IN VITRO ANTIMICROBIAL SCREENING OF SELECTED INDIAN MEDICINAL PLANTS

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ABSTRACT : We have examined antimicrobial activities of medicinal plants *Abultilon indicum, Adenocalymma alliaceum, Carica papaya, Crotolaria laburnifilia, Croton bonplandianum, Derris scandens, Eichornia crassipes, Iopomea hispida, Moringa heterohylla, Peltophorum pterocarpum* that have been popularly used as folk medicines. Scientific information on antimicrobial properties of various natural sources is still rather scarce. The antimicrobial activities of the organic solvent extracts on the various test microorganisms, including bacteria and fungi investigated using agar well diffusion technique. The length of inhibition zone was measured in millimeters from the edge of the well to the edge of the inhibition zone. Methanol extracts exhibited promising antimicrobial activity than chloroform and hexane extracts. The extracts from varies parts of plants were assessed in an effort to validate the medicinal potential of the herb. Our results showed plant extracts have significant levels of antimicrobial activity.

Keywords: *Abultilon indicum,* Medicinal plants, Agar well diffusion technique, Inhibition zone.

INTRODUCTION

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient. Medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth (antimicrobial activity) (Chopra et. al., 1992, Bruneton,1995)

Higher plants have been shown to be a potential source for new anti-microbial agents (Mitscher et. al., 1987). The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. The plants used, as drugs are fairly innocuous and relatively free from toxic effects or were so toxic that lethal effects were well known. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Saxena 1997, Nimri et. al., 1999, Saxena et. al., 1999).
Numerous studies have been carried out on various natural products screening their antimicrobial activity (Nita et. al., 2002, Ates et. al., 2003, Bhattacharjee et. al., 2006, Parekh and Chanda 2006).which can protect the human body against pathogens. Besides small molecules from medicinal chemistry, natural products are still major sources.

In this paper we report the results of such studies in order to orient future investigations towards the finding of potent and safe antimicrobial compounds.

MATERIALS AND METHODS

Solvents and chemicals used:

All chemicals were purchased from Merck, Qualigens fine Chemicals and SD fine chemicals, Mumbai.

Extraction procedure for antimicrobial:

Abultilon indicum, Adenocalymma alliaceum, Carica papaya, Crotolaria laburnifilia, Croton bonplandianum, Derris scandens, Eichornia crassipes, Iopomea hispid, Moringa heterohylla, Peltophorum pterocarpum medicinal plants material collected from various places of Andhra Pradesh they were taxonomically identified and the Voucher specimen is stored. The plant material were dried under shade with occasional shifting and then powdered with a mechanical grinder and stored in an airtight container. The powder obtained was subjected to successive soxhlet extraction with organic solvents with increasing order of polarity i.e. Hexane, Chloroform and Methanol respectively.

Test microorganisms: *Alternaria alternate* (MTCC 1362), *Aspergillus flavus* (MTCC 4633), *Fusarium oxysporum* (MTCC 1755), *Rhizoctonia solani* (MTCC 4633), *Xanthomonas compestries* (MTCC 2286), including both fungi and bacteria were procured from Microbial Type Culture Collection (MTCC), Chandigarh. Active cultures were generated by inoculating a loopful of culture in separate 100mL nutrient/potato dextrose broths and incubating on a shaker at 37°C overnight. The cells were harvested by centrifuging at 4000 rpm for 5 min, washed with normal saline, spun at 4000 rpm for 5 min again and diluted in normal saline to obtain 5 x 10^5cfu/mL.

Determination of antimicrobial activity: The crude extracts of the different plant parts of different species were subjected to antimicrobial assay using the agar well diffusion method of (Murray et al., 1995) modified by (Olurinola 1996) . 20 ml of nutrient agar was dispensed into sterile universal bottles these were then inoculated with 0.2 ml of cultures mixed gently and poured into sterile petri dishes. After setting a number 3-cup borer (6mm) diameter was properly sterilized by flaming and used to make three to five uniform cups/wells in each petri dish. A drop of molten nutrient agar was used to seal the base of each cup. The cups/wells were filled with 50µℓ of the extract concentration of 100 mg/ml and allow diffusing for 45 minutes. The solvents used for reconstituting the extracts were similarly analyzed. The plates were incubated at 37°c for 24 hours for bacteria. The above procedure is allowed for fungal assays but except the media potato dextrose agar instead of nutrient agar and incubates at 25°c for 48 hours. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in duplicates.

RESULTS

The results summarized in Table 1 and Fig 1 *A. indicum* (Malvaceae) known commonly as ‘‘Thuthi’’, is distributed throughout the hotter parts of India Chopra et. al., 1992) The leaves extractwas reported to contain Alkaloids, flavonoids, sterols, triterpenoids, and glycosides (Dhanalaksmi et. al., 1990, Sankara Subramanian et. al., 1972, Sharma et. al., 1989). In India Plant used for Diabetes, thirst, Painful menses, Hemorrhoids, Infusion, poultice or paste for Boils, and ulcers the extract (100 mg/ml concentration) was excellent against (17 mm) *A. alternate* and *F. oxysporum*.

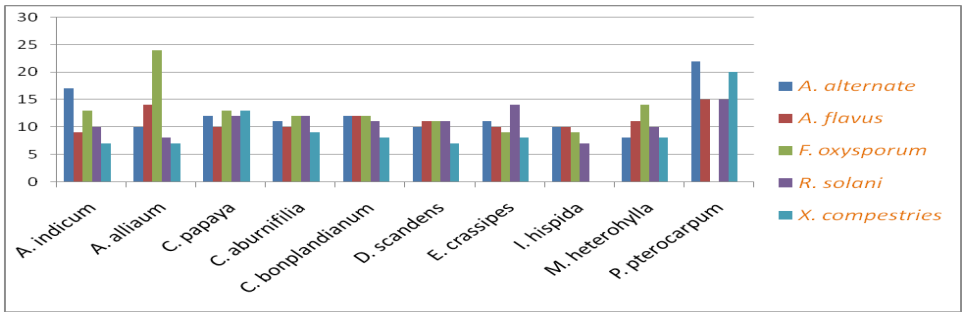

Figure 2

Table 1: Antimicrobial activity of methanol extracts of medicinal plants

Name of the pathogen	*Ai*	*Aa*	*Cp*	*Cl*	*Cb*	*Ds*	*Ec*	*Hi*	*Mh*	*Pp*
A. alternate	17	10	12	11	12	10	11	10	8	22
A. flavus	9	14	10	10	12	11	10	10	11	15
F. oxysporum	13	24	13	12	12	11	9	9	14	-
R. solani	10	8	12	12	11	11	14	7	10	15
X. compestries	7	7	13	9	8	7	8	-	8	20

Methanolic extract concentration of 100 mg/ml DMSO, Volume per well: 50µl,

Borer size used: 6mm

*All values indicates Zone of inhibition in mm *(-) Value indicates no activity

Ai= Abultilon indicum, *Aa = Adenocalymma alliaceum*, *Cp= Carica papaya*, *Cl= Crotolaria laburnifilia*, *Cb= Croton bonplandianum*, *Ds= Derris scandens*, *Ec= Eichornia crassipes*, *Hi= Iopomea hispida*, *Mh=Moringa heterohylla, Pp= Peltophorum pterocarpum*

The plant *A. alliaceum* (Bignoniaceae) garlic creeper leaves are used as astringent and extract showed highest (24 mm) activity against *F. oxysporum.*

P. pterocarpum (Fabaceae) bark is used for dysentery, tooth powder, eye lotion, embrocation for pains and sores. The bark also gives a dye of a yellow colour. Methanolic extract showed considerable inhibitory activity against *A. alternate* and *X. compestries.*
The data revealed that significant reduction in growth of *A. flavus* was observed with extracts of *A. alliaum, C. bonplandianum, D. scandens, M. heterohylla* and *P. pterocarpum.* No activity was found against *X. compestries* with *I. hispida* extracts and *P. pterocarpum* against *F. oxysporum.*

DISCUSSION

We found that medicinal plants are good sources of natural antimicrobial agents. Negative results do not indicate the absence of bioactive constituents, nor is that the plant inactive. Active compound(s) may be present in insufficient quantities in the crude extracts to show activity with the dose levels employed. Lack of activity can thus only be proven by using large doses. On the other hand, if the active principle is present in high quantities, there could be other constituents exerting antagonistic effects of the bioactive compounds. It is not surprising that there are differences in the antimicrobial activities of plant groups, due to the phytochemical differences between species. The present study was conducted to develop newer lead for better and safer chemotherapeutic agents from medicinal plants.

Although, the tested plant extracts may contain anti-microbial constituents, further phytochemical and pharmacological studies will be necessary to isolate the active constituents and evaluate the antimicrobial activity against a wide range of microbial population and also needed to establish the exact mechanism of action for antimicrobial action of the plant extract. However, the strength of the existing data is not enough to suggest a reasonable mode of action for antimicrobial effects. The data of this study may just enrich the existing comprehensive data of biological activity.

In particular, the authors may recommend that the methanolic extracts of *A. alliaum* to be used as potent biocide to treat diseases in caused by *F. oxysporum* as they showed maximum activity even at lower concentrations.

CONCLUSIONS

Extensive bioprocess parameter studies should under taken the methanolic extracts of *A. alliaceum* and *P. pterocarpum* showed strong antimicrobial activity among selected plant species. From the above results it can be concluded that plant extracts have greater potential as antimicrobial compounds against microorganisms and that they can be used in the treatment of infectious diseases caused by resistant pathogenic microorganisms.

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