Fungistatic activity of *Semecarpus anacardium* Linn. f nut extract

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Alcoholic extract of dry nuts of *S.anacardium* showed dose dependent antifungal activity in vitro against *Aspergillus fumigatus* and *Candida albicans*. At 400 mg/ml concentration, growth of both the fungi was inhibited and considerable reduction in size of cells and hyphae was observed. Sporulation also decreased.

*Semecarpus anacardium* L.f., a deciduous tree of family Anacardiaceae is found in Sub-Himalayan regions and hotter parts of India. The fruit, commonly called marking nut or Bhallataka, is also known as Dhobi nut as its liquid is used to mark laundry. The nut extract is used frequently as home remedy for inflammation, arthritis, warts and rheumatism. Its anticancer, immuno-modulatory and antibacterial activity has also been reported.

Not much work has been done on antifungal activity of the *S.anacardium* nut extract. In this view, a study on the effect of nut extract on two common fungal pathogens viz. *Aspergillus* and *Candida* was planned. *Aspergillus*, commonly found in soil, air and water is pathogenic to humans. It causes Aspergilloses—a disease of lungs which is quite common in patients with malignancies specially leukemia and lymphoma and in patients who receive immunosuppressants. Out of eight species of *Aspergillus*, *A.fumigatus* accounts for most infections. *Candida albicans* causes Candidiasis, skin and nail infection. Oral candidiasis is very common in new borns, debilitated and aged patients. Other diseases, physiological disorders, use of broad-spectrum antibiotics for a long duration creates conditions under which *C.albicans* is converted into a pathogen. It is also common in housewives, fruit canners, diabetics and people who are required to immerse their hands in water frequently for long durations. Pulmonary candidiasis leads to low grade fever, coughing, mucoid and blood streaked sputum, pleurisy and in severe cases pneumonia.

An upsurge in number of patients succumbing to fungal infections has resulted in demand for new herbal antifungal compounds. Present study reports the fungistatic activity of *S.anacardium* nut extract against *A.fumigatus* and *C.albicans*.

Material and Methods

The antifungal activity of crude alcoholic extract in vitro was determined using tissue method of biological assay technique. Extract preparation was done following modified method of Shadomy and Ingroff. Dried nuts of *S. anacardium* were crushed with silica and ground into fine powder. 20g powder was suspended in 100 ml absolute alcohol over night and subsequently centrifuged at 3000 rpm for 30 min. Supernatant was evaporated at 45°C and residue was re-dissolved in alcohol to obtain seven different concentrations of extract viz. 20, 40, 60, 80, 100, 200 and 400 mg/ml. Similarly aqueous extract was also prepared. One ml extract of each of the above mention concentrations was added to culture tubes containing 20 ml potato dextrose agar (PDA) medium. The tubes were autoclaved at 121°C and 15 psi to eliminate microbial growth. Autoclaving also results in evaporation of alcohol leaving only the extract in the medium. Autoclaved medium was then inoculated with pure cultures of *Aspergillus* and *Candida* and incubated at 36°C. Growth of fungus was observed on 3rd, 6th, 10th and 12th days. Tube dilution technique was used for determining the antifungal activity. Tubes containing extract free autoclaved PDA medium were used as control. Five replicates of each concentration were maintained and the experiment was repeated thrice.

Cultures of *A. fumigatus* and *C. albicans* were procured from RNT medical college, Udaipur and subcultured by serial dilution to get single spore culture.
Pure cultures thus produced were used for inoculation. Fungal mycelium and fungal cells were stained with cotton blue and mounted in lactophenol for microscopic observation.

Microscopic measurements of the spores were done using standard methods of micrometry at 100X. Standard deviation was calculated using computerized programme.

Results
No inhibitory activity was observed with aqueous extract. Abundant growth was visible in control as well as low concentrations of the alcoholic extract. Decrease in the growth was observed as concentration of the extract increased. 400 mg concentration proved inhibitory for both fungi. Inhibitory effect was noticed even after 12 days. Similar results were observed in all replicates and in successive repetitions, suggesting 100% inhibition.

Profuse growth of *C. albicans* was seen in control as it contains extract free medium (Fig. 1; a). No significant decrease in growth was observed at 20, 40, and 60 mg concentrations of extract (Fig. 1; b, c, d). Gradual decrease in growth was visible from 80 mg concentration onwards (Fig. 1; e, f, g, h). The order of decrease of growth was concomitant with the concentration of extract in these tubes. Maximum inhibition was observed at 400 mg concentration (Fig. 1; h) signifying that the inhibitory action is directly proportional to increasing concentration of extract.

*A. fumigatus* showed abundant growth at lower concentration of extract. Slight decrease in growth could be observed at 40 mg concentration although it was not very significant (Fig. 2; a, b). This suggests that the extract is ineffective at this and lower concentrations. Subsequently, progressive decrease in growth was observed at 80 mg concentration onwards, significant inhibition was noticed at 400 mg (Fig. 2; d, e, f, g). Concurrent with the inhibitory effect the colony colour also changed. Colony at 400 mg/ml extract was whitish (Fig. 2; g) whereas in all the other tubes the colony was dark green in colour (Fig. 2; a-f).

On microscopic examination, at 400 mg concentration, the hyphae and the cytoplasmic material of *A. fumigatus* appeared shrunken (Fig. 4) as compared to the control (Fig. 3). The average mycelium width at this concentration was 3.11 μ whereas in the control was 9.91 μ (Fig. 11; a). Spore number, size and sporangium size was also reduced as compared to control (Figs. 3, 4, 5 and 6). Sporangium diameter in the control was 49.45 μ but at 400 mg concentration, it was reduced to 20.54 μ (Fig. 11; a). Similarly the average spore diameter was reduced from to 2.97 μ in the control to 1.59 μ at 400 mg concentration (Fig. 11; a).

In *C. albicans*, pseudo mycelium, blastospores and chlamydospores were observed in control (Fig. 7,8). At 200 and 400 mg concentrations only chlamydospores could be seen (Fig 9,10). As compared to control, chlamydospores size decreased with increasing concentration of extract. The average chlamydospore diameter in control was 5.79 μ, at 200 mg it was 4.54 μ and at 400 mg it was further reduced to 2.54 μ (Fig. 11; b).
Figs 3 and 4—Mycelium and spores of *A. fumigatus* after 12 days of experimentation (3: control; 4: following 400 mg/ml treatment) × 10

Figs 5 and 6—Sporangiophore of *A. fumigatus* after 12 days of experimentation (5: control; 6: following 400 mg/ml treatment) × 20

Figs 7-10—Pseudomycelium, blastospores and chlamydospores of *C. albicans* after 12 days of experimentation (7 and 8: control; 9: following 200 mg/ml treatment; 10: following 400 mg/ml treatment) × 40 [M=mycelium; C=chlamydospores; sp= sporangium; S= spore]
Discussion

There was a remarkable decrease in growth and sporulation of *A. fumigatus* following treatment with *S. anacardium* extract. This deuteromycetous fungus reproduces mostly by producing spores. A reduction in their number as well as size, shrunken mycelium suggests that the extract may be fungistatic in nature. The spores are dark green and are responsible for the colour of the colony. Whitish colony in the tube containing 400 mg extract correlates with the microscopic observation of reduced sporulation. 53.75%, 58.56% and 46.46% reduction in mycelial width, sporangium and spore size respectively indicates inhibitory action of extract.

*C. albicans* normally occurs in two states. When the fungus grows as a commensal, it exists as unicellular yeast phase and when it becomes pathogenic, the thallus becomes pseudomycelial. As observed the thallus is pseudomycelial in the control (Figs 7, 8) but at 200 and 400 mg concentrations of the extract, only unicellular chlamydospores were seen (Figs 9, 10). Thus it can be said the fungus may have converted to the commensal nonpathogenic form. 21.59% and 57.68% reduction in the chlamydospore size at 200 mg and 400 mg extract concentration suggests that the extract is inhibitory.

The antimicrobial activity of the alcoholic extract can be attributed to the presence of naturally occurring antimicrobial phenolic compounds like catechols, oleoresins and secondary metabolites. Phenolic compounds alter membrane permeability and act as protein denaturing agents. Phenolic compounds are soluble in alcohol only, which explains the inactivity of aqueous extract. Reports of antitu merous activity of *S. anacardium* suggest that the extract and the oil of the nut are growth inhibitory. Antimicrobial activity of *S. anacardium* has also been reported.

The results of present study thus suggest that alcoholic extract of *S. anacardium* is fungistatic in nature as it inhibits growth and reproduction.

References

11. ShinY G & Cordell G A, Rapid identification of cytotoxic alkyl catechols in *S. anacardium* using bioassay-linked


