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In vitro Antimicrobial Activity of Methanolic Plant Extracts Against *Aspergillus flavus*

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***Aspergillus flavus* is common and widespread in nature it produces aflatoxins which are carcinogenic and immunosuppressive in human being. Bioassays for antimicrobial activities were carried out using stem, leaves and flowers of 10 medicinal plants were against *A. flavus* that have been popularly used as folk medicines using agar well diffusion technique. It is well known that plants containing active compounds are able to inhibit the microbial growth. Among all the tested plants *S.apetala* showed highest and significant activity with all three concentrations 100 mg/ml 250mg/ml and 500mg/ml DMSO. This study, has to some extent, validated the medicinal potential of the plants. Further studies are needed to establish the exact mechanism of action for antimicrobial action of the plant extract.**

Key words: *Aspergillus flavus*, Bioassays, Antimicrobial activity, Medicinal potential.

Since ages, man has been dependent on nature for curing various body diseases. From ancient civilization various parts of different plants were used to eliminate pain, control suffering and counteract disease. Most of the drugs used in primitive medicine were obtained from plants and are the earliest and principal natural source of medicines. Reported that the total number of plant chemicals may exceed 400,000 and out of it more than 10,000 are secondary metabolites whose major role in plant is defensive in nature. The nature has provided the storehouse of remedies to cure all ailments of mankind a large number of plants in different location around the world have been extracted and semi-purified to investigate individually their antimicrobial activity. India is endowed with a wealth of medicinal plants, which have been a valuable source of natural products for maintaining human health. Much work has been done on ethno medicinal plants in India [1,2]. Among the different plant derivatives, secondary metabolites proved to be the most important group of compounds that showed wide range of antibacterial and antifungal activity [3]. *Aspergillus flavus* is common and widespread in nature. Aflatoxins are carcinogenic and immunosuppressive metabolites produced by *A. flavus* these toxins have been incriminated as the cause of high mortality in livestock and some cases of death in human being [4] among all classes of aflatoxins, aflatoxin B1 is known to be the most significant in terms of animal and human health risk [5]. At least 13 different types of Aflatoxin are produced in nature with Aflatoxin B1 considered as the most toxic. The plants used, as drugs are fairly innocuous and relatively free from toxic

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effects or were so toxic that lethal effects were well known. Biologically active compounds present in the medicinal plants have always been of great interest to scientists working in this field. Researchers have shown that all different parts of the plants which include stem, root, flower, bark, leaves possess antimicrobial property. Bioassays for antimicrobial activities were carried out using stem, leaves and flowers of 10 medicinal plants were studied against *A. flavus* that have been popularly used as folk medicines. 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 antifungal compounds. The objectives of this research were to evaluate the fungitoxic effect of methanolic extracts of medicinal plants used in Traditional Indian medicine.

MATERIAL AND METHODS

Solvents and chemicals used

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

Extraction procedure for antimicrobial

Healthy, disease free, medicinal plant materials *Acanthus ilicifolius* Linn, *Centella asatica* Linn, *Clitoria ternatea* Linn, *Datura metel* L, *Lantana camara* L, *Lawsonia inermis* L, *Mimosa pudica* Linn, *Sonneratia apetala* (Buch - Ham), *Terminalia chebula* Retz, *Xylocarpus granatum* J. Koenig were collected from various places of Andhra Pradesh and they are taxonomically identified and the voucher specimens were deposited in the herbarium of the Department of Botany, Andhra University, Visakhapatnam. 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.

Microorganism used

An *Aspergillus flavus* was 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 Antifungal 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) [6] modified by (Olurinola 1996) [7]. 20 ml of potato dextrose agar (PDA) 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 500mg/ml, 250mg/ml and 100mg/ml and allow diffusing for 45 minutes. The solvents used for reconstituting the extracts were similarly analyzed. The plates were incubated at for fungal assays in the media potato dextrose agar 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. Hexane, Chloroform and methanolic extracts in different concentrations (100mg/ml, 300mg/ml, and 500mg/ml) to get the final drug concentration 5mg/well, 15mg/well, and 25mg/well respectively, control (DMSO) and standard (Bavistin 5µg/ml).

RESULTS AND DISCUSSION

Antifungal activity of 10 botanical extracts was assayed and data on effect of plant extracts on the growth of *A. flavus* presented in table 1

Table 1 summarizes the antimicrobial activities of zone of inhibition of various plants methanolic extracts (10 to 20 mm) with concentration of 100 mg/ml. All the extracts tested gave significant inhibition of mycelial growth of *A. flavus* except *A.ilicifolius*. Among all the tested plants *S.apetala* showed highest and significant activity with concentrations 100mg/ml 250mg/ml and 500mg/ml DMSO. In particular, the authors may recommend that the methanolic extract of

Table 1. Antimicrobial activity of methanol extracts of medicinal plants

S. No	Name of Plant Species	Parts used	*Dilutions (mg/ml)*		
			100	300	500
1	*Acanthus ilicifolius* Linn	Leaves	-	-	-
2	*Centella asatica* Linn	leaves	10	12	16
3	*Clitoria ternatea* Linn	Whole plant	12	13	16
4	*Datura metel* L.	Leaves and seeds	10	12	14
5	*Lantana camara* L	Leaves and flowers	11	14	16
6	*Lawsonia inermis* L.	Bark and leaves	12	20	25
7	*Mimosa pudica* Linn.	Whole plant	12	13	16
8	*Sonneratia apetala* (Buch - Ham)	Whole plant	20	24	30
9	*Terminalia chebula* Retz.	Fruit	18	20	22
10	*Xylocarpus granatum* J. Koenig	Bark and seed	11	13	17

S.apetala, L. inermis, T.chebula. The differences in antifungal activity is due to the potential difference in the susceptibility of conidia, germinated conidia and hyphae to antifungal compound and the time duration for the exposure of the compound. 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.

CONCLUSION

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. It was revealed in this study that increase in the antifungal activity of the extracts was enhanced by increase in the concentration of the extracts. Extensive bioprocess parameter studies should under taken the methanolic extract of *S.apetala* as a strong antifungal agent against *A. flavus.* The present study was conducted to develop newer lead for better and safer chemotherapeutic agents from medicinal plants. Further research is necessary for successful separation, purification and characterization of biologically active compounds using chromatographic methods and spectroscopic techniques. Further studies are needed to establish the exact mechanism of action for antimicrobial action of the plant extract.

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