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Screening and characterization of ethnopharmacological properties of selected flowers and spices against *Candida albicans*

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60 pus samples collected from Coimbatore Medical College (CMC) were processed for the isolation of *Candida albicans*, among which 24 (40%) were found to be positive. Anticandidal susceptibility pattern revealed that the strains were highly sensitive to fluconazole, ketoconazole; moderately sensitive to clotrimazole and but resistant to nystatin. When the same strains were evaluated for their susceptibility towards aqueous and diethyl ether herbal extracts from *Allium sativum, Punica granatum, Syzium aromaticum* and *Cassia auriculata* the results were interesting that they are highly sensitive. The maximum inhibition was found to be 49 mm with *Punica granatum* followed by 29mm with *Allium sativum*. The GC-MS analysis of both the extracts revealed the bioactive principles responsible for anticandidal activity.

Key words : *Allium sativum, Punica granatum, Syzium aromaticum, Cassia auriculata, Candida albicans*.

INTRODUCTION

There is an exigent need for the development of indigenous alternative antimicrobial molecules for the effective treatment of some serious diseases in the light of growing cases of microbial resistance to the time-honored antibiotics (Anand *et al*., 2008). Plants having medicinal property have been a major source of therapeutic agents for alleviation or complete cure of much human disease since times immemorial.

Although hundreds of plant species have been tested for antimicrobial activity, the vast majority has not yet been adequately evaluated (Cox and Balick, 1994). Plants are rich in a wide variety of secondary metabolites, such as tannins, alkaloids and flavonoids, which have been found *in vitro* to have antimicrobial properties.

Candidiasis is the commonest fungal disease found in human affecting mucosa, skin, nails and internal organs of the body. This is caused by several species of yeast like fungi belonging to genus Candida with *Candida albicans* as the representative species (Navarathna *et al.*, 2005).Plant produced compounds are of interest as a source of safer or more effective substitutes for synthetically produced antimicrobial agents (Cowan,1999). *Cassia* spp. finds number of application in controlling skin infections. The antimicrobial activity of *Cassia auriculata* L. has been reported against *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae* and *Proteus vulgaris* (Duraipandiyan and Ignacimuthu, 2000; Sivakumar *et al*., 2005).

Pomegranate (*Punica granatum*) has long been used as a natural intestinal parasite killer.Its pathogen fighting abilities have now begun permeate the medicinal literature. Pomegranates can inhibit numerous strains of bacteria and fungi. Garlic (*Allium sativum*) is one of the oldest, traditional and most widely used herbs through out the world. It is used as medicine and considered as antihyperlipidemic, antithrombotic, antibiotic, antiviral, antifungal and antihypertensive etc. Cloves are most famous for its antiinflammatory activities. Since previous reports highlights the antibacterial activity of these extracts, currently analyzed for the anticandidal activity.

MATERIALS AND METHODS

Collection of sample:

Vaginal and pus samples were collected in a sterile container from CMC and processed in Microbiological Laboratory to identify the etiological agents.

Culturing of Candida albicans:

The sample was microscopically examined and cultured on Mueller Hinton agar, Blood agar and Candida medium, then incubated at 37°C for 24 hours. Microscopic and macroscopic examination of budding yeast cell, germ tube, sugar fermentation, creamy colonies on Sabouraud's

Dextrose Agar confirmed the presence of C. albicans in the clinical sample.

Collection and processing of plants:

The flower of *Cassia auriculata*, the pericarp of *Punica granatum*, Allivum sativum, *Syzium aromaticum* were procured in and around Coimbatore. It was washed several times with distilled water and dried under shadow for 5 days, then made into coarse powder using homogenizer.

Extraction of bioactive compounds (Korem et al., 2005):

The extract was obtained using diethyl ether with soxhlet apparatus and then evaluated for its anticandidal property by Well Diffusion Method. Aqueous extract of the samples were obtained using cold percolation.

Antimicrobial assay (Adwan and Mhanna, 2008):

Various concentrations of extracts ranges from 100-200µl per well were used to assess the anticandidal activity. The potency of herbal extract was compared with standard antibiotics using Mueller Hinton Agar to assay the sensitivity pattern of *Candida albicans*.

Gas Chromatography-Mass Spectrophotometry:

To identify the bioactive principles responsible for candidal inhibition, GC-MS was performed.

RESULTS AND DISCUSSION

Candida albicans was reported to cause superficial and systemic infection and is the most common isolate in human disease (Buchheidt *et al.*, 2000). It causes disease in chickens too (Kuttin *et al.*, 1976). An increase in the prevalence of multidrug resistant fungus and diminish of drug availability makes the necessity to discover new antifungal compounds. This has led to a search for therapeutic alternatives particularly among medicinal plants and compounds isolated from them used for their empirically antifungal properties. In these natural sources, a series of molecules with antifungal activity have been found which are of great importance to humans and plants.

Antifungal drug resistance has been studied most extensively with the yeast *Candida albicans* (Cowen *et al.*, 2002). Since candidal species tend to develop resistance towards the time honored antifungal drugs there occur a genuine need to seek alternatives in the form of herbal medicine. Anticandidal activity of aqueous and solvent herbal extracts was shown in Figures (Fig. 1, 2, 3 and 4). A comparative analysis of herbal extracts and antibiotics spotlight the anticandidal potency of herbal extracts (Fig. 5).

Amphoterincin B and fluconazole are used to treat Spondylodiscitis caused by *Candida albicans*. Fluconazole at a concentration of 200 to 400 mg/day was feasible (Lee *et al.*, 2006; Yap and Low, 1999).

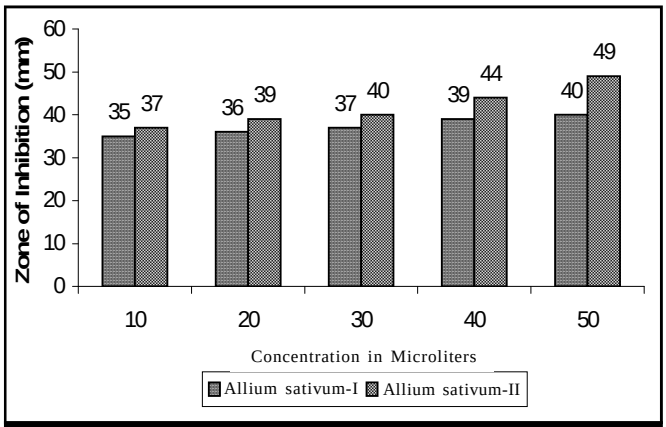


Fig. 1 : Anticandidal activity of aqueous extracts

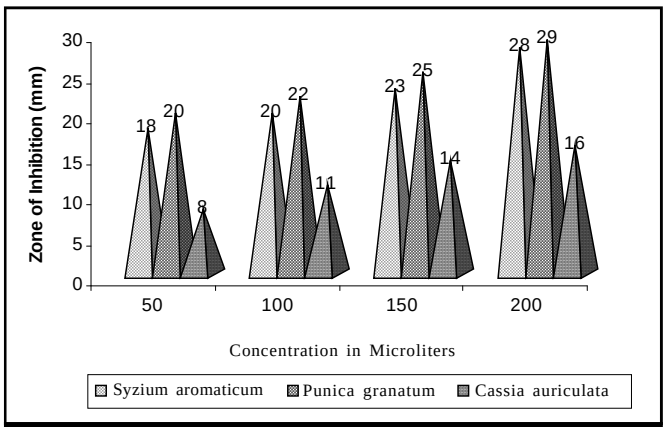


Fig. 2 : Anticandidal activity of aqueous extracts

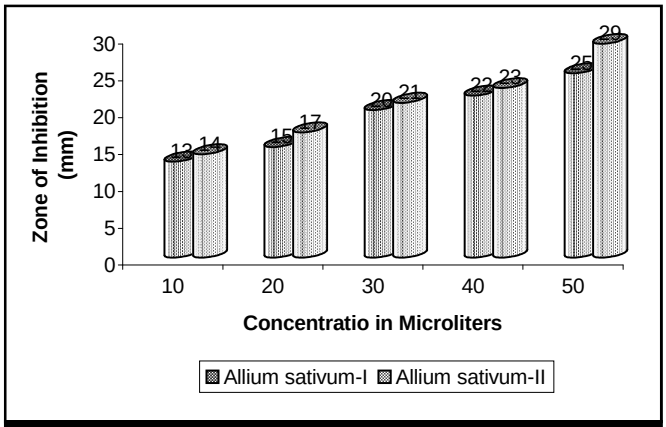


Fig. 3 : Anticandidal activity of solvent extracts

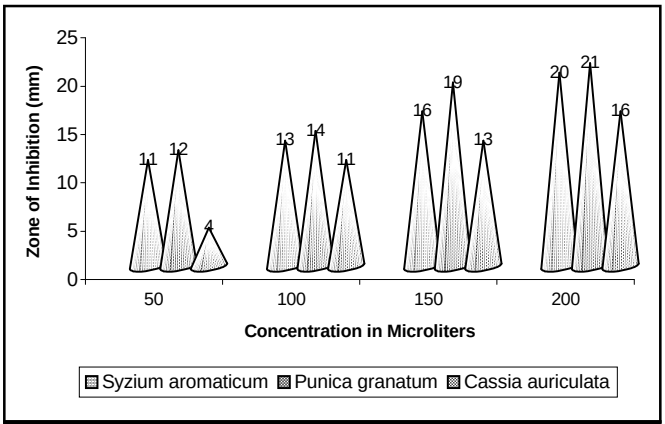
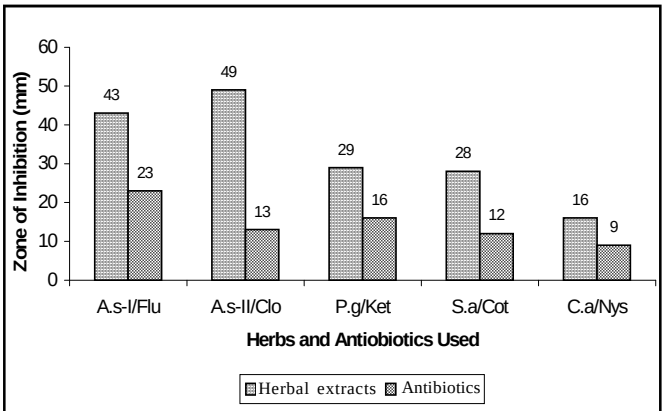


Fig. 4 : Anticandidal activity of solvent extracts



Flu – Fluconazole; Clo- Clotrimazole; Ket-Ketaconazole; Cot-Cotrimaxazole;
Nys-Nystatin; A.s-*Allium sativum*; P.g-*Punica granatum* & C.a-*Cassia auriculata*.

Fig. 5 : Comparision of herbal extracts and antibiotics

Ketoconazole was reported to produce side effects and hepatotoxicity (Stephen and Hernandez-Divers, 2005).

Fluconazole was reported to be the primary drug of choice for the treatment of *Candida albicans* (Martin, 1999). In correlation with this, the fluconazole was found to be the better drug of choice against *Candida albicans* in our study. Spikermann *et al*. (1976) suggested the use of clotrimazole as an effective antibiotic against *Candida albicans*. The present investigation showed an inhibition zone of 12mm against *Candida albicans*, a possible drug with therapeutic value.

Proline-biotin-buffer medium was recommended for the production of germ tube and germination of blastospores by *Candida albicans* (Dabrowa, 1976). Yet another media composed of cream of rice infusion, oxgall and tween 80 is described for the same purpose (Beheshti, 1975). Germ tube test form a key factor for the specific identification of *C.albicans* using animal serum.

Actinomycin A partially inhibits the growth rate of the organism (Kot *et al.*, 1976) and is not employed in our study, owing to its narrow spectrum and less antifungal activity. Carlone *et al*. (1976) reported that *C.albicans* was sensitive to nystatin, but in our investigation it is found to be resistant. *Candida albicans* was found to be sensitive to 5-flurocytosine (Stone *et al.*, 1975) Hence it could be considered as a drug of choice in future studies.

Agglutinin, precipitin and germ tube test were routinely employed for the diagnosis of *Candida albicans* from clinical sample (Oblack, 1976). Pseudohyphae produced by *Candida albicans* helps to penetrate the epidermal layer of skin and it causes cutaneous infection (Ray, 1976). The present work focused the surplus formation of germ tube, when incubated at room temperature in serum sample; possibly this germ tube, could account for the pathogenicity of the yeast.

Chromogenic media was employed by Baumgartner *et al.* (1996) for easy isolation and differentiation of *Candida* species based on pigmentation. In our investigation only Sabouraud Dextrose Agar was used. In future studies chromogenic media can be employed to aid easy identification of the organism.

Volatile oil of S.aromaticum was reported to possess antibacterial activity (Dorman and Deans, 2000). Jack *et al*. (2006) described another report in the same scenario. In our investigation aqueous extract was utilized. Future studies in this direction using oil extraction can elucidate the anticandidal activity of S. aromaticum in a better sense. Runyoro *et al.* (2006) reported that root barks of Balanites aegyptica and roots of Plectranthus barbatus were found to be active against *Candida albicans*.

Many authors reported anticandidal activity of fresh and solvent extracts of *Allium sativum* (Adetumbi *et al.*, 1986; Lemar *et al.*, 2002; Chung *et al.*, 2007). In our investigation two varieties of A.sativum were processed for its anticandidal activity, though both varieties showed better results, wild variety was found to be more promising.

GC-MS of *P.granatum* and *A.sativum* extracts revealed the presence of antimicrobial components responsible for candidal growth inhibition. The extract of A.sativum (Fig. 7) has capric acid, decanoic acid and thienyl ethanol that are found to possess antifungal activity and thus incorporated in antimicrobial pharmaceutics. Present results are parallel to early such reports (Nair *et al.*, 2005).

Similarly the extract of *P.granatum* (Fig. 6) revealed the presence of Etilefrin, 2-Azetidinone, 2-Furfuryl methanoate and Methyl hexacosanoate, which are anti-inflammatory and anti-degenerative substances forming a prime components of skin ointments. As reported in

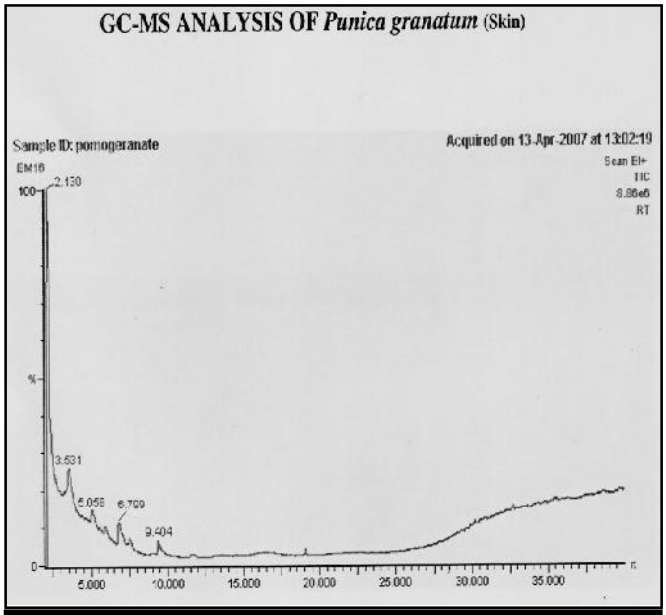


Fig. 6 : GC-MS Analysis of *Punica granatum*

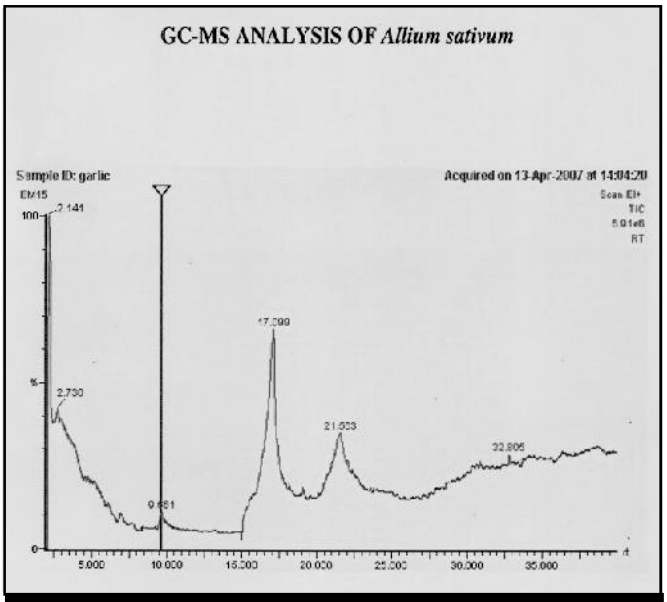


Fig. 7 : GC-MS Analysis of *Allium sativum*

earlier studies these compounds could probably involved in inhibition of cholesterol biosynthesis.

Rare medicinal plants hither to unidentified can also be brought to testing and analysis, which can form an interesting area of research in a country like India, which is nature's repository of valuable herbs (Anand *et al*., 2007). The study reveals that *P.granatum* and *A.sativum* were found to possess better anticandidal activity; hence these compounds could be formulated into suitable ointment and drugs for use as therapeutic against candidiasis, after suitable animal model experiments.

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