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In vitro Anticandidal Activity of *Cinnamomum verum*

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In recent years there has been an increasing interest in the use of natural substances. The medicinal properties of *Cinnamomum verum*, *Ferula asafoetida*, *Azadirachtin*, *Curcuma longa* and *Myristica fragrans* were known to mankind since antiquity. Therefore we have aimed at demonstrating the *in vitro* activity of these herbs at various concentrations against *Candida* species isolated from different clinical samples. A total of 57 isolates of *Candida* recovered from clinical sources, viz. sputum, blood, urine, pus, plastic devices (endotracheal tube), were studied in detail. Eight different species of *Candida* were identified. *Candida krusei* was the commonest species isolated and the *Candida* (non-albicans) species with 60.36% showed the major prevalence. The isolates included *C. krusei* (38.59%), *C. albicans* (33.33%), *C. parapsilosis* (8.7%), *C. guilliermondii* (1.75%), *C. glabrata* (7.09%), *C. tropicalis* (5.26%) and *C. pseudotropicalis* (5.2%). The data indicates that, Cinnamon was found to possess the strongest anticandidal property at concentrations 25 and 50 mg mL^{-1} using well diffusion method. This study provides practitioners with insight into the claims of anticandidal effect of these natural substances.

Key words: Candidaemia, cinnamon, anticandidal activity, well diffusion, clinical isolates

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INTRODUCTION

In recent years there has been an increasing interest in the use of natural substances. Essential oils, odorous and volatile products of plant secondary metabolism have a wide application in folk medicine, food flavouring and preservation as well as in fragrance industries. The antimicrobial properties of essential oils have been known for many centuries. The present study was undertaken to find out the anticandidal activity of *Cinnamomum verum*, *Ferula asafoetida*, *Azadirachtin*, *Curcuma longa* and *Myristica fragrans*.

The name cinnamon is derived from a Greek word, meaning sweet wood. It is derived from the inner bark of the cinnamon tree. There are two main varieties of *Cinnamon-Cinnamomum verum* (True or Ceylon *Cinnamon*) grown in Sri Lanka and Southern India and *Cinnamomum aromaticum* (also called Cassia), which are grown in China, Indonesia and Vietnam.

Cinnamon is used, as a flavoring agent in soft drinks, teas and bakery products *Cinnamon* is also a common ingredient in many Indian curries. Historically, *Cinnamon* was an ingredient of medicines for sore throat, cough, indigestion, abdominal cramps, intestinal spasms, nausea, flatulence. Was also an ingredient in appetite stimulants and anti-diarrhoeal medication. Its mild anti-inflammatory, anti-spasmodic and anti-clotting properties are believed to be due to its content of cinnamaldehyde. *Cinnamon* extracts are active against *Candida albicans*, the fungus responsible for vaginal infection and also against *Helicobacter pylori*; the bacterium responsible for gastric ulcers. The antimicrobial properties of *Cinnamon* are thought to be due to eugenol and a derivative of cinnamaldehyde. *Cinnamon* is also useful as a food preservative to inhibit the growth of common food-borne bacteria such as *Salmonella* and *Escherichia coli*. (Matan *et al*., 2006; Anupama *et al*., 2005; Ooi *et al*., 2006; Kalemba and Kunicka, 2003; Ahmad *et al*., 2005). *Cinnamon* has been granted. GRAS (generally recognized as safe) status as a food additive by the FDA.

Ferula asafoetida is a native of Iran. Asafoetida resin is used as spice. Asafoetida has certain medicinal uses and most commonly is used as a digestive aid. It is reputed to lessen flatulence and is often added to lentil or eggplant dishes in small quantities. It is also said to be helpful in cases of asthma and bronchitis It is used as a traditional remedy for cold in children. It is also used in medicines because of its antibiotic properties.

Azadirachta indica (neem) consists of 3 or 4 related compounds. One of the compound is *Azadirachtin*. *Azadirachtin* seems to be an ecdysone blocker. It blocks the production and release of these vital hormones in insects. Neem, as a vaginal contraceptive, inhibits the spread of micro-organisms including *Candida albicans*, *C. tropicalis*, *Neisseria gonorrhoeae*, *herpes* simplex-2 and HIV-1, as well as resistant strains of *E. coli* and *Staphylococcus aureus*, in part by boosting immune-system activity in the vagina. Neem has anti-inflammatory and antiseptic activity. *Myristica fragrans* (nutmeg), historically has been known to benefit circulation, muscles, joints, prevent nausea and help in fighting bacterial infection. It is also used as an anti-diarrhoeal agent. *Curcuma longa* is a member of the ginger family, Ziniberacee. It is commonly known as turmeric. The curcuminoids have demonstrated antiviral, anti-fungal, antibacterial, anticarcinogenic activity, anti-inflammatory activity and immunomodulating effects, mostly *in vitro*.

Candida infection starts as a local lesion on mucous membranes and disseminates later. An increase in immunosuppressive diseases such as AIDS, prolonged antibiotics therapy, immunosuppressive drug therapy, steroid therapy and increased usage of invasive procedures such as cardiac catheterization, are reflected in the increasing number of severe *Candida* infections (Pfaller, 1996; Arunaloke *et al*., 1999). The disease may be cutaneous, mucocutaneous, subcutaneous or systemic in nature. In immunosuppressed patients, *Candida* species have the potential to invade all host organs and cause severe systemic infection. Although *C. albicans* is the organism most often associated with serious fungal infections, other *Candida* species have also emerged as clinically important pathogens of opportunistic origin.

MATERIALS AND METHODS

Clinical samples were collected from hospitals and nursing homes, in and around Bangalore. The clinical samples that were collected included-9 from sputum, 27 from blood, 12 from urine, 4 samples from plastic devices (endotracheal tubes) and 5 from pus. All the respiratory specimens and exudates were examined in 10% KOH. In addition, the smears were Gram stained and examined. The samples were inoculated on to Sabouraud's dextrose agar as the main isolation medium. For blood samples, SDA biphasic medium with chloramphenicol and gentamicin was used. The culture medium was incubated at 37°C for a week or longer if required. The identification of the species was conducted by assessing the germ tube formation, chlamydospore formation, sugar fermentation and assimilation patterns. The comparison study was

done using the standard strains provided by PGIMER Chandigarh. The isolates include *C. albicans*, *C. tropicalis*, *C. krusei*, *C. glabrata*, *C. parapsilosis*, *C. guilliermondii* and *C. stellatoidea*. For checking the anticandidal activity of these herbs, their aqueous extracts were prepared. *Candida* species suspensions were inoculated into sabouraud's dextrose broth and incubated at 37°C for 24 h. Lawn culture of the isolates was done on Sabouraud's dextrose agar plates. Wells were punched using a sterile borer.

These wells were filled with 100 μL of the aqueous extracts. Two different concentrations (25 and 50 mg mL^{-1}) were used for the study. Dimethyle sulphoxide was run simultaneously. The antifungal agent Amphotericin B disc was used as positive control. These plates were incubated at 37°C for 24 h. The diameter of zones of inhibition was measured in millimeters (Geeta *et al*., 2001).

RESULTS AND DISCUSSION

The species spectrum of the isolate was as follows: of the 57 isolates, 22 were *C. krusei*, 19 *C. albicans*, 4 *C. glabrata*, 4 *C. parapsilosis*, 3 *C. tropicalis*, 3 *C. stellatoidea* and 1 was *C. guilliermondii*. *Candida* species distributions in different clinical samples are as shown in Table 1. Both concentrations of *Cinnamomum verum* were equally effective in inhibiting the growth of various species of *Candida*. *C. albicans* from urine and sputum were more sensitive to *Cinnamomum verum* than *C. albicans* from other sources. *C. tropicalis* and *C. glabrata* from blood samples were more sensitive. *C. stellatoidea* isolated from plastic devices also showed more sensitivity than any other sample. The zones of inhibition for *Cinnamomum verum* were as shown in Table 2. Higher concentrations showed better inhibitory zone. Ferula asafetida, *Azadirachtin*, *Curcuma longa* and *Myristica fragrans* did not show anticandidal activity at concentrations of 25 and 50 mg mL^{-1}.

Table 1: *Candida* species isolated from different clinical samples

Source of clinical isolates	Blood	Urine	Sputum	Plastic device	Pus	Total
Positive isolates	27	12	9	4	5	57
C. albicans	7	2	6	0	4	19
C. krusei	9	9	2	2	0	22
C. tropicalis	2	1	0	0	0	3
C. parapsilosis	4	0	0	1	0	5
C. guilliermondii	1	0	0	0	0	1
C. glabrata	4	0	0	0	0	4
C. stellatoidea	0	0	1	1	1	3

Candida species are yeast like fungi that can form true hyphae and pseudohyphae. *Candida* species are normal inhabitants of the skin and mucosa. Some alteration of the hosts' cellular defenses and physiology or alteration in normal flora must occur before colonization, infection and disease production by *Candida* (Odds, 1988). The clinical presentation can vary depending on the type of infection and the degree of immunosuppression. The importance of epidemiological monitoring of yeasts involved in pathologic processes is unquestionable due to the increase of these infections over the last decade, so are the changes observed in species causing candidiasis and empirical antifungal treatment (Anaissie *et al*., 2003).

The present study shows that the predominant species isolated were non-*Candida albicans* (Mujica *et al*., 2004). The most common isolate was *C. krusei*. Most catheters related septicemias are caused by micro organisms that invade the intracutaneous wound during catheter insertion or there after (Matsumato *et al*., 2001; D'Antonio *et al*., 2002). The proportion of such infection due to non-*Candida albicans* species is persistently rising. Urine samples yielded *C. tropicalis* (75.0%), followed by *C. albicans* (16.66.0%). These patients had symptoms of urinary tract infection. The isolates from pus and plastic device were predominantly *Candida* non-albicans.

Table 2: Effect of *Cinnamomum verum* on *Candida* species isolated from various clinical samples at two different concentrations

	C. albicans		*C. krusei*		*C. tropicalis*		*C. parapsilosis*		*C. glabrata*		*C. guilliermondii*		*C. stellatoidea*	
Source of clinical isolate	25	50	25	50	25	50	25	50	25	50	25	50	25	50
	mg													
Blood	++	+++	++	++	+++	+++	++	+++	+++	++++	++	+++	-	-
Urine	+++	++++	+	++	+	+	-	-	-	-	-	-	-	-
Sputum	+++	+++	++	++	-	-	-	-	-	-	-	-	+	+++
Pus	++	+++	-	-	-	-	-	-	-	-	-	-	++	++++
Plastic device	-	-	++	+++	-	-	++	+++	-	-	-	-	+++	+++
Standard strain	++	+++	++	++	++	+++	++	+++	+++	++++	-	-	-	-
DMSO control	-	-	-	-	-	-	-	-	-	-	-	-	-	-

D = Diameter of zone of inhibition, ++++: D>26 mm in diameter, +++: 21-25 mm in diameter, ++: 16-20 mm in diameter, +: 10-15 mm in diameter, DMSO = Dimethyl sulfoxido, -: No inhibition

CONCLUSION

The data in this study indicate that *Cinnamomum verum* is active against species of *Candida* isolated from different clinical sources. There was little inter species variation in susceptibility and all *Candida* species tested were uniformly susceptible. Emerging drug resistance is an important problem faced by clinicians today in treating the patients. This study provides practitioners with an insight into the claims of natural herbs' anticandidal effect.

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