

ANTICANDIDAL ACTIVITY OF SOME CULINARY HERBS

Sabahat Saeed, Nazia Masood and Perween Tariq

Department of Microbiology, University of Karachi, Karachi-75270, Pakistan;
e-mail:sabahatsaeed2003@yahoo.com

ABSTRACT

Twenty six (26) isolates belonging to 4 different species of *Candida*; *C.albicans* (16), *C.tropicalis* (6), *C.parapsilosis* (3) and *C.krusei* (1); isolated from clinical specimens were used for screening of anticandidal activities of aqueous infusions, decoctions and oils of cinnamon (*Cinnamomum zeylanicum*), clove (*Eugenia caryophyllata*), oregano (*Oreganum sativum*), kalonji (*Nigella sativa*) and poppy seed (*Papaver somniferum*) by standard disc diffusion method. Oil of cinnamon exhibited highest activity against all *Candida* species with maximum zone of inhibition 49.50mm against *C. albicans*. Decoction and infusion of cinnamon also exhibited good effect with 15.19mm and 11.15mm, respectively. Clove, oregano and kalonji oils were also effective against all isolates with 12.46mm, 13.31mm and 13.04mm, respectively while poppy seed oil had no effect on *Candida* species. In case of decoctions, only decoction of clove showed weak activity with 8.42mm while decoctions of oregano, kalonji and poppy seed did not inhibit the growth of *Candida*. Besides, all isolates were found resistant to aqueous infusions of clove, oregano, kalonji and poppy seed.

Key words: Antifungal activity, *C.albicans*, *C.tropicalis*, Cinnamon, Clove.

INTRODUCTION

Candida species have been emerged as important cause of invasive infections (Laupland *et al.*, 2005). Among *Candida* species *C. albicans* is a common cause of mucosal and blood stream infections (Badrane *et al.*, 2005). During 1990's, *C. parapsilosis* has been emerged as the most frequent yeast specie causing candidiasis in the non-albicans group. An increase in the infections caused by *C. krusei* during 1993-1998 was also noticed (Safdar *et al.*, 2002). Besides, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and other non-albicans *Candida* are also responsible for candidiasis (Horvath *et al.*, 2003).

Some common antifungal agents used to treat infections caused by *Candida* species are amphotericin B, nystatine, chlorhexidine and fluconazole (Chandra *et al.*, 2001). Substantial research has documented emergence of multi-drug resistance among *Candida* species which is spreading hazards in the world. Resistance to antifungal drugs have been emerged due to an increase in the incidence of systemic fungal infections, widespread use of antifungal agents and inappropriate prescribing of systemic antifungal agents. Use of combination antifungal therapy may be more effective in preventing development of resistance. The combination of amphotericin B and flucytosine is currently in use while combination of amphotericin B and azole, azole and flucytocine and azole and terbinafine are being tested (Hudson, 2001).

Another alternative to combat the problem of microbial resistance is the development of new antimicrobials for substitution with ineffective one (Bonjar, 2004). The use of higher plants to treat infections is an old age practice in a large part of the world population. Different parts of plants have been used for prevention of infections. These are easily available and can be used in domestic setting for self-medication. The present report gives an access of the anticandidal activities of aqueous infusions, decoctions and oils of some culinary spices viz., cinnamon (*Cinnamomum zeylanicum*), clove (*Eugenia caryophyllata*), oregano (*Oreganum sativum*), kalonji (*Nigella sativa*) and poppy seed (*Papaver somniferum*).

MATERIALS AND METHOD

A total of twenty six (26) clinical isolates belonging to 4 different *Candida* species; *C.albicans* (16), *C.tropicalis* (6), *C.parapsilosis* (3) and *C.krusei* (1); were obtained from culture collection of Microbiology Department, and maintained on sabouraud dextrose agar (SDA) (Oxoid).

Aqueous infusions of cinnamon, clove, oregano, kalonji and poppy seed; were prepared by steeping 20g in 100ml sterile distilled water in sterile flasks separately. The flasks were kept for two days with occasional shaking. The contents of flasks were filtered.

Aqueous decoctions of cinnamon, clove, oregano, kalonji and poppy seed; were prepared by boiling 20g in 100ml sterile distilled water for 15 minutes. The flasks were then plugged and removed from heat and allowed to cool. After cooling the contents of flasks were filtered.

McFarland Nephelometer Standard tube number 0.5 was prepared by mixing 0.5ml of 0.08M Barium chloride and 99.5ml of 0.36N Sulphuric acid. It was stored in dark at room temperature and was vortexed prior to use. The turbidity of the culture was compared to 0.5 McFarland Nephelometer Standard to get an inoculum of $0.5-2.5 \times 10^3$ CFU/ml (Graybill *et al.*, 1998). The standardized inoculum suspension was inoculated within 15-20 minutes.

A sterile cotton swab was immersed into standardized inoculum suspension. Excess broth was drained by pressing and rotating the swab against the inside wall of the suspension tube and streaked evenly on the surface of agar plates. With the help of sterile forcep the discs, soaked in infusions, decoctions and oils of cinnamon, clove, oregano, kalonji and poppy seed, were placed on the inoculated medium. After placement, the discs were pressed gently to the agar surface. The inoculated plates were incubated at 35°C for 18-24 hours and inhibition zone diameters were measured to the nearest millimeter (mm).

Table 1. Antifungal activity of oils of cinnamon, oregano, clove, kalonji and poppy seed.

<i>Candida</i> spp.	No. of Isolates	Mean zone of inhibition (mm \pm SD)				
		Cinnamon	Clove	Oregano	Kalonji	Poppy seed
<i>C. albicans</i>	16	49.50 \pm 4.09	13.00 \pm 1.37	13.50 \pm 1.70	13.00 \pm 2.09	---
<i>C. tropicalis</i>	06	49.33 \pm 3.73	11.83 \pm 1.46	13.00 \pm 1.15	13.33 \pm 1.89	---
<i>C. parapsilosis</i>	03	49.00 \pm 2.94	11.67 \pm 0.47	13.33 \pm 1.25	12.33 \pm 0.47	---
<i>C. krusei</i>	01	46.00	10.00	12.00	12.00	---
Total	26	49.27 \pm 3.87	12.46 \pm 1.50	13.31 \pm 1.54	13.04 \pm 1.95	---

Table 2. Antifungal activity of decoctions of cinnamon, oregano, clove, kalonji and poppy seed.

<i>Candida</i> spp.	No. of Isolates	Mean zone of inhibition (mm \pm SD)				
		Cinnamon	Clove	Oregano	Kalonji	Poppy seed
<i>C. albicans</i>	16	15.13 \pm 2.69	8.44 \pm 0.61	---	---	---
<i>C. tropicalis</i>	06	16.33 \pm 1.70	8.50 \pm 0.76	---	---	---
<i>C. parapsilosis</i>	03	13.33 \pm 0.94	8.33 \pm 0.47	---	---	---
<i>C. krusei</i>	01	15.00	8.00	---	---	---
Total	26	15.19 \pm 2.43	8.42 \pm 0.63	---	---	---

Table 3. Antifungal activity of infusions of cinnamon, oregano, clove, kalonji and poppy seed.

<i>Candida</i> spp.	No. of Isolates	Mean zone of inhibition (mm \pm SD)				
		Cinnamon	Clove	Oregano	Kalonji	Poppy seed
<i>C. albicans</i>	16	11.57 \pm 2.50	---	---	---	---
<i>C. tropicalis</i>	06	10.33 \pm 1.02	---	---	---	---
<i>C. parapsilosis</i>	03	11.00 \pm 1.63	---	---	---	---
<i>C. krusei</i>	01	9.00	---	---	---	---
Total	26	11.15 \pm 2.11	---	---	---	---

RESULTS AND DISCUSSION

Essential oils and extracts of plants are frequently used as an active ingredient in certain medicines. These are also used for the purification of environment since they possess antimicrobial properties. Several investigations have been directed towards their antifungal properties (Singh *et al.*, 1995). In view of this the present study gives an account on the anticandidal activities of aqueous infusions, decoctions and oils of some culinary spices viz., cinnamon, clove, oregano, kalonji and poppy seed.

Cinnamon has antiparasitic (Oishi *et al.*, 1974), antibacterial (Nir *et al.*, 2000) and antifungal properties (Singh *et al.*, 1995). The major antimicrobial components in cinnamon have been reported to be eugenol and cinnamic aldehyde (Davidson *et al.*, 1983). In present study, the oil of cinnamon revealed the highest activity against *C. albicans* with mean zone of inhibition 49.50 mm \pm 4.09 standard deviation (SD) (Table 1). It also exhibited good

effect on *C. tropicalis*, *C. parapsilosis* and *C. krusei* with 49.33 mm±3.73 SD, 49.00 mm±2.94 SD and 46.00 mm, respectively (Table 1). Our results are in fair correlation with the study carried out by Abdel-Mallek *et al.* (1994). They studied *in vitro* anti-yeast activity of some essential oils and found that cinnamon oil was the most effective oil against all species tested. Decoction of cinnamon was also found effective against *C. albicans*, *C. tropicalis*, *C. parapsilosis* and *C. krusei* with 15.13 mm±2.69 SD, 16.33 mm±1.70 SD, 13.33 mm±0.94 SD and 15.00 mm, respectively (Table 2). These results are also in harmony with a study in which decoction of cinnamon bark has an inhibitory action against fungi *in vitro* (Chang and But, 1986). Infusion of cinnamon was found weakly effective against *C. albicans*, *C. tropicalis*, *C. parapsilosis* and *C. krusei* with 11.57 mm±2.50 SD, 10.33 mm±1.02 SD, 11.00 mm±1.63 SD and 9.00 mm, respectively (Table 3).

In the present study, the effect of clove was found next to cinnamon. Cloves are sun-dried flower buds of *Eugenia caryophyllata*. It has broad spectrum antibacterial activity. It is also found to be antiseptic, antiviral and antifungal. The principle constituent of clove oil is the volatile oil, eugenol (Dominique, 2002). Clove oil also has toxic activity for fungal spores and mycelial growth of *Aspergillus niger*, *Alternaria alternata* and *Fusarium* species. It has also been reported that mycelial growth and aflatoxin production by *A. flavus* are completely inhibited by the clove oil (Mabrook and El-Shayeb, 1980). Furthermore, the growth and aflatoxin production by two mycotoxigenic fungi, *A. parasiticus* and *F. moniliforme* has also been successfully inhibited by clove oil (Juglal *et al.*, 2002). It is also documented in the literature that clove oil inhibits the growth of dermatophytes (El-Naghy *et al.*, 1992). In the present study, the clove oil exhibited anticandidal activity against *C. albicans*, *C. tropicalis*, *C. parapsilosis* and *C. krusei* with 13.00 mm±1.37 SD, 11.83 mm±1.46 SD, 11.67 mm±0.47 SD and 10.00 mm, respectively (Table 1). The decoction of clove also exhibited weak activity against *C. albicans*, *C. tropicalis*, *C. parapsilosis* and *C. krusei* with 8.44 mm±0.61 SD, 8.50 mm±0.76 SD, 8.33 mm±0.47 SD and 8.00 mm, respectively (Table 2). While infusion of clove did not exhibit anticandidal activity. Our results are in correlation with the study in which clove extract completely inhibited yeast growth with in 5 h (Arora and Kaur, 1999).

Another spice used in the present study was oregano. Oil of oregano is a rich source of volatile oils, thymol and carvacol, which have been shown to be responsible for its antimicrobial properties (Meschino, 2005). Aqueous infusion and decoction of oregano did not exhibit anticandidal activity. While oil of oregano was found to be effective against all *Candida* spp. viz., *C. albicans*, *C. tropicalis*, *C. parapsilosis* and *C. krusei* with 13.50 mm±1.70 SD, 13.00 mm±1.15 SD, 13.33 mm±1.25 SD and 12.00 mm, respectively (Table 1). These results are in fair correlation with a previous study in which good inhibitory effect of oregano oil was found on experimental oral candidal infections, *in vitro* and *in vivo* (Chami *et al.*, 2005). In another study oil of oregano inhibited 30 different strains of pathogenic bacteria such as *Staphylococcus aureus* as well as other microorganisms including corona viruses (Meschino, 2005).

In the present study antifungal effect of infusion, decoction and oil of kalonji was also evaluated. Kalonji has been used in traditional medicine for the treatment of a variety of diseases including diarrhea and asthma (Mashhadian and Rakhshandeh, 2005). Kalonji oil exhibited moderate anticandidal effect on *C. albicans*, *C. tropicalis*, *C. parapsilosis* and *C. krusei* with 13.00 mm±2.09 SD, 13.33 mm±1.89 SD, 12.33 mm±0.47 SD and 12.00 mm, respectively (Table 1). While its decoction and infusion did not show inhibitory effect on *Candida* species. Our results are in harmony with the study carried out by Mashhadian and Rakhshandeh (2005), who assessed the inhibitory effects of aqueous, methanol and chloroform extracts of oregano against *C. albicans*, *S. aureus*, and *Pseudomonas aeruginosa* and found that aqueous extract did not show any effect, but other extracts showed high inhibitory effects against all microorganisms.

In the present study the anticandidal activity of poppy seeds was also investigated. Poppy seeds have a large number of medicinal usages. One of the best known usages of poppy seeds is as a cure for insomnia, sleeplessness and dysentery. However, none of its medicinal forms should be taken for more than three subsequent days. Being a powerful narcotic, it might induce symptoms of addiction (Goswami, 2005). Information about its antibacterial activity is lacking in the literature. It was found that oil, decoction and infusion of poppy seeds did not possess any anticandidal activity.

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