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Antifungal activity of selected plant extracts against phytopathogenic fungi *Aspergillus niger* F2723

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Abstract: In this present study forty nine different plants used in traditional Indian medicine were examined against *Aspergillus niger* using agar well diffusion method. The methanolic extracts of 43 plants exhibited varying degrees of inhibition activity against the fungi. Among the forty nine plants studied 86% of the plants had antifungal activity while the remaining 14% had no antifungal activity. The extract from *Grewia arborea* showed maximum activity. *Emblica officinales, Heldigordia populipolia, Hyptis sueolences, Moringa heterophylla, Strychnos nuxvomica* and *Vitex negundo* did not exhibit antifungal activity at the condition studied.

Keywords: *Aspergillus niger*, Antifungal, medicinal plants

Introduction

Medicinal plants represent a rich source of antimicrobial agents (Mahesh & Satish, 2008). Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine (Mann *et al*., 2008). Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine (Ibrahim, 1997; Ogundipe *et al*., 1998). The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world (Reddy *et al*., 2001; Ateb & ErdoUrul, 2003). Much work has been done on ethno medicinal plants in India (Maheshwari *et al*., 1986; Negi *et al*., 1993). Interest in a large number of traditional natural products has increased (Taylor *et al*., 1996). Plants are the sources of natural pesticides that make excellent leads for new pesticide development (Arokiyaraj *et al*., 2008; Gangadevi *et al*., 2008; Satish *et al*., 2008; Brinda *et al*., 2009; Jagadish *et al*., 2009; Milind Pande *et al*., 2009; Shanmugavalli *et al*., 2009; Swarna Latha & Neelakanta Reddy, 2009; Vetrivel Rajan *et al*., 2009).

Aspergillus niger as a saprophyte in soil causes black mould of onion, garlic and shallot; stem rot of *Dracaena;* root stalk rot of Sansevieria; and boll rot of cotton; spoilage of cashew kernels, dates, figs, vanilla pods and dried prune. Crown rot of groundnut is the most serious plant disease caused by *A. niger.* The main objective of this study was to investigate the inhibitory effects of different organic solvent extracts from forty nine medicinal plant species against *A. niger* and to evaluate the potential application of medicinal plant based treatments to control diseases caused by *A. niger*.

Materials and Methods

Plant material and extracts preparation

The plant materials of forty nine plant species (Table1) were collected from different places in Visakhapatnam district, Andhrapradesh. The collected plants were identified and authenticated by a botanist in the Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh. The selected parts of different medicinal plants were cut into small pieces and shade dried at room temperature for fifteen days, finely powdered plant materials were successively extracted with organic solvent methanol basing on order of polarity using soxhlet apparatus. The different extracts obtained were subsequently concentrated under reduced pressure to get their corresponding residues. 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), were transferred to the cups of each agar plate, incubated at room temperature (28^0C) and examined for inhibition zones after 36 hours of incubation to screen for antifungal activity.

Microbial cultures and growth conditions

The plant extracts were assayed for antifungal activity against the fungal strain *A. niger,* F2723 obtained from Microbial Type Culture Collection & Gene Bank (MTCC), Chandigarh. This fungus was grown on PDA plate at 28^oC and maintained with periodic sub-culturing at 4^oC.

Antifungal activity

The methanolic extracts of forty nine different plant extracts (Table 1) were screened for antifungal activity by agar well diffusion method (Perez *et al*., 1990) with sterile cork borer of size 6.0mm. The cultures of 48 hours old grown on potato dextrose agar (PDA) were used for inoculation of fungal strain on PDA plates. An aliquot (0.02ml) of inoculum was introduced to molten PDA and poured in to a petri dish by pour plate technique. After solidification, the appropriate wells were made on agar plate by using cork borer. In agar well diffusion method 0.05ml of methanolic extracts of forty nine different plant extracts were introduced serially after successful completion of one plant analysis. Incubation period of 24-48hours at 28^0C was maintained for observation of antifungal activity of plant extracts. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth surrounding the plant extracts. The complete antifungal analysis was carried out under strict aseptic conditions. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in triplicates.

Minimum inhibitory concentration (MIC) assay

Based on the preliminary screening (Fig.1, 2) chloroform and methanolic extracts revealed potent

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Table 1. List of Investigated Medicinal Plants

Botanical Name	Parts used	Uses / Ailments treated
Acacia farnesiana (L.) Willd	Bark, roots	Astringent, demulcent, poultice, stomachic.
Acalypha indica Linn.	Aerial parts	Skin diseases, ulcers, bronchitis, head ache, snake bite
Acanthus ilicifolius Linn.	Leaf extract	Relieve rheumatism
Adenocalymma alliaceum (Lam.)	Leaves	Astringent,
Adhatoda vasica Nees.	Leaves, whole plant	Cough, chronic bronchitis, rheumatism, asthma and asthma.
Andrographis paniculata Nees.	Whole plant	Anti-biotic, anti-viral, anti-parasitic and immune system stimulant.
Avicennia officinalis L.	Seed	Relieving ulcers
Boerhaavia diffusa Linn.	Whole plant	Scabies, myalgia, aphrodisiac
Bridelia montana (Roxb.) Willd	Leaf	Stomach pains, sore eyes and headaches.
Cassia occidentalis Linn.	Whole plant	Boils, spasm. hysteria, whooping cough
Catharanthus roseus Linn.	Leaves and roots	Anti-mitotic and anti-microtubule agents
Centella asiatica Linn.	Whole plant	Diuretic, treatment of leprosy, use as brain tonic and stimulates hair growth.
Cleome viscosa Linn.	Leaves and seeds	Anthelmintic, carminative, diaphoretic and rubefacient.
Coleus forskohlii (Willd.).	Roots	Treat heart & lung diseases, intestinal spasms, insomnia & convulsions.
Coriandrum sativum Linn.	Fruits	Colic, laxative, blood purifier, indigestion, sore throat
Derris scandens (Roxb.) Benth	Stem	Arthritis, anti-inflammatory
Eichhornia crassipes (C.Mart.)	Whole plant	Biomass, soil reclamation
Emblica officinalis Gaertn.	Fruit	Aperient, carminative, diuretic, aphrodasiac, laxative, astringent .
Gmelina arborea Linn.	Roots	Gonorrhea, cough, insanity, epilepsy, fevers, indigestion, nerve tonic.
Gynandropsis gynandra (L.)	Leaf	Anti-irritant
Hildegardia populifolia (Roxb.)	Stem bark	Dog bite, Malaria.
Holarrhena antidysenterica Foxh.	Bark and seeds	Dysentery, piles, leprosy, colic, dyspepsia, chronic chest complaints, , spleen diseases, jaundice, bilious, calculi
Hiptage benghalensis (L.) Kurz.	Leaves and bark	Insecticidal, cough, inflammation; skin diseases and leprosy
Hyptis suaveolens (L.) Poit.	Leaves	Antispasmodic,antirheumatic and antisoporific
Kyllinga nemaralis Rottb.	Whole Plant	Promotes action of liver, and relief prunitus
Lantana camara Linn.	Whole Plant	Antidote to snake venom, Malaria, wounds cuts ulcers, Eczema, Tumours
Melia azedarach L.	Leaf, seed oil, flower	Vermifuge, insecticide, astringent, antiseptic, antidiabitic, antiviral, antibiotic
Mimosa pudica Linn.	Whole Plant	Menorrhagia, piles, diarrhoea, hydrocele, whooping cough, filiriasis
Moringa heterophylla L.	Roots,Seeds,	Antibiotic, anti-inflammatory and diabetes
Muntinga calabria Linn.	Leaves	Antiseptic
Marraya Koenigii (L.) Spreng.	Leaves	Skin diseases, heminthiasis, hyperdipsia, pruritus, etc.
Ocimum sanctum Linn.	Leaves, Seeds	Malaria, bronchitis, colds, fevers, absorption, arthritis.
Peltophorum pterocarpum (DC.)	Whole plant	Reclamation
Phyllanthus niruri L.	Leaves	Jaundice, diabetes
Plumeria rubra Linn.	Leaves	Ulcers, leprosy, inflammations, rube facient.
Pongamia pinnata (L.) Pierre.	Bark, seeds	Anti malaria , skin disease, rheumatic and leprous sores
Ricinus communis Linn.	Leaves	Jaundice, sores,
Salvadora persic, Linn.	Roots	Antimicrobial and dental diseases
Sesbania grandiflora (L.)	Flowers	Treat gonorrhea and for curing infection of the cornea.
Strychnos - nux - vomica Linn.	Seeds	Cholera, chronic wounds, ulcers, paralysis, diabetes
Suaeda maritima (L.) Dumort.	Whole plant	Bioremediation
Tephrosia pumila (Lamk.) *Persoon.*	Root	Rheumatism, bladder disorders, coughing, hair loss, reproductive disorders
Tephrosia tinctoria Pers.	Root	Ant syphilitic
Tephrosia villosa (L.) Pers.	Root, leaves, bark	Cure for leprosy, ulcers, ailments of liver, spleen, heart, blood, asthma etc.
Terminalia chebula Retz.	Fruit	Antimicrobial, mouthwash/gargle, astringent, douche for vaginitis.
Tinospora cordifolia (Willd.)	Stem	Analgesic and anti-inflammatory.
Tridax procumbens Linn.	Whole plant	Antimicrobial, anti-oxidant and anti-inflammatory,
Vitex pentaphylla Linn.	Aerial parts	Foetid discharges, febrifuge, rheumatism, catarrhal
Withania somnifera (L.) Dunal	Leaves	Sore eyes, febrifuge, ulcers, cure sterility of women, sedative

antimicrobial activity. The Minimum Inhibitory Concentrations (MIC) of the extracts were determined according to Elizabeth *et al.,* (1999). A final concentration of 0.5% (v/v) Tween-20 (Sigma) was used to enhance crude extract solubility. A series of two fold dilution of each extract, ranging from 0.2 to 100 mg/ml, was prepared. After sterilization, the medium was inoculated with 3µl aliquots of culture containing approximately 105 CFU/ml of each organism of 24 hours slant culture in aseptic condition and transferred into sterile 6 inch diameter petri dishes and allowed to set at room temperature for about 10 minutes and then kept in a refrigerator for 30 minutes. After the media solidified 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. Different plant crude extracts ranging from 0.2 to 100 mg/ml were added to the cups/wells of each petri dish and the control plates without plant extract. Inhibition of organism growth in the plates containing test crude extracts was judged by comparison with growth in blank

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Plant methanolic extracts inhibition on Aspergillus niger
(Volume per well: 50µl, 'x' axis = Medicinal plant extracts concentration A: 100 mg/ml = 5 mg/well, B: 300 mg/ml =15 mg/well, C: 500 mg/ml= 25 mg/well, Borer size used: 6mm, 'y' axis =0-40 = Zone of inhibition in mm)

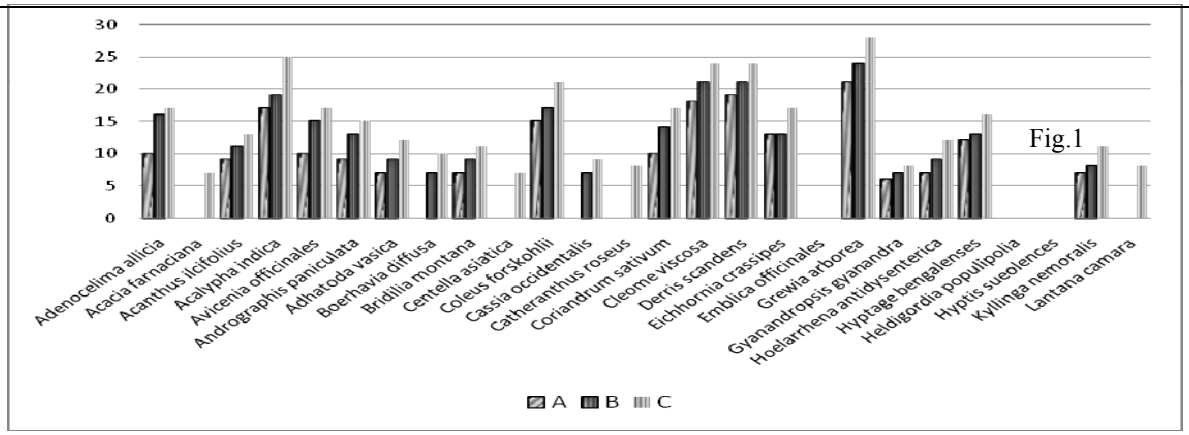


Fig.1

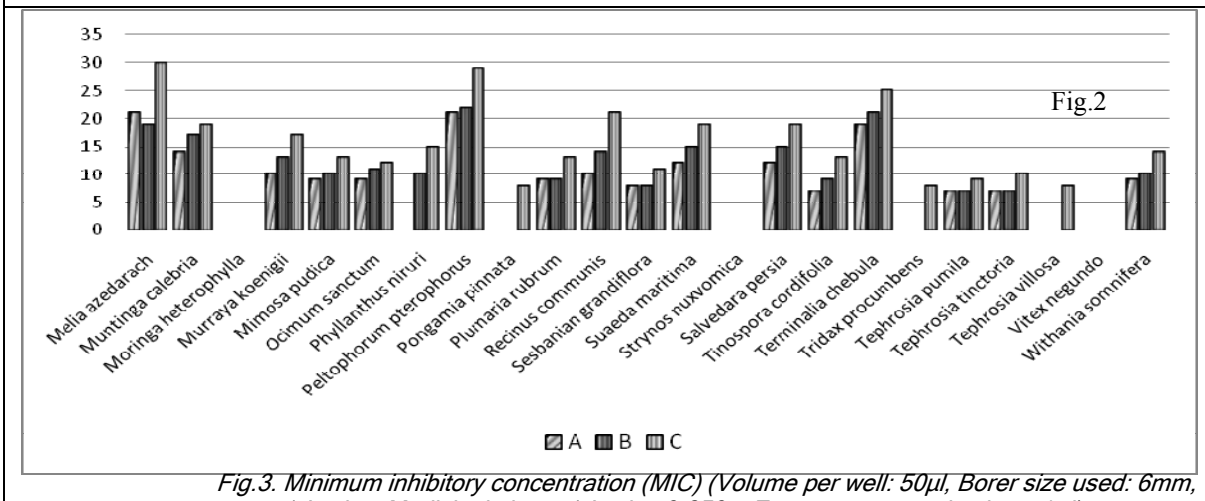
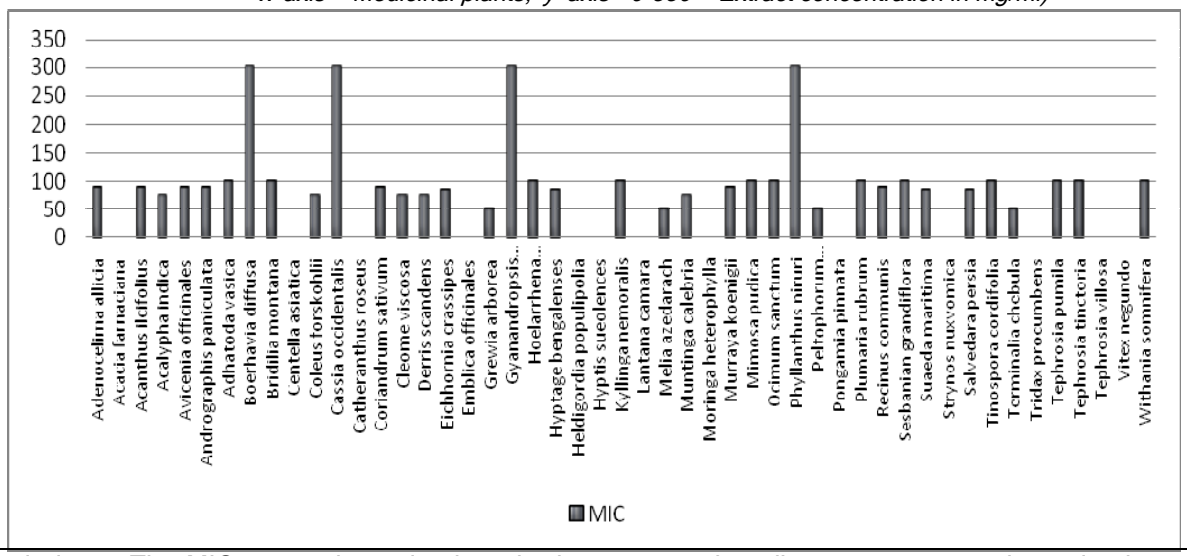


Fig.2

Fig.3. Minimum inhibitory concentration (MIC) (Volume per well: 50µl, Borer size used: 6mm, 'x' axis = Medicinal plants, 'y' axis =0-350 = Extract concentration in mg/ml)



control plates. The MICs were determined as the lowest concentration of extracts inhibiting visible growth of each organism on the agar plate. Similarly the MICs of methanolic extracts were determined against all other microorganisms. The results were given in Fig.3.

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Results and discussion

Antifungal activity of forty nine botanical extracts was assayed and data on effect of plant extracts on the growth of *A. niger* presented in Fig.1, 2. The data revealed that significant reduction in growth of *A. niger* was observed with extracts of forty three medicinal plants and the extracts showed significant differences in their efficacy. Among all the forty nine plant methanolic extracts, 86% plants showed inhibition of mycelial growth of *A. niger* over control and four plants *Grewia arborea, Melia azedarach, Peltophorum pterophorus, Terminalia chebula,* showed exceptionally prominent activity. The extract of plant *Grewia arborea* showed maximum activity even at lower concentrations. The following six plants, viz, as *Emblica officinales, Heldigordia populipolia, Hyptis sueolences, Moringa heterophylla, Strychnos nux-vomica L.,* and *Vitex negundo* did not exhibited the antifungal activity against *A. niger.* Therefore, this study suggests that methanolic extracts of screened plants would be helpful in treating diseases in plants caused by *A. niger*. The control plate representing DMSO did not exhibit inhibition on the tested fungi where as standard antifungal drug Bavistin have antifungal activity even at 5µg/well. In particular, the authors may recommend that the methanolic extract of *G. arborea* to be used as potent biocide to treat diseases in plants caused by *A. niger* as it showed maximum activity even at lower concentrations nearly equal to the standard antifungal agent. It was revealed in this study, that the antifungal activity of the extracts was enhanced by increase in the concentration of the extracts. It also supports the earlier investigation (Banso & Adeyemo, 2007) that the tannins isolated from the medicinal plants possess remarkable toxic activity against bacteria and fungi and may assume pharmacological importance. Extensive bioprocess parameter studies should be undertaken for the methanolic extract of *G. arborea* as a strong antifungal agent against *A. niger* causing plant diseases.

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