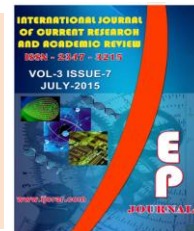




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Anti-Candidal Activity of Leaf Extracts of Certain Medicinal Plants from Tamil Nadu, India

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KEYWORDS

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A B S T R A C T

Ethanol, acetone and benzene leaf extracts of the medicinal plants, *Euphorbia heterophylla*, *Tamilnadia uliginosa*, and *Capparis sepiaria* have been tested for anti-fungal activity against *Candida albicans* using broth dilution technique. Clotrimazole was used as positive control. The minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) of the different extracts ranged between 800-1400 µg/ml and 1500-2000 µg/ml respectively, whereas clotrimazole showed 10 µg/ml MIC and 30 µg/ml MFC. Among the different extracts tested, ethanolic extract of *Tamilnadia uliginosa* leaves showed higher activity. A least MIC of 800 µg/ml was observed in ethanolic leaf extract followed by 900 µg/ml in benzene leaf extract of *Tamilnadia uliginosa*.

Introduction

Medicinal plants are vital for the treatment of many diseases including fungal-borne diseases. There are plenty of reports on different medicinal plant extracts showing anti-fungal, more specifically anti-candidal activity are available through published works.

In the present study, three different medicinal plants, *Euphorbia heterophylla* L. (Euphorbiaceae), *Tamilnadia uliginosa*

(Retz.) Tirveng. & Sastre (Rubiaceae) and *Capparis sepiaria* L. (Capparidaceae) have been selected due to their medicinal utility (Chaudhari et al., 2004; Thirumalaikumaran and Reddy, 2011; Okeniyi et al., 2012; Kalpana and Prakash, 2015). The overgrowth of *Candida* species in oral candidiasis causes oral lesions and among the species, *Candida albicans* is the most common associate of many fungal infections (Williams and Lewis, 2000).

There are reports available with regard to the antifungal activity of medicinal plants against *Candida albicans*. Thirach et al. (2003) reported that the minimum inhibitory concentrations (MIC) of clove, sweet flag and eugenol against *Candida albicans* were 17.41, 28.8 and 12.16 respectively. Citral, a plant derivative showed *in vitro* antifungal potential against strains of *Candida albicans* (Leite et al., 2014). However, only sporadic reports are available for the plants selected in the present study, *Euphorbia heterophylla*, *Tamilnadia uliginosa* and *Capparis sepiaria*. Hence the study has been designed to study the anti-candidal activity of ethanol, acetone and benzene extracts of the leaves.

Materials and Methods

The leaves of the medicinal plants selected for the present study were collected from Sirumalai hills (Eastern Ghats), Dindigul, Tamil Nadu and the identification was confirmed using standard local floras (Gamble and Fischer, 1957; Matthews, 1983). The names of the plants identified were *Euphorbia heterophylla* L. (Euphorbiaceae) and *Tamilnadia uliginosa* (Retz.) Tirveng. & Sastre (Family: Rubiaceae) and *Capparis sepiaria* L. (Capparidaceae). The leaves collected were transported to the laboratory for further processing.

The cold extraction procedure was used for extracting leaves with solvents as per the procedure given below (Prakash and Karmegam, 2012; Vigneshwari et al., 2014). The leaves of the plants collected were individually washed with tap water, blotted with filter paper and spread over news paper for air drying under shade. After complete dryness, the leaves of individual plants were powdered using a mixer grinder. A known quantity of leaf powder (100 g) of each plant was taken in a 250 ml conical flask and

added with 100-200 ml of acetone, ethanol and benzene individually. The solvent-leaf powder mixtures were kept at room temperature for 48 hrs and rapidly stirred using glass rod every 8 hrs. After 48 hrs, the extract of each plant was filtered through Whatmann No.1 filter paper to exclude the leaf powder. Then each filtrate was kept in beaker on a water bath at 45°C until the solvent gets evaporated. A greasy final material (crude extract) obtained for each plant was transferred to screw cap tubes and stored under refrigerated condition till use.

The culture of the fungus, *Candida albicans* maintained in the Department of Microbiology, Kanchi Shri Krishna College, Kanchipuram was used for anti-fungal assay. The culture was revived in Sabouraud Dextrose Agar (SDA) broth and used for dilution studies. In the culture tubes with SDA containing various leaf extract concentrations (500-2500 µg/ml) and clotrimazole (10-60 µg/ml), *Candida albicans* at approximately 5×10^6 cfu/ml were inoculated. The susceptibility of the fungus was expressed as minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) by adopting the method of Hindler (2000).

Results and Discussion

The MIC and MFC of clotrimazole, 10 and 30 µg/ml was observed against *Candida albicans* respectively. Among the different extracts used, the lowest MIC of 800 µg/ml was shown by ethanolic extracts *Tamilnadia uliginosa* leaves followed the benzene extract of the same plant (Table 1), and the next lowest MIC was recorded again in acetone extract of *Tamilnadia uliginosa* leaves (1000 µg/ml). The MIC values of 1400 µg/ml were found in *Euphorbia heterophylla* and *Capparis sepiaria* leaf extracts against *Candida albicans*. In all the

solvent extracts, *Tamilnadia uliginosa* showed lowest MIC values.

The MFC values of leaf extracts of selected medicinal plant showed a range of 1200-2000 µg/ml where the lowest value was recorded for ethanolic leaf extract of *Tamilnadia uliginosa* and the higher was recorded for ethanolic leaf extract and benzene leaf extract of *Euphorbia heterophylla*. The fungicidal activity as measured in MFC was 1200 µg/ml in ethanol extract of *Tamilnadia uliginosa* followed by 1400 and 1500 µg/ml of acetone and benzene extracts of *Tamilnadia uliginosa* leaves respectively (Table 2). The comparison of MIC and MFC activities is

given in Fig. 1, which clearly indicates the parallel MIC and MFC activities of plant extracts.

Many studies indicated that the plant extracts are possessing antifungal activity against a range of fungal organisms. Satish et al. (2007) studied fifty-two plants including aqueous extract of *Acacia nilotica*, *Achras zapota*, *Datura stramonium*, *Emblica officinalis*, *Eucalyptus globules*, *Lawsonia inermis*, *Mimusops elengi*, *Peltophorum pterocarpum*, *Polyalthia longifolia*, *Prosopis juliflora*, *Punica granatum* and *Syngium cumini* have recorded significant antifungal activity against one or the other *Aspergillus* species tested.

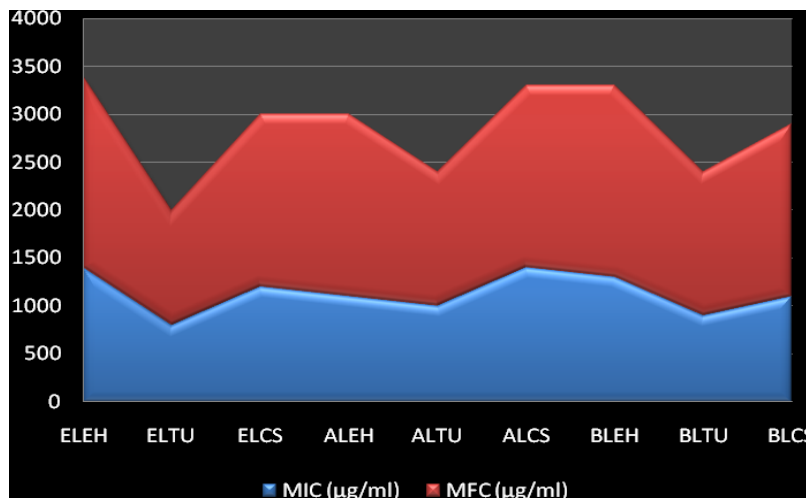
Table.1 Minimum Inhibitory Concentration (MIC) in Ethanol, Acetone and Benzene Extracts of the Leaves of Selective Medicinal Plants

Extract used		MIC (µg/ml)
Ethanolic leaf extract	<i>Euphorbia heterophylla</i> L.	1400
	<i>Tamilnadia uliginosa</i> (Retz.) Tirveng. & Sastre	800
	<i>Capparis sepiaria</i> L.	1200
Acetone leaf extract	<i>Euphorbia heterophylla</i> L.	1100
	<i>Tamilnadia uliginosa</i> (Retz.) Tirveng. & Sastre	1000
	<i>Capparis sepiaria</i> L.	1400
Benzene leaf extract	<i>Euphorbia heterophylla</i> L.	1300
	<i>Tamilnadia uliginosa</i> (Retz.) Tirveng. & Sastre	900
	<i>Capparis sepiaria</i> L.	1100

Table.2 Minimum Fungicidal Concentration (MFC) in Ethanol, Acetone and Benzene Extracts of the Leaves of Selective Medicinal Plants

Extract used		MFC (µg/ml)
Ethanolic leaf extract	<i>Euphorbia heterophylla</i> L.	2000
	<i>Tamilnadia uliginosa</i> (Retz.) Tirveng. & Sastre	1200
	<i>Capparis sepiaria</i> L.	1800
Acetone leaf extract	<i>Euphorbia heterophylla</i> L.	1900
	<i>Tamilnadia uliginosa</i> (Retz.) Tirveng. & Sastre	1400
	<i>Capparis sepiaria</i> L.	1900
Benzene leaf extract	<i>Euphorbia heterophylla</i> L.	2000
	<i>Tamilnadia uliginosa</i> (Retz.) Tirveng. & Sastre	1500
	<i>Capparis sepiaria</i> L.	1800

Fig.1 Comparison of MIC and MFC Recorded for Different Solvent Extracts of Selected Plant Leaves against *Candida Albicans*. Ethanolic Leaf Extracts of *Euphorbia Heterophylla* (ELEH), *Tamilnadia uliginosa* (ELTU) and *Capparis Sepiaria* (ELCS); Acetone Leaf Extracts of *Euphorbia Heterophylla* (ALEH), *Tamilnadia Uliginosa* (ALTU) and *Capparis Sepiaria* (ALCS); Benzene Leaf Extracts of *Euphorbia Heterophylla* (BLEH), *Tamilnadia Uliginosa* (BLTU) and *Capparis Sepiaria* (BLCS)



The results of the study conducted by Thirach et al. (2003) showed that *Candida albicans* was significantly ($p < 0.01$) more susceptible to the extract of clove than sweet flag, whereas *Cryptococcus neoformans* was significantly susceptible to the clove extract ($p > 0.05$); the extract of clove showed significantly ($p < 0.01$) more potent inhibitory activity against *Cryptococcus neoformans* than eugenol, whilst it showed significantly ($p < 0.01$) less inhibitory activity against *Candida albicans* than eugenol. Another study by Mathur et al. (2011) revealed that the hydroalcoholic extracts of *Andrographis paniculata* and *Achyranthes aspera* showed maximum potency against *Aspergillus niger* and *Candida albicans* with the MIC values of 0.5 and 0.3 mg/ml respectively. Kalpana and Prakash (2015) reported that ethanolic fruit extracts of *Capparis sepiaria* showed higher antibacterial activity than ethanolic leaf extracts and the results showed that in 1000 ppm leaf extract, a maximum of 2.1 cm ZI was observed against *Bacillus subtilis*

followed by 2.0 against *Enterococcus faecalis*. The maximum ZI of 2.4 cm was recorded in 1000 ppm ethanolic fruit extract against *Pseudomonas aeruginosa* followed by 2.3 cm and 2.1 cm respectively against *Escherichia coli* and *Enterococcus faecalis*. This might be due to the presence of various phytochemical components present in the plant parts (Chaudhari et al., 2004). Our findings also fall in the line of the works cited above. In the present study also the antifungal activity of *Euphorbia heterophylla*, *Tamilnadia uliginosa*, and *Capparis sepiaria* extracts measured in terms of MIC and MFC showed quantifiable activity against *Candida albicans*. Hence these plant extracts could be used against *Candida albicans* after thorough screening and application protocols.

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