

**CASSIA ESSENTIAL OIL: EFFECTIVE ANTICANDIDAL
AND POSSIBLE THERAPEUTIC AGENT****VILAS A. KAMBLE***

*Department of Microbiology, Adarsha Science, J. B. Arts & Birla Commerce
Mahavidyalaya, Dhamangaon Rly, Di. Amravati - 444 709, M. S., India*

ABSTRACT

The antimicrobial property of volatile aromatic oils from medicinal as well as other edible plants has been recognized since antiquity. *Candida* species are an important cause of opportunistic infections in the oral cavity of immunocompromised patients and vaginal candidiasis. The antifungal activity of the essential oil of *Cinnamomum cassia* (cassia oil) was investigated against 75 clinical isolates of *Candida albicans* and non-*albicans Candida*. Disc diffusion method was used to evaluate the sensitivity profile of clinical isolates to undiluted and diluted (3:1, 2:2 and (1:3) cassia oil. Minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) were evaluated by broth microdilution and broth macrodilution method. . Cassia essential oil strongly inhibited all clinical isolates of *C. albicans* and non-*albicans Candida* with growth inhibition zones ranging from 40 to 72 mm. Cassia oil inhibited *C. albicans* growth with mean minimum inhibitory concentration (MIC) of 0.10 $\mu\text{l/ml}$ (v/v) and 0.07 $\mu\text{l/ml}$ (v/v) by broth micro dilution and broth macrodilution method, respectively. The clinical isolates of *C. albicans* required as high as 0.15 $\mu\text{l/ml}$ (v/v) concentration of cassia oil for its inhibition by both methods. The isolates of non-*albicans Candida* showed MIC range of 0.02 – 0.62 $\mu\text{l/ml}$ (v/v) by broth microdilution and broth macrodilution method.

KEYWORDS: Antiyeast activity, Medicinal plant, Essential oil, *Cinnamomum cassia*, Pathogenic yeast

**VILAS A. KAMBLE**

Department of Microbiology, Adarsha Science, J. B. Arts & Birla Commerce
Mahavidyalaya, Dhamangaon Rly, Di. Amravati - 444 709, M. S., India

*Corresponding author

1. INTRODUCTION

Around 80 % of the world's population relies on medicinal plants as their primary healthcare source. Application of medicinal plants especially in traditional medicine is currently well acknowledged. In the developing countries, drugs are not only expensive but also have many side effects during treatment for any disorders that is why now in this era it is being emphasized to search medicinally valuable plants and predict their biological activity. Scientific evidence is accumulating and many of the plants have medicinal properties that alleviate symptoms or prevent diseases¹. In present investigation interest has focused on *Cinnamomum cassia* (also known as *Laurus Cassia* and *Cinnamomum aromaticum*) which is an ancient spice used in many countries. It possess anti-inflammatory activity, antioxidant, hepatoprotective, antiulcer, antibacterial, antifungal, insecticidal, anticancer, anti-HIV, anti-diabetic, antiallergic, anti-diarrhea, anti-emetic, antispasmodic, immunomodulatory, nematocidal and skin whitening activity². It is also documented for atherosclerosis, arteriosclerosis, cataracts, colds, flu, ringworm, viral infection, influenza, fevers, arthritis and rheumatism. It contains medicinally important essential oil called cassia oil in leaves, fruits, inner and outer bark. Much of cinnamon's bioactivity resides in its oil, which is about 90% cinnamaldehyde. It is used mainly in medicine, foods and cosmetics³, and is employed in aromatherapy as a rub to promote blood circulation. It also contains both anti-fungal and anti-bacterial principles that can be used to prevent food spoilage due to bacterial contamination⁴. Essential oils are one of the most important groups of plant constituents responsible for biological activity of herbs and spices, and especially for their medicinal and antimicrobial properties. Numerous *in vitro* studies have demonstrated activity of different essential oils against bacteria, moulds and yeast⁵. The increased incidence of fungal infections in the past two decades has been overwhelming. Fungi cause both superficial and internal mycoses. Systemic infections caused by fungi constitute a major public health problem in

many parts of the world, both in developed and developing countries. *Candida* spp. is an important cause of bloodstream infections and opportunistic infections in the oral cavity of immuno-compromised patients⁶. *Candida* spp. is also the most common cause of vaginal candidiasis or thrush and approximately 80-90% of thrush cases are caused by *Candida albicans* with other species⁷. *Candida* spp. accounts for 8-15% of nosocomial blood stream infections and fourth most common isolate of patients of intensive care unit⁸. *Candida albicans* has accounted for virtually all mucosal candidiasis and responsible for about 60% of both superficial and systemic mycoses⁹. However, in recent years this picture is complicated by the emergence of non-albicans *Candida* (NAC) species such as *Candida glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* which cause serious oropharyngeal candidiasis and occasionally esophageal candidiasis^{10,11}. Antibiotic and multi-drug resistance is a world-wide problem in hospitals, long-stay residential centres and in the community. There are a limited number of antifungals available, most just providing fungistatic but not fungicidal effects. Continuous exposure to the antibiotics and synthetic chemical drugs results in the development of resistance in the organisms. Fluconazole, itraconazole, voriconazole are the primary drugs used for treatment of serious fungal infections. However, decreased susceptibility among yeast and molds to these prime antifungal drugs have been noted and prompted a search for new drugs that may be effective in the management of patients with mycoses due to a wide range of filamentous fungi and yeast pathogens^{12,13}. The increasing ineffectiveness of these drugs and unavailability of alternative antimicrobials in developing countries causing spread of major infectious diseases. This necessitated the search of new antimicrobial substances from other sources such as medicinal plants. Currently, very little information is available on its comparative antifungal activity on the growth and physiology of human pathogenic yeasts either *in vitro* or *in vivo*. There needs a high-quality studies investigating the use of

cassia for any indication. The objective of the present study was to evaluate the *in vitro* inhibitory activity of cassia oil against clinical isolates of *Candida albicans* and non-*albicans Candida*.

2. MATERIALS AND METHODS

2.1 Essential Oil

The cassia oil used in the present investigation was kindly provided by Kancor Flavours and Extracts Limited, Angamally (Kerala, India) in sealed glass bottle. The sterility of the oil was checked by inoculating a loopful of oil on potato dextrose agar and nutrient agar slants, and then assessing the growth. The essential oil was stored in the dark at 25°C when not in use. Different concentrations of cassia oil with DMSO (25%, 50% and 75%) were prepared for experiments.

2.2 Organisms

75 clinical isolates of the pathogenic yeasts including *Candida albicans* (n = 28), *C. krusei* (n = 7), *C. glabrata* (n = 9), *C. parapsilosis* (n = 7), *C. tropicalis* (n = 12), *C. pseudotropicalis* (n = 6), *C. guilliermondii* (n = 3) and *C. stellatoidea* (n = 3) were isolated from various clinical specimens such as oral swab, vaginal discharge and blood. Each isolate was originated from a different patient with clinical manifestations. Clinical isolates were identified to the species level based on morphological criteria, carbohydrate assimilation profile, and germ tube test in serum and chlamyospores production on corn meal agar¹⁴. Isolates were maintained on Sabouraud dextrose agar (Hi-Media, Mumbai) at 4°C in refrigerator until used in the study. Prior to testing, each isolate was checked for purity and viability.

2.3 Inoculum Preparation

To prepare inoculum, a small amount of growth was taken from 24 hrs old culture of the respective organism grown on SDA slant and inoculated into 5 ml of 0.85% sterile saline. The resulting inoculum density was adjusted to 0.5 McFarland standard to yield a suspension of 1×10^6 to 5×10^6 cells/ml¹⁵.

2.4 Anticandidal Sensitivity Testing by Disc Diffusion Method

Disc diffusion assay of cassia oil was performed as described by Bauer *et al*¹⁶. In short 90 mm diameter plates containing Sabouraud dextrose agar (Hi-Media, Mumbai) at a depth of 4 mm were used. The agar surface was seeded with inoculum by using a sterile swab. Sterile disc (SD 067, Hi-Media, Mumbai) of 6 mm diameter was impregnated with 20 µl of undiluted oil (100%) and diluted oil (25%, 50%, 75% in DMSO) was placed at center of seeded agar surface. The plates were then left undisturbed for 30 min to allow diffusion of the essential oil into the agar and incubated at 35 °C for 24 h. The inhibition zone was measured in millimeter and the assay was carried out three times for each isolate tested. Isolates with zone size ≥ 28 mm were classified as strongly sensitive, with a zone diameter of < 28 to 16 mm as moderately sensitive, with a zone diameter of < 16 to 12 mm as weakly sensitive and isolates with zone diameter of < 12 mm as resistant¹⁷.

2.5 MIC & MFC Studies

Minimum inhibitory concentrations (MIC) and Minimum fungicidal concentrations (MFC) of cassia oil were determined by broth microdilution and broth macrodilution method¹⁵ with some modifications.

2.5.1 Broth Microdilution Method

Stock solution of cassia oil (10 µl/ml) was prepared in sterile Sabouraud dextrose broth (Hi-Media, Mumbai). 0.15% (w/v) bacteriological agar (Hi-Media, Mumbai) was added as a stabilizer¹⁸ of the oil water mixture. Serial two fold dilutions of stock solution of essential oil was prepared over the range of 0.02 – 10 µl/ml (v/v) with a final oil concentration range 0.01 – 5.0 µl/ml (v/v) in 96-well microtitre plates. A freshly grown yeast suspension in Sabouraud dextrose broth was standardized to 1×10^6 cells/ml (0.5 McFarland standard). A working yeast inoculum suspension of 1×10^4 cells/ml was prepared by diluting the stock inoculum (1×10^6) 1:100 with sterile Sabouraud dextrose broth. Sabouraud dextrose broth containing 0.15% agar without essential oil served as growth control. 100 µl yeast suspension was

added to each well. Well containing only the Sabouraud dextrose broth with 0.15% agar without microorganism was used as sterility control. The lowest concentration of oil that inhibited visible growth after incubation at 35°C for 48 ± 2 hrs without shaking was taken as minimum inhibitory concentration (MIC). Minimum concentration of oil that inhibited 50%, 70% and 90% of the isolates tested were defined as MIC₅₀, MIC₇₀ and MIC₉₀, respectively. To determine the minimum fungicidal concentration (MFC), a loopful of broth was removed from each well and spot inoculated onto SDA plate and after incubation at 35°C for 48 hrs, the lowest concentration of oil that inhibited the complete growth was considered as MFC. The concentration of cassia oil fungicidal for 90% of the clinical isolates tested was defined as MFC₉₀.

2.5.2 Broth Macrodilution Method

A range of doubling dilutions of cassia oil from 0.02 – 10 µl/ml (v/v) with a final oil concentration range 0.01 – 5 µl/ml (v/v) was prepared in Sabouraud dextrose broth in round bottom sterile glass tubes (12 × 75 mm). Bacteriological agar was included at a concentration of 0.15% (w/v) to enhance oil solubility. A working inoculum suspension of 1 × 10⁴ cells/ml was added to each tube except sterility control. Sabouraud dextrose broth containing 0.15% agar without essential oil served as growth control. The tubes were then

incubated at 35°C for 48 ± 2 hrs without agitation and observed for the presence or absence of visible growth. The MIC was defined as the lowest concentration of oil inhibiting visible growth.

3. RESULTS

3.1 Anticandidal activity by disc diffusion

In the present study, anticandidal properties of cassia oil *in vitro* by disc diffusion method were evaluated. The mean inhibition zones (MIZ) and inhibition zone range (IZR) obtained against eight different *Candida* species is shown in Table 1. All the clinical isolates of *Candida* were strongly inhibited by undiluted cassia oil with 42 – 72 mm inhibition zone range with average inhibition zone of 62 mm. Oil of cassia was highly potent against the isolates of *Candida albicans* and *C. krusei* (MIZ = 65 mm) and was comparatively least potent to the isolates of *C. parapsilosis* (MIZ = 52 mm). Isolates of *C. tropicalis*, *C. guilliermondii*, *C. stellatoidea* and *C. pseudotropicalis* were inhibited with mean inhibition zone of 62 mm, while the isolates of *C. glabrata* exhibited a 44 – 47 mm inhibition zone range, with mean inhibition zone of 57 mm. All the clinical isolates were strongly sensitive even to the 3:1, 2:2 and 1:3 diluted cassia oil. Only one isolate of *C. glabrata* showed moderate sensitivity to 1:3 diluted cassia oil.

Table 1
Inhibition zones obtained by disc diffusion method of the cassia oil assayed against eight different *Candida* species.

Species (No. of isolates)	Inhibition Zones (in mm)							
	4:0 (100% oil)		3:1 (75% oil)		2:2 (50% oil)		1:3 (25% oil)	
	MIZ	IZR	MIZ	IZR	MIZ	IZR	MIZ	IZR
<i>Candida albicans</i> (n = 28)	65	44 – 72	58	39 – 67	53	36 – 62	46	32 – 54
<i>C. glabrata</i> (n = 9)	57	44 – 70	51	37 – 61	45	30 – 57	39	24 – 47
<i>C. tropicalis</i> (n = 12)	62	44 – 72	56	40 – 66	50	37 – 57	43	30 – 52
<i>C. parapsilosis</i> (n = 7)	52	42 – 61	49	39 – 55	45	32 – 51	37	28 – 45
<i>C. pseudotropicalis</i> (n = 6)	62	56 – 70	56	51 – 60	51	46 – 56	42	40 – 44
<i>C. krusei</i> (n = 7)	65	62 – 68	59	58 – 60	55	53 – 56	48	46 – 49
<i>C. guilliermondii</i> (n = 3)	62	60 – 64	53	52 – 54	47	46 – 48	41	40 – 42
<i>C. stellatoidea</i> (n = 3)	62	60 – 64	49	47 – 51	45	44 – 46	39	38 – 40
Total (n = 75)	62	42 – 72	55	39 – 67	49	30 – 62	43	24 – 54

MIZ, Mean Inhibition Zone; IZR, Inhibition Zone Range; 4:0 represents undiluted oil; 3:1 represents 3 parts oil and 1 part DMSO solvent; 2:2 represents 2 parts oil and 2 parts DMSO solvent; 1:3 represents 1 part oil and 3 parts DMSO solvent. Data are means of triplicates determinations.

3.2 Evaluation of Anticandidal Activity by Broth Microdilution Method

In our study, various concentrations of cassia have been used to determine the MIC₉₀ and MFC₉₀ by broth microdilution assay (Table 2). The effective MIC range for inhibiting all the *Candida* species was observed to be 0.02 – 0.62 µl/ml. *C. pseudotropicalis* and *C. stellatoidea* was found to be the most susceptible among the *Candida* species, requiring lowest amount of cassia oil for its inhibition, with its MIC₉₀ of 0.04 µl/ml. The least susceptible of the *Candida* species to cassia oil was the *C. glabrata* and *C. tropicalis*, with MIC₉₀ of 0.31 µl/ml. MIC range

for the isolates of *C. albicans* was found to be 0.08 - 0.15 µl/ml cassia oil with MIC_{90s} at 0.15 µl/ml. Much of the isolates of *C. albicans* showed good consistency with respect to MIC requirement, with exception of few isolates that showed 2 fold variations in their MIC values. However, cassia oil showed optimum inhibitory activity against *C. krusei* and *C. guilliermondii* at 0.08 µl/ml concentration. MIC₉₀ of *C. glabrata*, *C. tropicalis*, *C. krusei* and *C. stellatoidea* were two fold greater than MIC₅₀ and MIC₇₀, while MIC₉₀ of *C. parapsilosis*, *C. pseudotropicalis* and *C. guilliermondii* were observed to be equal to MIC₅₀ and MIC₇₀.

Table 2
Minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) obtained by broth microdilution of the cassia oil assayed against eight different *Candida* species.

Species (No. of isolates)	MIC (µl/ml)					MFC (µl/ml)
	MIC Range	MIC ₅₀	MIC ₇₀	MIC ₉₀	Mean MIC	MFC ₉₀
<i>Candida albicans</i> (n = 28)	0.08 – 0.15	0.08	0.15	0.15	0.10	0.15
<i>C. glabrata</i> (n = 9)	0.02 – 0.62	0.15	0.15	0.31	0.21	0.31
<i>C. tropicalis</i> (n = 12)	0.08 – 0.31	0.15	0.15	0.31	0.16	0.31
<i>C. parapsilosis</i> (n = 7)	0.02 – 0.15	0.15	0.15	0.15	0.10	0.15
<i>C. pseudotropicalis</i> (n = 6)	0.04 – 0.04	0.04	0.04	0.04	0.04	0.04
<i>C. krusei</i> (n = 7)	0.04 – 0.08	0.04	0.04	0.08	0.05	0.08
<i>C. guilliermondii</i> (n = 3)	0.08 – 0.08	0.08	0.08	0.08	0.08	0.08
<i>C. stellatoidea</i> (n = 3)	0.02 – 0.04	0.02	0.02	0.04	0.03	0.04
Total (n = 75)	0.02 – 0.62	0.15	0.15	0.31	0.09	0.31

MIC, Minimum Inhibitory Concentration; MFC, Minimum Fungicidal Concentration ;(MIC and MFC values are expressed in µl /ml). Data are means of triplicates determinations.

3.3 Evaluation of Anticandidal Activity by Broth Macrodilution Method

Table 3 illustrated the MIC and MFC *in vitro* susceptibility of anticandidal activity by cassia oil assessed by broth macrodilution at different concentrations. The broth macrodilution study also showed that the *C. stellatoidea* was most susceptible to cassia oil at a minimum inhibitory concentration of 0.02 µl /ml, whereas *C. parapsilosis* was least susceptible to cassia oil with MIC₉₀ of 0.62 µl/ml. The wide MIC range for the isolates of *C. parapsilosis*

was observed to be 0.02 – 0.62 µl/ml and this MIC range was found to be most effective for inhibiting all the species of yeast. All the 28 isolates of *C. albicans* were inhibited at 0.15 µl/ml concentration of oil. MIC_{90s} of *C. albicans*, *C. glabrata* and *C. tropicalis* were found to be two fold greater than MIC₅₀, while MIC₉₀ of *C. parapsilosis* was found to be four fold greater than MIC₅₀. On the other hand MIC_{90s} of *C. pseudotropicalis* *C. krusei*, *C. guilliermondii* and *C. stellatoidea* were observed to be similar to MIC₅₀.

Table 3
Minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) obtained by broth microdilution of the cassia oil assayed against eight different *Candida* species.

Species (No. of isolates)	MIC (μ /ml)					MFC (μ /ml)
	MIC Range	MIC ₅₀	MIC ₇₀	MIC ₉₀	Mean MIC	MFC ₉₀
<i>Candida albicans</i> (n = 28)	0.02 – 0.15	0.08	0.08	0.15	0.07	0.15
<i>C. glabrata</i> (n = 9)	0.02 – 0.15	0.08	0.15	0.15	0.09	0.15
<i>C. tropicalis</i> (n = 12)	0.04 – 0.62	0.08	0.15	0.15	0.15	0.15
<i>C. parapsilosis</i> (n = 7)	0.02 – 0.62	0.15	0.15	0.62	0.19	0.62
<i>C. pseudotropicalis</i> (n = 6)	0.04 – 0.08	0.08	0.08	0.08	0.07	0.08
<i>C. krusei</i> (n = 7)	0.02 – 0.04	0.04	0.04	0.04	0.03	0.04
<i>C. guilliermondii</i> (n = 3)	0.08 – 0.08	0.08	0.08	0.08	0.08	0.08
<i>C. stellatoidea</i> (n = 3)	0.02 – 0.02	0.02	0.02	0.02	0.02	0.02
Total (n = 75)	0.02 – 0.62	0.15	0.15	0.62	0.08	0.62

MIC, Minimum Inhibitory Concentration; MFC, Minimum Fungicidal Concentration ;(MIC and MFC values are expressed in μ / ml). Data are means of triplicates determinations.

4. DISCUSSION

C. albicans is a harmless commensal dimorphic yeast-like fungus in healthy humans, which can cause superficial as well as life threatening systemic infections under immunocompromised states¹⁴. *C. albicans* can infect virtually all body sites because of its high adaptability to different host niches by the activation of appropriate sets of genes in response to complex environmental signals¹⁹. A strong immune system and a healthy inner ecology keep the *Candida* yeast in check and prevent it from taking over its surrounding environment. Unfortunately, overuse of antibiotics and a diet full of processed foods, sugar, and gluten limit the body's ability to keep *Candida* in check. Because *Candida* is an opportunistic microorganism, it multiplies when it has the chance and cause issues like thrush, vaginal yeast infections, and systemic candidiasis. In this context, our aim was to evaluate the possible therapeutic potential of cassia oil against this human commensal organism, which can become a facultative pathogen under altered physiological situations. In the light of this fact and with an intension to manage the *C. albicans*, our previous investigation²⁰ assessed the antifungal activity of several essential oils and reported the strong antifungal activity of cassia oil against reference strains of *C. albicans* (MTCC-227, MTCC-3017 & NCIM- 3100), *C. glabrata* (MTCC-3019), *C. krusei* (MTCC-231),

C. blanki (MTCC-624), *C. cylindracea* (MTCC-1908), *C. tropicalis* (MTCC- 184) with inhibition zones ranging from 40 to 66 mm. In the present investigations therapeutic potential of cassia oil is assessed directly against the *Candida* spp. isolated from different patient with clinical manifestations. The results were extremely promising and consistent, with strong inhibitory potential towards the clinical isolates of *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. stellatoidea* and *C. tropicalis* with inhibition zones ranging from 42 to 72 mm. The results showed a good correlation between broth microdilution and broth macrodilution methods, however in some cases 4 to 8 fold higher MICs were found in broth microdilution method. Previous studies to assess the antifungal effects of cassia essential oil against plant pathogenic fungi which are big problem in agriculture have demonstrated a significant antifungal effect of cassia oil against *Sclerotinia sclerotiorum* which is a plant pathogenic fungus and can cause a disease called white mold, cottony rot, watery soft rot, stem rot, drop, crown rot and blossom blight²¹. Essential oil of *C. cassia* and its pure cinnamaldehyde was found to have broad-spectrum inhibitory activity against bacteria and fungi, including yeasts (*C. albicans*, *C. tropicalis*, *C. glabrata* and *C. krusei*), filamentous moulds (*Aspergillus* spp and *Fusarium* spp), and dermatophytes (*Microsporum gypseum*, *Trichophyton rubrum* and *Trichophyton mentagrophytes*)²².

Essential oil of cassia reported to potentiate the antifungal action of amphotericin B, a widely used drug for most indications, against *C. albicans* suggests possible utilization of this essential oil in addition to antifungal drugs for the treatment of mycoses. The potentiating activity of amphotericin B *in vitro* may show promise for the development of less toxic and more effective therapies²³. Systemic Aspergillosis is the second most common invasive fungal infections. Previous investigations^{20, 24} reported strong inhibitory activity of cassia oil against fungus *Aspergillus niger* and suggested solution for possible application of essential oil of *C. cassia* in different food systems. Cassia oil also reported to be significantly inhibitory to Aflatoxin producing fungi *Aspergillus flavus*. Oropharyngeal candidiasis, a fungal disease of the oral mucosa and tongue, is the most common intraoral lesion among persons infected with HIV. As antimicrobial agents, essential oils may be appropriate in HIV/AIDS for specific opportunistic infections. Essential oil could be explored for potential use in HIV/AIDS focusing on opportunistic infections caused by *Candida albicans* and others²⁵. Although there is no known cure for HIV, the immune system can be strengthened and secondary infection can be prevented through the use of essential oils. There is limited information available about the use of essential oils in the care of AIDS. In an experiment²⁶ testing the effects of various plants used in traditional Indian medicine, an extract of *Cinnamomum cassia* had an effect on HIV – 1. Almeida²⁷ studied antifungal activity *Cinnamomum cassia* oil against fifteen clinical samples of *Candida albicans* isolated from HIV- positive patients using microdilution technique and recorded 64 $\mu\text{g mL}^{-1}$ MIC for 80% of the strains. In present investigation Cassia oil strongly inhibited all the isolates of *Candida* species isolated from various clinical specimens such as oral thrush, vaginal discharge and blood, with MICs as high as 0.62 $\mu\text{l/ml}$. For *C. albicans* MIC range was found to be 0.08 – 0.15 $\mu\text{l/ml}$ by broth microdilution method, while the range was 0.02 – 0.15 $\mu\text{l/ml}$ by broth macrodilution method. The high activity of cassia oil was displayed against *C. stellatoidea*, *C. krusei*, and *C. pseudotropicalis*, while comparatively

lower activity was observed against *C. tropicalis* and *C. glabrata* by broth microdilution method. MFC values for cassia oil were similar to MIC results. MFC₉₀ range for different candida species was found to be 0.04 – 0.31 $\mu\text{l/ml}$ by broth microdilution and 0.02 – 0.62 $\mu\text{l/ml}$ by broth macrodilution method. MIC values reported in this study could be much significant as concentration below the MIC values inhibits germ tube formation, which is an important virulence factor in the pathogenesis of *C. albicans*. Evidences suggest that cassia oil have significant activity against *Candida*. However, well-designed trials are needed before a firm conclusion can be made. Cinnamaldehyde is major volatile and divers constituent present in cassia oil and also has a variety of active components viz., eugenol, cinnamic acid, diterpenes, proanthocyanidins. These active constituents posses both antifungal and antibacterial properties that could be used as a medicine to prevent the human health effecting disorders³. Previous literature has reported that cinnamaldehyde kills 80% bacteria and fungi²⁸. In the present study, essential oil of *Cinnamomum cassia* inhibited 100% isolates of *Candida* species. The oil was found highly effective against *C. albicans*, *C. stellatoidea*, *C. krusei*, *C. pseudotropicalis*, *C. tropicalis* and *C. glabrata* isolated from specimens such as oral swab, vaginal discharge and blood. This strong inhibitory activity of cassia oil could be related to the presence of Cinnamaldehyde in oil. Antibacterial activity of essential oil of *Cinnamomum cassia* is also documented in literature²⁹. Ates *et al.*³⁰ observed antimicrobial effect of essential oil of *Cinnamomum cassia* and found remarkable inhibition against variety of tested bacterial and fungal strains. Reports of Hammer *et al.*³¹ reveals that cassia oil inhibit *C. albicans* at a low MIC, indicating more effectiveness of cassia oil than clove oil, mint oil, citronella oil, juniper oil, frankincense oil and sandalwood oil. In a hospital setting, *Candida* biofilms are a serious health hazard. Patients that receive implants such as catheters or prosthetic heart valves end up with hospital-acquired *Candida*. Moreover, *Candida* biofilms are incredibly resistant to common antifungal drugs, like amphotericin B and fluconazole³². Recently

Mathur *et al.*³³ reported reduction in the biofilm formation by essential oils. Results reported in the present study contribute to the knowledge of the anticandidal effect of *Cinnamomum cassia*, as well as confirm their potential in the treatment and prevention of diseases caused by *Candida*. With this background, cassia oil may especially be effective at busting through the tough biofilm that *Candida* hides behind. Cassia Oil could be an excellent way to fight internal *Candida* infections and boost your immune system. This study also represents an inexpensive or cost effective source of natural mixtures of antifungal compounds that exhibit potential for use in controlling *Candida* spp. which is an important cause of bloodstream infections, vaginal candidiasis or thrush, superficial and systemic mycoses and opportunistic infections in the oral cavity of immuno-compromised patients. It is expected that further investigations will lead to a better understanding of some other roles that *C. cassia* plays in preventing and treating diseases.

7. REFERENCES

- Lai P. K. and Rov J., Antimicrobial and chemopreventive properties of herbs and spices. *Curr Med Chem*, 47(2): 234-238, (2004)
- Bansode V. J., A review on pharmacological activities of *Cinnamomum cassia* Blume. *International J. of Green Pharmacy*, 6(2):102-108 (2012)
- Bown D., *The Royal Horticultural Society Encyclopedia of herbs and their uses*. Dorling Kindersley Ltd. London. pp. 424 (1995)
- Fabio A., Corona A., Forte E. and Quaglio P., Inhibitory activity of spices and essential oils on psychrotrophic bacteria. *Microbiol.*, 26(1): 115-120, (2003)
- Kalemba D., Matla M., Smętek A., Antimicrobial Activities of Essential Oils Dietary Phytochemicals and Microbes , pp 157-183 ebook ed Amlan K. Patra published by springer (2012)
- Pfaller M. A., Diekema D. J., Role of sentinel surveillance of candidemia: Trends in species distribution and antifungal susceptibility. *J Clin Microbiol*, 40:3551-7 (2002)
- Hammer K. A., Carson C. F. and Riley T. V., *In vitro* activity of essential oils, in particular *Melaleuca alternifolia* (tea tree) oil and tea tree oil products against *Candida* spp. *J Antimicrob Chemother*, 42:591-595 (1998)
- Chakrabarti A., Shivaprakash M. R., Microbiology of systemic fungal infections. *J Postgrad Med* , 51(5):16-20 (2005)
- White T. C., Marr K. A. and Bowden R. A., Clinical, cellular and molecular factors that contribute to antifungal drug resistance. *Clin Microbiol Rev*, 11:382-402 (1998)
- Wingard J. R., Importance of *Candida* species other than *C. albicans* as pathogens in oncology patients. *Clin Infect Dis*, 20:115-25 (1995).
- Vazquez J. A., Arganoza M. T., Boikov D., Vaishampayan J. K. and Akins R. A., *In vitro* susceptibilities of *Candida* and *Aspergillus* species to *Melaleuca alternifolia* (tea tree) oil. *Rev Iberoam Micol*, 17:60-63 (2000)
- Espinel-Ingroff A., *In vitro* activity of the new triazole Voriconazole (UK- 109,496) against opportunistic filamentous and dimorphic fungi and common and emerging yeast pathogens. *J Clin Microbiol*, 36:198-202 (1998)
- Pina-Vaz C., Goncalves R. A., Costa-de-Oliveira S., Ricardo, Per-Ander M., Potent synergic effect between ibuprofen and azoles on *Candida* resulting from blockade of efflux

5. CONCLUSION

In conclusion, cassia oil is an effective natural anticandidal agent that shows significant promise as a potential therapeutic agent for the treatment of superficial and mucosal candidiasis including vaginal candidiasis caused by *Candida* spp. Thus, cassia oil can be effectively utilized for the control of *Candida* yeast. *In vitro* results indicate anticandidal efficacy of cassia oil at low concentration. But certain clinical trials are needed to determine the usefulness of cassia oil *in vivo*.

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- pumps as determined by FUN-1 staining and flow cytometry. J Antimicrob Chemother, 56:678- 685 (2005)
14. Koneman E. W. and Roberts G. D. (eds). Practical Laboratory Mycology, 3rd edn. The Williams and Wilkins Co., Baltimore, (1985)
 15. National Committee for Clinical Laboratory Standards. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeast: Approved standard. 2nd eds. (Document M27-A2) Villanova, PA: National Committee for Clinical Laboratory Standards, (2002).
 16. Bauer A. W., Kirby W. M. M., Sherris J. C. and Turok M., Antibiotics susceptibility by standardized single disc method. Am. J Clin Patho, 45: 493 – 496 (1966)
 17. Elgayyar M., Draughon F. A., Golden D. A. and Mount J. R., Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. J Food Protect, 2001; 64: 1019-1024 (2001)
 18. Mann C. M. and Markham J. L., A new method for determining the minimum inhibitory concentration of essential oils. J Appl Microbiol, 84: 538 – 544(1998)
 19. Vijaya M., Cass I., Gray J., Nadeem A. T., Echard B. W., Bagchi D. and Preuss H. G., Antifungal activities of origanum oil against *Candida albicans*. Molecular and Cellular Biochemistry, 228: 111–117 (2001)
 20. Kamble V. A. and Patil S. D., Spice-Derived Essential Oils: Effective Antifungal and Possible Therapeutic Agents. Journal of Herbs, Spices & Medicinal Plants, 14(3-4):129 – 143 (2008)
 21. Zhenhua J., Hong J. and Pengfei X., Antifungal activities against *Sclerotinia sclerotiorum* by *Cinnamomum cassia* oil and its main components. J Essential Oil Research, 25 (6): 444-451 (2013)
 22. Ooi L. S., Li Y., Kam S. L., Wang H., Wong E. Y., Ooi V. E., Antibacterial activities of cinnamon oil and cinnamaldehyde from the Chinese medicinal herb *Cinnamomum cassia* Blume. Am J Chin Med, 34:511-22 (2006)
 23. Giordani R., Regli P., Kaloustian J., Portugal H., Potentiation of antifungal activity of amphotericin B by essential oil from *Cinnamomum cassia*. Phytother Res, 20:58-61 (2006)
 24. Kocevski D., Du M., Kan J., Jing C., Lačanin I., Pavlović H., Antifungal effect of *Allium tuberosum*, *Cinnamomum cassia*, and *Pogostemon cablin* essential oils and their components against population of *Aspergillus* species. J. Food Sci, 78(5):M731-7 (2013)
 25. Buckle J., Clinical aromatherapy and AIDS. J Assoc Nurses AIDS Care, 13(3):81-99 (2002)
 26. Premanathan M., Rajendran S., Ramanathan T., Kathiresan K., Nakashima H., Yamamoto N., A survey of some Indian medicinal plants for anti-human immunodeficiency virus (HIV) activity. Indian Journal of Medical Research, 112: 73–7. (2000)
 27. Almeida L. F. D., Cavalcanti Y. W., Castro R. D. and Lima E. O., Antifungal activity of essential oils against clinical samples of *Candida albicans* isolated from HIV-positive patients. Rev. Bras. Plantas Med, 14(4): 649-655 (2012)
 28. McCann, J. Herbal Medicine Handbook, 2nd Edition. Philadelphia: Lippincott. (2003)
 29. Chaudhry N. M. A. and Perween T., Anti-microbial activity of *Cinnamomum cassia* against diverse microbial flora with its nutritional and medicinal impacts. Pak J Bot, 38(1): 169-174 (2006).
 30. Ates, D. A. and Erdogru O. T., Antimicrobial activities of various medicinal and commercial plant extracts. Turk J Biol, 27:157-162 (2003)
 31. Hammer K. A., Carson C. F. and Riley T. V., Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol, 86 : 985 – 990 (1999)
 32. Mohd Sajjad A. K. and Iqbal A., Biofilm inhibition by *Cymbopogon citratus* and *Syzygium aromaticum* essential oils in the strains of *Candida albicans*. Journal of Ethnopharmacology, 140 (2): 416-423 (2012)
 33. Mathur S., Udgire M. and Khambhupati A., Effect of essential oils on biofilm formation by *Proteus mirabilis*. Int J Pharm Bio Sci 4(4): (B) 1282 – 1289 (2013).