

## Antifungal activity of *Lantana camara* L. and *Syzygium aromaticum* L. against *Candida albicans*

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### Abstract

*Candida* is a commensal organism found in 40-80% of normal humans. Candidiasis is observed in immunocompromised individuals such as HIV-positive patients, it may also occur in blood and genital tract. The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases in immunocompromised, AIDS and cancer patients. The *in vitro* antimicrobial activity was performed by Agar disc diffusion and Agar well diffusion method. The crude aqueous fractions and Methanolic extracts of leaves of *Lantana camara* and buds of *Syzygium aromaticum* were investigated for their antifungal activity against yeast *Candida albicans*. Methanolic extract of *Syzygium* was found to be effective against *Candida* while aqueous extract showed no activity. Both aqueous and methanolic extract of *Lantana camara* showed no antifungal activity. These observations could be the basis for the usefulness of the buds of *Syzygium* in treatment remedies for candidiasis or microbial infections.

**Keywords:-** *Candida albicans*, *Syzygium aromaticum*, *Lantana camara*, Antifungal activity

### Introduction

Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people everyday. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world (Pidcock and Wise, 1989; Singh *et al.*, 1992; Mulligen *et al.*, 1993; Davis, 1994; Robin *et al.*, 1998). However, the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics. The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases in immunocompromised, AIDS and cancer patients (Rinaldi, 1991; Diamond, 1993). In the present scenario, emergence of multiple drug resistance to human pathogenic organisms, has necessitated a search for new antimicrobial

substances from other sources including plants. The increasing resistance to antifungal compounds and the reduced number of available drugs led us to search therapeutic alternatives among aromatic plants and their essential oils, empirically used by antifungal properties (Fontenelle *et al.*, 2007). The use of traditional medicine for the treatment of various diseases has been practiced for generation and a large number of populations in the country use traditional medicines for day to day healthcare needs. (Moshi *et al.*, 2006; Hamza *et al.*, 2006). Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as microbes, plants and animals. (Janovska *et al.*, 2003). Fungi are opportunistic organisms, which are ubiquitous in nature. Prolonged antibiotic therapy, invasive therapeutic procedures, immunosuppressive therapies and AIDS have all contributed to the rise in systemic fungal infections. (Advani *et al.*, 1992). Ayurveda is the system of traditional medicine prevalent in India since 2000 B.C. Herbal plants from nature

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provide rational means for the treatment of many diseases, which are considered to be obstinate and incurable in other systems of medicine. It is a form of treatment by natural remedies, which makes use of the power of nature to restore human beings to a state of balance. Genuine and pure powdered medicinal herbs of Indian origin are used in herbal medicines, herbal extracts, herbal cosmetics and nutrition foods and other alternative medicines and herbal remedies for natural healings (Heinerman, 1996).

Plants that are traditionally used in treatment of bacterial and fungal infections or related ailments could be a good source for new, safe and biodegradable antimicrobial drugs (Hamza *et al.*, 2006) and could offer potential lead towards

development of novel compounds that are active against pathogenic microbes (Runyoro *et al.*, 2006). In the present communication, an attempt has been made to explore antimycotic principles, which involves an investigation on the efficacy of essential oil against *Candida*. Ethnobotanical data is given in Table 1.

## Materials and Method

### Test Organisms

The strain of *Candida albicans* ATCC 0231 was obtained from American type culture Collection from IMTECH, Chandigarh and was biochemically and serologically characterized by standard methods. The culture was maintained at  $4 \pm 1^\circ\text{C}$  on slants, plates of *Candida* Agar and corn meal agar (HiMedia) and overnight grown culture in sterile glycerol (15%).

**Table 1: Ethnobotanical data of the plants used in the present study**

Botanical name	Family	Common name	Part used	Traditional uses (Chopra <i>et al.</i> , 1992)
<i>Lantana camara</i> L.	Verbenaceae	Ghaneri	Leaves	Decoction used for sore throat
<i>Syzygium aromaticum</i> L.	Myrtaceae	Laung	Bud	Stimulant, carminative used in dyspepsy

### Plant Materials

Leaves of *Lantana camara* were collected from Garhwal region and bud of *Syzygium aromaticum* was procured from local market of Dehradun.

### Preparation of Plant Extracts

Plant extracts were prepared by the methods of Alade and Irobi (1993) with minor modification. Both aqueous as well as alcoholic extracts were used to check the antifungal activity. The leaves of *L. camara* and bud of *Syzygium aromaticum* were air dried, ground and soaked in water for aqueous extract and in methanol for alcoholic extract for 72 hrs with continuous shaking and homogenized using high speed homogeniser (REMI RQ 127 A/D). It was filtered (Whatman No. 1) and extracts were obtained using

soxhlet assembly. The extracts were concentrated in check at  $30^\circ\text{C}$  and stored at  $4^\circ\text{C}$  for further use.

### Inoculum preparation

For the preparation of inoculum, the tested fungus was cultured in mycological broth (HiMedia) at  $37^\circ\text{C}$  for 24hrs and standardized for the same absorbency, number 0.5 of the McFarland Standard, which corresponds to the order of  $10^8$  CFU/ml (Barry and Thornsberry, 1985).

### Antifungal susceptibility testing

The antifungal assay was performed by two methods viz. agar disc diffusion method (Bauer *et al.*, 1986) for aqueous extract and agar well diffusion method (Perez *et al.*, 1990) for solvent extract. The





molten PDA (at 45°C) was inoculated with 100 µl of the inoculum ( $10^8$ cfu/ml) and poured into petri dishes. The plates were allowed to gel for an hour. 50 mg of both extracts were dissolved in 100 µl of sterile ethanol (15%) separately and sterilized by passing through 0.45 µm millipore syringe filter. For agar disc diffusion method, the sterile disc (0.7 cm) Hi-Media, was saturated with 100 µl of both extract, allowed to dry and was introduced on the upper layer of the seeded agar plates separately. Sterile paper disc treated with 15% of sterile ethanol was used as control. For agar well diffusion method, a well was prepared in the plates with the help of a cork-borer (0.85cm). 100 µl of the both extracts was introduced into the well in different plates. The plates were incubated overnight at 37 °C. Microbial growth was determined by measuring the diameter of zone of inhibition (ZOI). Pure solvents instead of extract were used as Control. The result was obtained by measuring zone diameter. The experiment was done three times and the mean values were taken.

#### Minimum inhibitory concentration

MIC method using broth dilution method was applied on extracts that proved their high efficacy against test microorganism by disc diffusion method and agar well diffusion method. The dried plant extract was dissolved in 15% ethanol and serially diluted to fungal broth in order to observe their activities at low concentration. *Syzygium* extract was evaluated against *Candida* by dilution of stock to various concentrations from 100 mg/ml to 6.25 mg/ml. Equal volume of stock and fungal broth was mixed in a test tube. Specifically 0.1ml of standardized inoculum ( $10^8$  CFU/ml) was added to each tube. The tubes were incubated aerobically at 37°C for 18-24 h. Two control tubes were maintained for each batch as antibiotic control (tube containing extract and the growth medium without inoculum) and organism control (the tube containing the growth medium, physiological saline and the inoculum). The lowest concentration (highest

dilution) of the extract that produced no visible fungal growth (no turbidity) when compared with the control tubes were regarded as MIC. However, MFC was determined by sub -culturing the test dilution on to a fresh drug-free medium and incubated further for 18-24 h. It is defined as the highest dilution that yielded no single fungal colony.

#### Results and Discussion

The antifungal activity of the extracts and their potency was quantitatively assessed by the presence or absence of inhibition zone and zone diameter respectively as shown in Table 2. Alcoholic extract was found to be a better solvent for extraction of antimicrobial active substances compared to water and hexane as also shown by Ahmad *et al.* (1998). The antifungal activity of the extracts and their potency was quantitatively assessed by the presence or absence of inhibition zone and zone diameter, respectively as shown in Table 3. The results showed that the growth of *C. albicans* was inhibited by *S. aromaticum*. Methanolic extract was found effective against *Candida* and displayed potent antifungal activity but *Candida* was not inhibited by aqueous extract (Fig. 1 and 2). Zone of inhibition of *Syzygium* against *Candida* was 26 mm obtained after 24 hrs of incubation. MIC was observed at 6.25 mg/ml and MFC was observed at 12.50 mg/ml (Table 4). *Syzygium* extract evaluated against *Candida* by serial two fold dilution of stock concentration (100mg/ml) showed inhibition upto 6.25 mg/ml (Fig. 3). *Syzygium* was more potent and maximum zone of inhibition was observed. Aqueous and methanolic extract of *L. camara* showed no activity, confirming that *Candida* is resistant to this extract. A drug resistant strain of *C. albicans* was found to be sensitive to the tested plant extract. This indicated that antibiotic resistance does not interfere with antifungal action of plant extract. Similar reports on anticandidal activities of these



**Table 2- Antifungal Activity of *Lantana camara* and *Syzygium aromaticum* Zone of Inhibition (mm)**

	<i>Lantana camara</i>		<i>Syzygium aromaticum</i>	
	Aqueous	Methanolic	Aqueous	Methanolic
<i>C. albicans</i>	NI	NI	NI	25

NI: No Inhibition

**Table 3: Antifungal Activity of *Syzygium aromaticum* Zone of Inhibition (mm)**

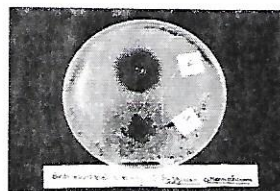
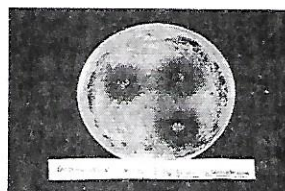
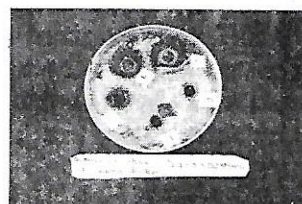
Stock (100 mg/ml)	50.00 mg/ml	25.00 mg/ml	12.50 mg/ml	6.25 mg/ml
25.00	19.00	13.00	10.00	9.00

Note: ZOI in mm; &lt; 10mm- Low activity; 10-20mm- Moderate activity; &gt;20mm- High activity

**Table 4: Minimum Inhibitory concentration (MIC) and Minimum Fungicidal Concentration (MFC) (*Syzygium aromaticum*)**

Organism	MIC (mg/ml)	MFC (mg/ml)
<i>C. albicans</i>	6.25	12.50

plants were also reported by Ahmad *et al.*, 2001 with varying degrees of potency. The difference in potency may be due to the stage of collection of the plant sample, different sensitivity of the test strains and method of extraction (Nimri *et al.*, 1999). Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers or practitioners make use of water primarily as a solvent, but our studies showed that

**Fig.1: Antifungal activity of *Syzygium aromaticum* against *C. albicans***  
A: Methanolic extract B: Aqueous extract**Fig.2: Antifungal activity of *Syzygium aromaticum* (methanolic) against *C. albicans* showing zone of inhibition****Fig.3: Antifungal activity of *Syzygium aromaticum* in various dilutions against *C. albicans***

Well A: 100mg/ml; Well B: 50mg/ml; Well C: 25mg/ml; Well D: 12.5mg/ml; Well E: 6.25mg/ml

methanolic extracts of these plants were certainly much better and powerful. This may be due to the better solubility of the active components in the organic solvent (De Boer *et al.*, 2005). These observations can be rationalized in terms of polarity of the compounds being extracted by each solvent and in addition to their intrinsic bioactivity, by their ability to dissolve or diffuse in the different media used in the assay. The growth media also seem to play an important role in the determination of the antibacterial activity. (Lin *et al.*, 1999).

The results of the present study supports the traditional usage of the studied plants and suggests that some of the plant extracts possess compounds with antifungal properties that can be used as antifungal agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic antimicrobials and carry out further pharmacological evaluation.



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