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 officinalis, Helidigoria populipolia, Hptis sueolences, Moringa heterophylla, Strychnos nuxvomica and Vitex negundo did not exhibit antifungal activity at the condition studied.

Keywords: Aspergillus niger, Antifungal, medicinal plants

Materials and Methods
Plant material and extracts preparation

The plant materials of forty nine plant species (Table 1) were collected from different places in Visakhapatnam district, 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°C) 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°C and maintained with periodic sub-culturing at 4°C.

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°C 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...
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 added to the cups/wells of each petri dish

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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 µg/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 nutrient agar.
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.
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 materials, 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 officinalis, Hedligordia populipolia, Hyptis suoeolences, Moringa heterophylla, Strychnos nux-vomica and Vitex negundo did not exhibit 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 whereas standard antifungal agent 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.

Reference