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Original Research Paper

IN VITRO ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACT OF SELECTED INDIAN MEDICINAL PLANTS

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ABSTRACT

Antimicrobial activities have been studied with the methanolic plant extracts of *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. 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 extract exhibited promising antimicrobial activity than chloroform and hexane extracts. The extracts from various parts of plants were assessed in an effort to validate the medicinal potential of the herb. Our results showed that *A. alliaceum* and *P. pterocarpum* plant extracts have higher levels of antimicrobial activity.

Keywords: *Abultilon indicum,* Medicinal plants, Agar well diffusion technique, *P. pterocarpum,* 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, appropriate steps needs to 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).[1,2] Higher plants have been shown to be a potential source for new anti-microbial agents.[3] 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.[4,5,6] Numerous studies have been carried out on various natural products screening their antimicrobial activity.[7,8, 9, 10] Besides small molecules from medicinal chemistry, natural products are still major sources. In India plant *Abultilon indicum* (*Malvaceae*) used for *Diabetes*, *thirst*, *Painful menses*, *Hemorrhoids*, *Infusion*, *poultice* or *paste for Boils*, and *ulcers*. *Adenocalymma alliaceum* (*Bignoniaceae*) garlic creeper leaves are used as astringent. *Carica papaya* L. (*Caricaceae*) latex from the leaves has been used as *antihelmints* and for the treatment of infections of bacterial origin.[11] *Crotolaria laburnifilia* (*Fabaceae*) infusion used as gargle. *Derris scandens* (*Fabaceae*) tender stems and twigs crushed, warmed and applied on painful parts for relief of rheumatic pains. *Eichhornia crassipes* (*Pontideraceae*) commonly known as water hyacinth is warm water aquatic plant belonging to the family. Water hyacinth is listed as one of the most productive plant on earth and is considered the world's worst aquatic plant. *Peltophorum pterocarpum* (*Fabaceae*) bark is used for *dysentery*, *tooth powder*, *eye lotion*, *embrocation for pains* & *sores* and also gives a dye of a yellow colour. *Moringa heterohylla* (*Moring*aceae) roots and seeds used as antibiotic, anti-inflammatory and diabetes. *Peltophorum pterocarpum* (*Fabaceae*) whole plant used for reclamation.

In this paper the results of such antimicrobial activities of the plants have been reported 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 plant materials collected from various places of Andhra Pradesh and were taxonomically identified and the Voucher specimen were stored in the Department library of Andhra University. 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 of each plant 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^{o}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 5×10^{5} cfu/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)[12] modified by (Olurinola, 1996)[13] 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 and their average results are reporting here.

RESULTS

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.

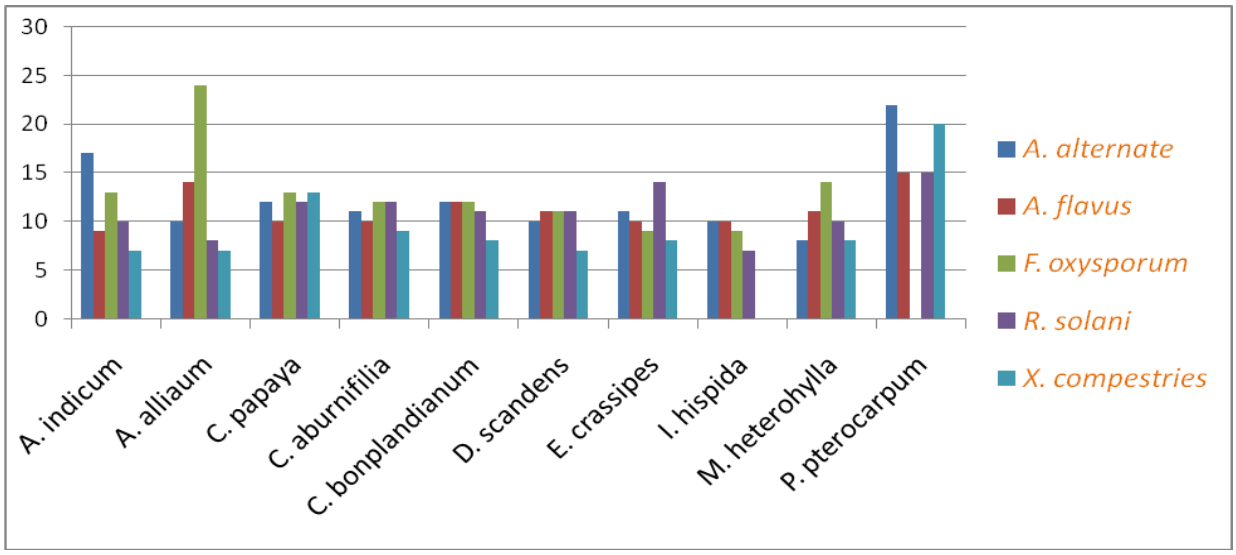

Figure 1

The results summarized in Table 1 and Fig 2 *A. indicum* (*Malvaceae*) known commonly as ‘‘Thuthi’’, is distributed throughout the hotter parts of India. The leaf extracts were reported to contain Alkaloids, flavonoids, sterols, triterpenoids, and glycosides.[14,15,16] The extract of the plant (100 mg/ml concentration) is known to be good antifungal activity against (17 mm) *A. alternate* and *F. oxysporum*. The plant *A. alliaceum* extract showed highest (24 mm) activity against *F. oxysporum, P. pterocarpum* 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. alliaceum, 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 while working with *I. hispida* and *P. pterocarpum* extracts respectively. 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. 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. alliaceum* to be used as potent biocide to treat diseases in caused by *F. oxysporum* as they showed

maximum activity even at lower concentrations.

CONCLUSION

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 mentioned 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. Further pharmacological evaluations, toxicological studies and possible isolation of the therapeutic antimicrobial substances from these plants are the future challenges.

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