fungi and used for further experiment.

Extraction of leaves

For extraction of powdered leaves sample, the solvents as water, ethanol, Butanol, Petroleum ether were taken individually and 50% dilution with distilled water were taken. About 10 g of leaf samples were mixed with 90 ml of solvent and cold extracted with rotary shaker for 12 hours at ambient temperature. The extracts were filtered through Whatman No.1 filter paper and concentrated near dryness and labeled for further assay.

Determination of Antimicrobial properties

Antibacterial activity

Antibacterial activity of crude extracts of all the samples were individually tested by an agar plate method by using Mueller Hinton agar at various concentrations like 250 µl, 500 µl and 5000 µl. The agar medium was inoculated with different species of bacteria selected in the present study and incubated at 37°C for 24 hours period. After incubation period, observation was made for inhibition zone and compared with control without plant extracts in solvent.

Antifungal activity

The antifungal activity of the extracts was tested with potato dextrose agar by using agar well method. The agar medium inoculated with different species of fungi by spore suspension method. The inoculated agar plates were made with wells and the plant extracts were placed with 250 µl, 500 µl and 1000 µl quantity. Then the agar

were incubated at 30°C for 3 to 5 days and observed for any inhibitory effect and compared with control without plant extracts.

RESULTS

Seven plant species were taken for extraction with 7 solvent systems and the effect of concentrated crude solvent extracts were studied for its antimicrobial and antifungal activity. In the agar plate assay method, the effectiveness of plant extracts on the growth and inhibition on microorganisms for preliminary screening to find out whether the plant compounds active against particular organism. The table 1 shows the effect of plant extracts obtained by using ethanol and butanol on different bacterial organisms used. In the ethanol and butanol extract, few plants exhibited the inhibitory effect on selective bacterial species. Among the plant extracts used, the ethanol extract from *Abutilon indicum* and *Aristolochia bracteata* showed inhibitory effect from the butanol extract as 0.7 cm and 0.5 cm diameter inhibitory zone on the bacterial species *Erwinia caratovora* respectively. The plant species *Pongamia glabra* showed inhibitory effect extracted by using ethanol and butanol on only

Erwinia caratovora as 0.7 and 0.8 cm diameter respectively. The significant observation is the plant species *Cleome viscosa* extracted with both the solvent systems like ethanaol and butanol showed remarkable effect on all the bacteria used as *Psuedomonas fluerescens*, *Xanthomonas campustris* and *Erwinia caratovora*. Overall, the results shows as the extract from *Abutilon indicum*, *Cleome viscosa* and *Pongamia glabra* exhibited inhibitory effect on different bacterial species. However, the other solvent systems prepared from water, petroleum ether and the solvent mixed with water did not show an inhibitory effect on microorganisms.

The table 2 shows the effectiveness of plant extracts from different solvents system on few selective fungal species. Among the plant species used for antifungal activity, *Cleome viscosa* extracted from ethanol and butanol inhibited the growth of *Alternaria solani* and *Fusarium graminearum*. *Pongamia glabra* inhibited the growth of *Fusarium graminearum* and *Thespesia populanea* effected the growth against *Alternaria solani*. However, none of the plant species used in the present study observed to inhibit the growth of *Helminthosporium solani*.

Table 1. Antibacterial activity of different plant species from Ethanol and Butanol extracts

S. No.	Name of the plant	Solvents used	Name of the Bacteria tested		
			<i>P.f</i>	<i>X.c</i>	<i>E.c</i>
01	<i>Abutilon indicum</i>	EE	-	-	+
		BE	-	-	+
02	<i>Aristolochia bracteata</i>	EE	-	-	-
		BE	-	-	-
03	<i>Gynandropsis pentaphylla</i>	EE	+	+	+
		BE	+	+	+
04	<i>Morinda tinctoria</i>	EE	-	-	-
		BE	-	-	-
05	<i>Pongamia glabra</i>	EE	-	+	+
		BE	-	+	+
06	<i>Solanum trilobatum</i>	EE	-	-	-
		BE	-	-	-
07	<i>Thespesia populanea</i>	EE	-	-	-
		BE	-	-	-

EE- Ethanol extract

+ Effective

BE – Butanol extract

- Not effective

P.f - pseudomonas flourescens X.c - Xanthomonas campasteris and

E.c - Erwinia caratovora

Table 2. Antifungal activity of different plant species from Ethanol and Butanol extracts

S. No.	Name of the plant	Solvents used	Name of the Bacteria tested		
			A.s	F.g	H.s
01	<i>Abutilon indicum</i>	EE	-	-	-
		BE	-	-	-
02	<i>Aristalochia bracteata</i>	EE	-	-	-
		BE	-	-	-
03	<i>Cleome viscosa</i>	EE	+	+	+
		BE	+	+	+
04	<i>Morinda tinctoria</i>	EE	-	-	-
		BE	-	-	-
05	<i>Pongamia glabra</i>	EE	-	-	-
		BE	-	-	-
06	<i>Solanum trilobatum</i>	EE	-	-	-
		BE	-	-	-
07	<i>Thespesia populanea</i>	EE	+	+	+
			+	+	+

EE- Ethanol extract

BE – Butanol extract

A.s - *Alternaria solani*F.g - *Fusarium graminearum*H.s - *Helminthosporium solani*

+ Effective

- No effective

DISCUSSION

Among the dilutions of plant extracts, higher concentrations like more than 500 µl of 1:10 extraction were effective for inhibition on the growth of microorganism. Of the solvents used in the study, ethanol and butanol were the suitable solvents recover some active plant compounds which are possessing with antimicrobial properties. The present study is the preliminary screening of few plants which are already known to be as medicinally important for antimicrobial properties and the usage of these plants is studied for control on plant pathogens. Antimicrobial properties of many plants were studied by various authors in *Ocimum basilicum* (Adiguzel *et al.*, 2005), *Heliotropium simtatum* (Modak *et al.*, 2004), *Allium sativum* and *Allium cepa* against *Xanthomonas campestris* (Sathish *et al.*, 1999), *Oxalis* spp. Against *Fusarium* spp. Shiraishi *et al.*, 2003), Piper beetle against phytopathogenic fungi (Shitut *et al.*, 1999); *Abutilon indicum* (Srividya *et al.*, 2009); *Aristalochia bracteata* (Negi *et al.*, 2004) and *Morinda tinctoria*, (Sivaraman and Muralidharan, 2010). The effect of various herbal extracts by using different solvent system

against human pathogens and phytopathogenic microorganisms were well documented (Mahesh and Satish, 2008; El Astal *et al.*, 2005; Martin and Earst, 2003; Culter, 2000; and Ashebir and Ashenafi, 1999). Though much number of reports is available for the antimicrobial effect of various plant species on various types of microorganisms, the application of plant extract to control on disease control on phytopathogenic fungi and bacteria is require more attention.

The present study is limited to concentrate on phyto-pathogenic microorganisms in a preliminary way which may lead to plant disease control after characterizing and standardizing particular effective compounds isolated and purified. It is concluded that from the present study as the plant *Cleome viscosa* might be possess with potential bio-compounds which involve in various antimicrobial properties (Borgio *et al.*, 2008; Dabur *et al.*, 2007).. However, *Pongamia glabra* also found to have antimicrobial effects on few species of Bacteria like *Xanthomonas campestris* and *Ervinia caratovora* at considerable level which is supported by literature studies (Sathish *et al.*, 1999). The present study shows encouraging results on these

selective species of plants in line with earlier studies reported by many scientists (Yasmin *et al.*, 2008; Angalaparameswari *et al.*, 2010). However, it leads the need of further work in this area to make a detailed study. Therefore, further study is planned to carry out on the plant compounds in an elaborate way by various phyto-chemical techniques and its application on crop plants. Overall, the present study concludes the possibility of exploring the role of antimicrobial compounds isolated from different plants in control of various plant diseases which minimize the usage of various agrochemicals which are hazardous and expensive.

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