

In Vitro: Evaluation of Inhibitory Activity of Some Plant Extracts Against Oral Candidiasis

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Abstract: Six ethanolic extracts from plants of Ginger (*Zingiber officinale*), Cinnamon (*Cinnamomum verum*), Black Cumin (*Nigella sativa*), Clove (*Syzygium aromaticum*), Black Pepper (*Piper nigrum*), and Chamomile (*Anthemis nobilis*) were purchased from local market in Hail province, Saudi Arabia were assayed for the in vitro inhibitory activity against some oral Candidal isolates. The prevalence of oral candidal infections among poor control diabetes patients was higher (56%) than fair control (30%) and good control (7%) diabetes patients. Statistically, our data indicated that a higher significant difference between the prevalence of oral candidiasis and diabetic control (p -value = 0.001). Cinnamon and Clove plant extracts were the most effective of all pathogenic yeasts studied. *Candida albicans*, *C. parapsilosis* and *C. tropicalis* showed greatest degree of sensitivity to Cinnamon and Clove plant extracts. The inhibition zone diameter recorded 34.6, 31.5; 45, 31; 45.5, 29.5 mm, respectively at 100 µg/ml concentration. Based on paired t-test, there is no significant difference between the mean values of the inhibition zone size of Amphotericin B, Black cumin, Chamomile and plant extracts were reported (P -value < 0.05). Both Cinnamon and Clove extracts showed remarkable effect on *C. albicans* and *C. tropicalis* at very low concentration MIC, 15, 18 and 15, 19 µl/ml, respectively.

[Mohamed A. Fareid. **In Vitro: Evaluation of Inhibitory Activity of Some Plant Extracts Against Oral Candidiasis**. *N Y Sci J* 2014;7(12):66-76]. (ISSN: 1554-0200). <http://www.sciencepub.net/newyork>. 9

Keywords: Inhibitory activity, Plant extracts, Oral, Candidiasis.

1. Introduction

Due to the increasing development of drug resistance in human pathogens as well as the appearance of undesirable effect of certain antimicrobial agents, there is a need to search for new antifungal agents without toxicity and side effects. Therefore it is necessary to search for more effective and less toxic novel antifungal agents that would overcome these disadvantages. Infection with the yeast like fungal organism *Candida albicans* is termed as candidiasis (Navejesh and Brightman, 1995). In recent years, there has been a significant increase in the incidence of human fungal infections (Lass-Flörl, 2009). A number of factors have been implicated with this increase, but it is generally accepted that the main influences relate to the more widespread provision of new medical practices, such as immunosuppressive therapy and use of broad spectrum antibiotics, and invasive surgical procedures such as solid organ or bone marrow transplantation. Infections may either be superficial, affecting the skin, hair, nails and mucosal membranes, or systemic, involving major body organs (Ruping *et al.*, 2008). Of the fungi regarded as human pathogens, members of the genus *Candida* are amongst the most frequently recovered from disease.

Oral candidiasis is one of the common fungal infections affecting the oral mucosa. Oral fungal disease commonly encountered within general dental practice is oral candidiasis (Farah *et al.*, 2010). These

infections are most frequently caused by *Candida albicans*, but other species such as *Candida glabrata* *Candida tropicalis* *Candida parapsilosis* *Candida krusei* and *Candida dubliniensis* are also responsible (Thompson *et al.*, 2010; Akpan and Morgan 2002). Diabetes mellitus is a chronic disease frequently associated with many risk factors (Lorenzo and Haffner, 2010; Craig *et al.*, 2009; Dodds *et al.*, 2000). It is a syndrome of abnormal carbohydrate, fat and protein metabolism due to the decreased insulin secretion or/and disturbed insulin activity. Diabetes mellitus has been related to numerous oral complications, such as periodontal disease, decreased function of salivary glands (xerostomia), burning mouth sensation and oral candidiasis (Lamster *et al.*, 2008; Samaranayake and Leung, 2000; Guggenheimer *et al.*, 2000).

Amphotericin B has been provided for the standard treatment of the most systemic fungal infections (Medoff and Kobayashi, 1980). Unfortunately, treatment with Amphotericin B, especially for long-term periods, can lead to adverse effects in patients or to the development of resistant organisms during the course of therapy (Kovacicova *et al.*, 2001). In the quest for new antifungal agents, low toxicity and broad spectrum fungicidal activities are needed for effective management of the infections. Triazole antifungal agents, such as Fluconazole and Itraconazole, are considered to be first-choice agents for treatment and prevention

because of their relatively low side effects and high effectiveness on mucosal infections. However, with prolonged exposure to azoles, drug resistance becomes a challenge for clinicians and patients alike.

Due to the presence and increase of numerous drug resistant strains, there is an urgent need to develop novel antimicrobial agents; hence, much attention has been made on medicinal plants during the last decade (Fani *et al.*, 2007; Pesewu *et al.*, 2008; Aqil *et al.*, 2005; Martin and Ernst, 2003). Crude extracts from a number of medicinal herbs have been shown to exhibit antifungal activities *in vitro* (Liu *et al.*, 2011; Alsaïdy, 2014; Doudi *et al.*, 2014). The oil extract of green tea (*Camellia sinensis*) (Hirasawa *et al.*, 2002), garlic (*Allium sativum*) (Fani *et al.*, 2007; Martin and Ernst, 2003), Cinnamon (*Cinnamomum zeylanicum*) (Senhaji, *et al.*, 2007), *Eucalyptus globulus* (Salari *et al.*, 2006), *Zataria multiflora* (Khosravi *et al.*, 2008), *Artemisia feddei* (Cha *et al.*, 2007), and Rosmary (*Rosmarious officinalis*) (Luqman *et al.*, 2007), were the most extensively researched medicinal plants which have been reported to have inhibitory activity on various bacterial, fungal and viral agents. The current study aimed to investigate the frequency of oral candidiasis in diabetic and non-diabetic patients, as well as the association between the frequency of oral candidiasis and some risk factors. In addition to evaluate the inhibitory activity of some plant extracts against the isolated *Candida* species.

2. Materials And Methods

2.1. Sample collection and cultivation

Sixty five *Candida* isolates were obtained from one hundred seventy five patients (sixty two diabetics and one hundred thirteen non-diabetics) who had attended private dental clinics, in H'ail province, Saudi Arabia. Samples were mainly collected from the buccal mucosa, tongue and hard palate by using sterile cotton swabs. These swabs were cultivated on the Sabouraud dextrose agar (SDA) plates and placed in the incubator for 48-72 hr at 37 °C. The growth was stained and identified.

2.2. Identification of isolates

Candida isolates were identified by classical methods using the following biochemical tests: Fermentation of D-glucose, assimilation of carbohydrates D-galactose, maltose, sucrose, cellobiose, trehalose, raffinose, melezitose, soluble starch, L-arabinose and germ tube formation (Brown and Gow, 1999), hyphae/pseudohyphae (Kurtzman and Fell, 1998) and chlamydospores production (Taschdjian, 1957). Carbohydrate fermentation and urea hydrolysis were carried out by subculture of 2-3 representative colonies on CHROMagar *Candida* medium (CHROMagar, Paris, France). The plates

were incubated at 37°C for 48-72 hr. Also, the identification of *Candida* isolates was confirmed by using the API 20C *Candida* identification system (Bio-Merieux, Marcy l'Etoile, France).

2.3. HbA_{1c} level

HbA_{1c} level was determined in capillary whole blood or venous whole blood using an immunoturbidimetric assay (DCA 2000 HbA_{1c} System, Bayer. Denmark). The validity of this assay has previously been tested (Mortensen *et al.*, 1994).

2.4. Plant material

Six plants were used Ginger (*Zingiber officinale*), Cinnamon (*Cinnamomum verum*), Black Cumin (*Nigella sativa*), Clove (*Syzygium aromaticum*), Black Pepper (*Piper nigrum*), and Chamomile (*Anthemis nobilis*) they were purchased from a local market in H'ail city, Saudi Arabia. The plants were brought to the laboratory and thoroughly washed in distilled water and dried in shade at room temperature, then stored in a plastic bag at 4 °C until use.

2.5. Preparation of plant extract

Ethanol extract of Ginger (*Zingiber officinale*), Cinnamon (*Cinnamomum verum*), Black Cumin (*Nigella sativa*), Clove (*Syzygium aromaticum*), Black Pepper (*Piper nigrum*), and Chamomile (*Anthemis nobilis*) plants were extracted according to the method described by Okogun (2000) with slight modifications. A 50 g sample of each plant was air-dried, ground into powder using an electric blender (National MX4911V, Matsushita electronics). The blended material was transferred into a beaker and 10 ml of 95% ethanol was added at ambient temperature (28 ±2°C). The mixture was extracted by agitation on a rotary shaker. Extraction was allowed to proceed for 48 hr. The mixture was decanted and the solvent was removed by evaporation at room temperature (28 ±2°C) to obtain the extract. The extracts were kept in sterile bottle at 4 °C until use.

2.6. In vitro antifungal assay

The antimicrobial assay was performed using the agar diffusion method of Collins *et al.* (1995) with slight modifications. The test organisms were inoculated on Sabouraud dextrose agar (SDA) plates and spread uniformly using a sterile glass spreader. Wells of 5 mm diameter were made on the Sabouraud dextrose agar using a sterile cork borer. The cut agar disks were carefully removed by the use of sterilized forceps. To each well was introduced various concentrations (0, 25, 50, 75 and 100 µg/ml) of the extracts. Control experiments comprising inoculums without plant extract were set up. The plates were allowed to stand for one hour at room temperature (25 ±2°C) for diffusion of the substances to proceed before the growth of organisms commenced. The

plates were incubated at 37°C for 48-72 hr. The zones of inhibition were then measured and recorded in mm diameter.

2.7. Determination of minimum inhibitory concentration (MIC) of ethanolic plant extracts on Candidal growth.

Plant extracts that inhibited the growth of Candida isolates (*Candida albicans*, *C. glabrata*, *C. krusei*, *C. guilliermondii*, *C. parapsilosis* and *C. tropicalis*) were investigated to determine the MICs using a broth-microdilution method. The Candida isolates were cultured overnight on mueller-hinton agar and then re-suspended in 1 ml mueller-hinton broth (OXOID CM 405) to obtain a final concentration of 100 CFU ml. Each extract was serially diluted with muellerhinton broth using methods approved by the National Committee for Clinical Laboratory Standards (M27-A) (NCCLS, 1997). After incubation, the MIC was determined as the lowest concentration of extract for which there was no visible.

2.8. Statistical analysis

The statistical SPSS version 15 was used in data analysis. All the parameters measured were expressed

as means ± standard deviation. The difference between mean values was analyzed by Student's *t* test at the 5 % significance level. The confidence interval used for all statistical analyses was 95%. *P*-values less than 0.05 were significant.

3. Results

Sixty five Candida isolates were obtained from one hundred seventy five patients (sixty two diabetics and one hundred thirteen non-diabetics) who had attended private dental clinics, in H'ail province, Saudi Arabia. The isolated pathogens were *Candida albicans*, *C. glabrata*, *C. krusei*, *C. guilliermondii*, *C. parapsilosis* and *C. tropicalis*. Out of a total six ethanolic plant extracts (Ginger, Cinnamon, Black cumin, Clove, Black pepper and Chamomile) (Table 1) were tested for their *in vitro* inhibitory activities against all isolated Candida species. Our results indicated that *Candida albicans* was in higher frequency between the isolated pathogens and followed by *C. tropicalis*. They recorded 38.46% and 24.62%, respectively, while *C. guilliermondii* was in lower frequency. It recorded 3.08% of the total percentage of the isolated pathogens (Figure 1).

Table (1): Common and scientific names of some plants used to detect their antifungal activities in vitro.

Common name	Scientific name	Family	Used part
Ginger	<i>Zingiber officinale</i>	Zingiberaceae	Dried rhizome
Cinnamon	<i>Cinnamomum cassia</i>	Lauraceae	Dried barks
Black cumin	<i>Nigella sativa</i>	Myrtaceae	Dried seeds
Clove	<i>Syzygium aromaticum</i>	Myrtaceae	Dried buds
Black pepper	<i>Piper nigrum</i>	Piperaceae	Dried fruits
Chamomile	<i>Anthemis nobilis</i>	Compositae	Dried Flowers

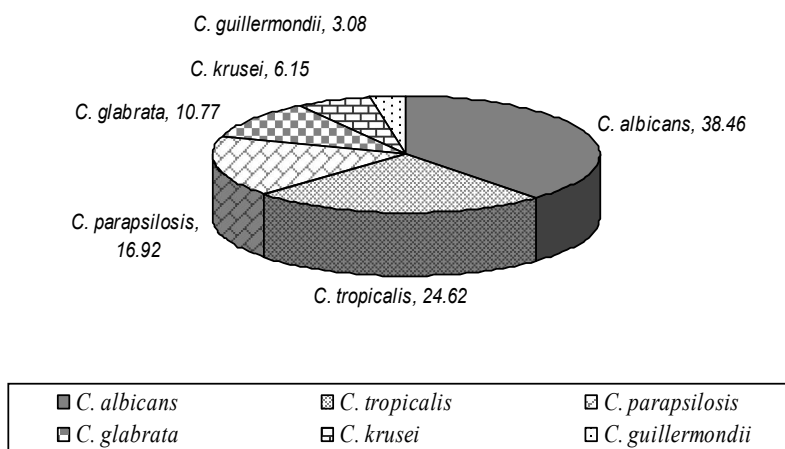


Figure 1. Frequency percentage of isolated pathogens.

3.1. Association between the frequency of oral candidiasis and some risk factors.

Table (2) represents the incidence of oral candidiasis in relation to the risk factors. A higher

percentage of oral candidiasis (41.5%) was found within the age brackets of 30-39 years while age groups 20-29 years had the least percentage (13.8%). Also, highest number of oral candidiasis was obtained from smoking (68%) than from non-smoking patients (32%). In addition to smoking, diabetic control level showed a lower number of oral candida infection in HBA₁C good control patients

(14%), while a higher number of oral candida infections were detected in HBA₁C poor control patients (56%). Statistically, our results were showed a highly significant difference between prevalence of oral candidiasis and all tested risk factors (P-value < 0.05) except in sex factor there is no significant difference (P-value > 0.05).

Table (2): Association between the frequency of oral candidiasis and some risk factors.

Characteristics	Frequency of Oral Candidiasis		P-value
	No.	%	
Age group (years)			0.004
20-29	9	13.8	
30-39	27	41.5	
40-49	19	29.2	
50-59	10	15.4	
Sex			0.709
Male	38	52.31	
Female	37	47.69	
Smoking			0.010
Current	34	68	
Never/ Former	16	32	
HBA₁C level			0.001
Good control (6.5-7.5 %).	7	14	
Fair control (8 -9.5 %).	15	30	
Poor control (> 9.5 %).	28	56	

3.2. Susceptibility of *C. albicans* to different concentrations of some plant extracts.

Out of a total 6 ethanolic plant extracts (Ginger, Cinnamon, Black cumin, Clove, Black pepper and Chamomile) were tested for their *in vitro* inhibitory activities against *Candida albicans*. Our results showed that Cinnamon and Clove were recorded higher inhibitory activity at 100 µg/ml concentration.

The inhibition zone recorded 34.6 and 31.5 mm, respectively, while Black cumin and Chamomile were recorded a lower inhibitory activity at the same concentration. The inhibition zone recorded 20.3 mm (Table 3). Statistically, our data exhibit a highly significant difference between all concentrations of all plant extracts (P-value < 0.05).

Table (3): Susceptibility of *C. albicans* to different concentrations of some plant extracts.

Plant name	Inhibition zone (mm) ± SD at different concentrations of plant extracts					
	Control	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	t-Test
Ginger	0.0	14.1 ±0.2	16.6 ±0.4	19.1 ±0.5	24.0 ±0.6	0.003
Cinnamon	0.0	15.6 ±0.4	20.0 ±0.6	27.5 ±0.3	34.6 ±0.4	0.010
Black cumin	0.0	10.3 ±0.4	13.0 ±0.6	16.6 ±0.4	20.3 ±0.8	0.006
Clove	0.0	12.0 ±0.6	16.6 ±0.8	22.5 ±0.3	31.5 ±0.3	0.014
Black pepper	0.0	11.6 ±0.4	17.3 ±0.4	23.6 ±0.4	30.0 ±0.6	0.016
Chamomile	0.0	10.6 ±0.4	13.0 ±0.6	17.6 ±0.8	20.3 ±0.4	0.006

3.3. Susceptibility of *C. glabrata* to different concentrations of some plant extracts.

Table (4) represents the inhibitory activity of tested ethanolic plant extracts (Ginger, Cinnamon, Black cumin, Clove, Black pepper and Chamomile) against *C. glabrata*. Cinnamon and Clove were recorded higher inhibitory activity at 100 µg/ml concentration. The inhibition zone recorded 31.5 and

24.3 mm, respectively, while Black cumin and Chamomile recorded a lower inhibitory activity at the same concentration, the inhibition zone recorded 19.5 and 18.5 mm, respectively. Statistically, our results showed that there is a highly significant difference between all concentrations of all plant extracts (P-value < 0.05).

Table (4): Susceptibility of *C. glabrata* to different concentrations of some plant extracts.

Plant name	Inhibition zone (mm) \pm SD at different concentrations of plant extracts					
	Control	25 μ g/ml	50 μ g/ml	75 μ g/ml	100 μ g/ml	t-Test
Ginger	0.0	12.5 \pm 0.3	14.3 \pm 0.5	17.0 \pm 0.6	23.1 \pm 0.5	0.005
Cinnamon	0.0	13.0 \pm 0.6	17.6 \pm 0.4	24.8 \pm 0.2	31.5 \pm 0.3	0.013
Black cumin	0.0	9.5 \pm 0.7	11.1 \pm 0.5	15.5 \pm 0.3	19.5 \pm 0.3	0.009
Clove	0.0	11.6 \pm 0.5	16.5 \pm 0.3	20.1 \pm 0.5	24.3 \pm 0.7	0.007
Black pepper	0.0	10.5 \pm 0.3	13.6 \pm 0.2	16.5 \pm 0.3	22.6 \pm 0.2	0.009
Chamomile	0.0	8.6 \pm 0.2	11.5 \pm 0.3	15.6 \pm 0.2	18.5 \pm 0.3	0.008

3.4. Susceptibility of *C. krusei* to different concentrations of some plant extracts.

Table (5) represents the susceptibility of *C. krusei* to different concentrations of some plant extracts. Our results showed that all tested ethanolic plant extracts (Ginger, Cinnamon, Black cumin, Clove, Black pepper and Chamomile) exhibit antifungal effects against *Candida krusei*. Cinnamon and Clove were recorded higher inhibitory activity at

100 μ g/ml concentration. The inhibition zone recorded 35.8 and 26 mm, respectively, while Black cumin and Chamomile recorded a lower inhibitory activity at the same concentration, the inhibition zone recorded 18.7 and 19.5 mm, respectively. Statistically, our data showed that there is a highly significant difference between all concentrations of all plant extracts (P-value < 0.05).

Table (5): Susceptibility of *C. krusei* to different concentrations of some plant extracts.

Plant name	Inhibition zone (mm) \pm SD at different concentrations of plant extracts					
	Control	25 μ g/ml	50 μ g/ml	75 μ g/ml	100 μ g/ml	t-Test
Ginger	0.0	13.5 \pm 0.3	15.6 \pm 0.2	17.5 \pm 0.3	22.6 \pm 0.4	0.003
Cinnamon	0.0	11.6 \pm 0.2	19.5 \pm 0.3	26.6 \pm 0.2	35.8 \pm 0.5	0.020
Black cumin	0.0	7.1 \pm 0.5	11.5 \pm 0.3	15.6 \pm 0.2	18.7 \pm 0.5	0.013
Clove	0.0	12.0 \pm 0.3	16.0 \pm 0.6	20.8 \pm 0.5	26.0 \pm 0.3	0.009
Black pepper	0.0	8.5 \pm 0.3	14.1 \pm 0.2	18.1 \pm 0.5	25.9 \pm 0.6	0.020
Chamomile	0.0	7.6 \pm 0.2	10.4 \pm 0.2	14.1 \pm 0.5	19.5 \pm 0.3	0.015

3.5. Susceptibility of *C. guilliermondii* to different concentrations of some plant extracts.

Table (6) represents the susceptibility of *C. guilliermondii* to different concentrations of some plant extracts. Our results showed that a highly inhibitory activity to Cinnamon, Clove and Black pepper against *C. guilliermondii* at 100 μ g/ml

concentration. The inhibition zone recorded 27.8, 28.5 and 27.5 mm, respectively, while Black cumin recorded a lower inhibition zone (18.8 mm) at the same concentration. Statistically; our data exhibit a highly significant difference between all concentrations of all plant extracts (P-value < 0.05).

Table (6): Susceptibility of *C. guilliermondii* to different concentrations of some plant extracts.

Plant name	Inhibition zone (mm) \pm SD at different concentrations of plant extracts					
	Control	25 μ g/ml	50 μ g/ml	75 μ g/ml	100 μ g/ml	t-Test
Ginger	0.0	12.6 \pm 0.6	15.5 \pm 0.3	17.1 \pm 0.2	22.0 \pm 0.6	0.003
Cinnamon	0.0	10.8 \pm 0.5	16.5 \pm 0.3	22.8 \pm 0.5	27.8 \pm 0.2	0.013
Black cumin	0.0	8.5 \pm 0.3	11.8 \pm 0.2	15.5 \pm 0.3	18.8 \pm 0.2	0.009
Clove	0.0	12.8 \pm 0.2	17.8 \pm 0.5	23.5 \pm 0.3	28.5 \pm 0.3	0.009
Black pepper	0.0	11.8 \pm 0.5	15.8 \pm 0.2	22.0 \pm 0.6	27.5 \pm 0.3	0.011
Chamomile	0.0	7.8 \pm 0.2	10.5 \pm 0.3	16.0 \pm 0.6	22.1 \pm 0.7	0.021

3.6. Susceptibility of *C. parapsilosis* to different concentrations of some plant extracts.

Table (7) represents the susceptibility of *C. parapsilosis* to different concentrations of some plant extracts. Our data exhibit higher inhibitory activity to Cinnamon and Clove against *C. parapsilosis* at concentration 100 μ g/ml. The inhibition zone

recorded 45 and 31 mm, respectively, while Black cumin recorded a lower inhibitory activity at concentration 25 and 100 μ g/ml. Its inhibition zone recorded 8.5 and 19.7 mm, respectively. Statistically, our data exhibit a highly significant difference between all concentrations of all plant extracts (P-value < 0.05).

Table (7): Susceptibility of *C. parapsilosis* to different concentrations of some plant extracts.

Plant name	Inhibition zone (mm) \pm SD at different concentrations of plant extracts					
	Control	25 μ g/ml	50 μ g/ml	75 μ g/ml	100 μ g/ml	t-Test
Ginger	0.0	11.8 \pm 0.2	15.1 \pm 0.5	18.5 \pm 0.3	22.5 \pm 0.3	0.005
Cinnamon	0.0	14.6 \pm 1.5	22.3 \pm 0.4	31.8 \pm 0.5	45.0 \pm 0.6	0.023
Black cumin	0.0	8.5 \pm 0.3	10.7 \pm 0.1	15.5 \pm 0.3	19.7 \pm 0.1	0.012
Clove	0.0	11.7 \pm 0.1	15.5 \pm 0.3	22.8 \pm 0.5	31.0 \pm 0.6	0.018
Black pepper	0.0	11.5 \pm 0.3	15.5 \pm 0.3	19.0 \pm 0.6	27.3 \pm 0.4	0.012
Chamomile	0.0	8.5 \pm 0.3	11.0 \pm 0.6	16.6 \pm 0.2	21.5 \pm 0.3	0.016

3.7. Susceptibility of *C. tropicalis* to different concentrations of some plant extracts.

Table (8) represents the susceptibility of *C. tropicalis* to different concentrations of some plant extracts. Our data showed that the inhibitory activity of tested ethanolic plant extracts (Ginger, Cinnamon, Black cumin, Clove, Black pepper and Chamomile) against *C. tropicalis*. Our results exhibit higher inhibitory activity to Cinnamon and Clove at

concentration 100 μ g/ml. The inhibition zone recorded 45.5 and 29.5 mm, respectively, while Black cumin and Chamomile were recorded a lower inhibitory activity at the same concentration, the inhibition zone recorded 22.1 and 21.1 mm, respectively. Statistically, our data exhibit a highly significant difference between all concentrations of all plant extracts (P-value < 0.05).

Table (8): Susceptibility of *C. tropicalis* to different concentrations of some plant extracts.

Plant name	Inhibition zone (mm) \pm SD at different concentrations of plant extracts					
	Control	25 μ g/ml	50 μ g/ml	75 μ g/ml	100 μ g/ml	t-Test
Ginger	0.0	12.8 \pm 0.5	16.0 \pm 0.6	19.5 \pm 0.3	24.6 \pm 0.4	0.005
Cinnamon	0.0	15.2 \pm 0.8	22.0 \pm 0.6	40.0 \pm 0.3	45.5 \pm 0.3	0.023
Black cumin	0.0	6.8 \pm 0.2	11.0 \pm 0.6	14.8 \pm 0.5	22.1 \pm 0.5	0.024
Clove	0.0	11.1 \pm 0.5	16.0 \pm 0.6	21.3 \pm 0.4	29.5 \pm 0.3	0.014
Black pepper	0.0	10.0 \pm 0.6	16.0 \pm 0.6	21.0 \pm 0.6	24.8 \pm 0.5	0.011
Chamomile	0.0	7.1 \pm 0.2	11.1 \pm 0.8	16.5 \pm 0.3	21.1 \pm 0.2	0.020

3.8. The inhibition zone diameters of the tested isolates against different antifungal agents.

Table (9) represents the inhibition zone diameters shown by the antifungal agents on the *Candida* isolates, there were varying degree of inhibition of all the isolates used in the study. The species of *C. albicans*, *C. tropicalis* were shown

resistance towards Clotrimazole and Voriconazole, respectively. Whereas, *C. krusei* and *C. glabrata* were sensitive towards all the azoles except itraconazole and fluconazole, respectively. *Candida parapsilosis* was exhibit sensitive to all tested antifungal agents. None of the *Candida* species was shown resistance towards Amphotericin B.

Table (9): The inhibition zone diameters of the tested isolates against different antifungal agents.

Candida isolates	Inhibition zone of tested <i>Candida</i> isolates (mm)				
	Fluconazole	Itraconazole	Amphotericin B	Clotrimazole	Voriconazole
<i>C. albicans</i>	16.6 \pm 0.8 S	16.1 \pm 0.2 S	20.3 \pm 0.4 S	14.0 \pm 1.3 R	26.0 \pm 0.6 S
<i>C. glabrata</i>	12.0 \pm 0.6 R	16.0 \pm 0.6 S	22.0 \pm 0.6 S	15.6 \pm 1.7 S	23.3 \pm 1.7 S
<i>C. krusei</i>	15.0 \pm 0.6 S	12.5 \pm 0.3 R	17.6 \pm 0.4 S	22.6 \pm 0.4 S	14.0 \pm 0.6 S
<i>C. guilliermondii</i>	10.5 \pm 0.3 R	16.3 \pm 0.4 S	22.3 \pm 0.4 S	17.6 \pm 1.5 S	23.0 \pm 1.3 S
<i>C. parapsilosis</i>	24.0 \pm 0.3 S	15.0 \pm 0.6 S	19.0 \pm 0.6 S	21.0 \pm 1.3 S	29.3 \pm 1.1 S
<i>C. tropicalis</i>	15.6 \pm 0.4 S	14.0 \pm 0.3 S	20.8 \pm 0.5 S	18.0 \pm 0.6 S	12.0 \pm 1.3 R

R, Resistance; S, Sensitive.

3.9. Paired Samples t-Test of anti-fungal agents and ethanolic extracts of some plant species.

To test the hypothesis of no difference or no relationship between the size of inhibition zone of antifungal drugs and all tested ethanolic plant extracts paired t-test was performed (Table 10). For nine pairs, 30% of pairs rejected null hypothesis in favor of alternate hypothesis, there is no significant

difference between the mean values of Amphotericin B, Black cumin and Chamomile inhibition zone (P-value > 0.05). Itraconazole was significantly related to all tested ethanolic plant extracts (P-value < 0.05), while Voriconazole was showed no significant difference to all tested ethanolic plant extract except with cinnamon and clove.

Table (10): Paired Samples t-Test of anti-fungal agents and ethanolic extracts of some plant

Paired Samples Test					
			t	Sig. (2-tailed)	Decision ($\alpha = 0.05$)
Pair 1	Fluconazole	Ginger	3.886	0.012	Significance
Pair 2	Fluconazole	Cinnamon	11.315	0.000	Significance
Pair 3	Fluconazole	Black cumin	2.255	0.074	Non-significance
Pair 4	Fluconazole	Clove	8.530	0.000	Significance
Pair 5	Fluconazole	Black pepper	5.637	0.002	Significance
Pair 6	Fluconazole	Chamomile	2.621	0.047	Significance
Pair 7	Itraconazole	Ginger	10.703	0.000	Significance
Pair 8	Itraconazole	Cinnamon	6.647	0.001	Significance
Pair 9	Itraconazole	Black cumin	6.751	0.001	Significance
Pair 10	Itraconazole	Clove	11.317	0.000	Significance
Pair 11	Itraconazole	Black pepper	10.600	0.000	Significance
Pair 12	Itraconazole	Chamomile	7.419	0.001	Significance
Pair 13	Amphotericin B	Ginger	3.461	0.018	Significance
Pair 14	Amphotericin B	Cinnamon	4.897	0.004	Significance
Pair 15	Amphotericin B	Black cumin	0.585	0.584	Non-significance
Pair 16	Amphotericin B	Clove	5.634	0.002	Significance
Pair 17	Amphotericin B	Black pepper	4.325	0.008	Significance
Pair 18	Amphotericin B	Chamomile	0.194	0.854	Non-significance
Pair 19	Clotrimazole	Ginger	3.241	0.023	Significance
Pair 20	Clotrimazole	Cinnamon	6.866	0.001	Significance
Pair 21	Clotrimazole	Black cumin	1.105	0.319	Non-significance
Pair 22	Clotrimazole	Clove	5.553	0.003	Significance
Pair 23	Clotrimazole	Black pepper	4.623	0.006	Significance
Pair 24	Clotrimazole	Chamomile	1.761	0.139	Non-significance
Pair 25	Voriconazole	Ginger	0.630	0.556	Non-significance
Pair 26	Voriconazole	Cinnamon	3.513	0.017	Significance
Pair 27	Voriconazole	Black cumin	0.473	0.656	Non-significance
Pair 28	Voriconazole	Clove	2.764	0.040	Significance
Pair 29	Voriconazole	Black pepper	2.014	0.100	Non-significance
Pair 30	Voriconazole	Chamomile	0.279	0.791	Non-significance

3.10. Minimum inhibitory concentration ($\mu\text{l/ml}$) of ethanolic plant extracts on *Candida* isolates growth.

MICs of the six plant extracts (Ginger, Cinnamon, Black cumin, Clove, Black pepper and Chamomile) were calculated by using a broth-microdilution method (Table 11). Our result showed that the MIC for all plant extracts was ranged from

15-28 $\mu\text{g/ml}$. The MIC for Cinnamon recorded 15 $\mu\text{g/ml}$ on *Candida albicans*, *C. parapsilosis* and *C. tropicalis*, while on *C. krusei* and *C. guilliermondii* were recorded 24 and 25 $\mu\text{g/ml}$, respectively. Also, the MIC for Ginger, Clove and Black Pepper were recorded 20 $\mu\text{g/ml}$ on *C. guilliermondii* and *C. parapsilosis*, while recorded 20, 19 and 24 $\mu\text{g/ml}$, respectively on *C. tropicalis*, respectively.

Table (11): Minimum inhibitory concentration ($\mu\text{l/ml}$) of ethanolic plant extracts on Candidal isolates growth.

Candidal isolates	Minimum inhibitory concentration (MIC) ($\mu\text{l/ml}$)					
	(1)	(2)	(3)	(4)	(5)	(6)
<i>C. albicans</i>	18	15	25	18	19	26
<i>C. glabrata</i>	20	20	25	24	24	28
<i>C. krusei</i>	18	24	28	22	22	28
<i>C. guilliermondii</i>	20	25	28	20	20	24
<i>C. parapsilosis</i>	20	15	28	20	20	25
<i>C. tropicalis</i>	20	15	26	19	26	25

(1), Ginger; (2), Cinnamon; (3), Black cumin; (4), Clove; (5), Black Pepper; (6), Chamomile.

4. Discussion

Oral candidiasis is one of the common fungal infections affecting the oral mucosa. In the current study we isolated six types of candida species in oral cavity; they are *Candida albicans*, *C. glabrata*, *C. krusei*, *C. guilliermondii*, *C. parapsilosis* and *C. tropicalis*. Similar work carried out by Gravina *et al.* (2007) he found that there are nearly five types of candida species which are seen in the oral cavity. They are *Candida albicans*, *C. tropicalis*, *C. krusei*, *C. parapsilosis* and *C. guilliermondii*. Batool, *et al.* (2011) found that the majority of yeast isolates from oral cavity swabs were *C. albicans* (75%), but it was often recovered in association with other yeasts, and the second most common yeast isolated in this survey was *C. glabrata*. Similarly, our results found that *Candida albicans* was in higher frequency between all isolated pathogens and followed by *C. tropicalis*. They recorded 38.46% and 24.62%, respectively. The prevalence of oral candida infections among poor control diabetes patients was higher (56%) than fair control (30%) and good control (7%) diabetes patients. Statistically, our data indicated that a higher significant difference between the prevalence of oral candidiasis and diabetic control (p- value = 0.001).

The current study is consistent with numerous previous studies, which have shown that diabetes mellitus is a major predisposing factor to symptomatic candidiasis, oral or otherwise (Aly *et al.*, 1995; Abu-Elteen and Abu-Alteen 1998; Leon *et al.*, 2002; Khaled *et al.*, 2006). This is also in agreement with numerous previous studies, which have all indicated that diabetes mellitus enhances *Candida* colonization and proliferation (Darwazah *et al.*, 1990; Peer *et al.*, 1993; Kumar *et al.*, 2005; Gupta *et al.*, 2007; Khovidhunkit *et al.*, 2009; Mohammad *et al.*, 2009; Sashikumar *et al.*, 2010; Radmila *et al.*, 2011). In addition to diabetes mellitus, the prevalence of oral *Candida* infections is influenced primarily by smoking (Rindum *et al.*, 1994; Neville *et al.*, 1995; Abu-Elteen and Abu-Alteen 1998; Khaled *et al.*, 2006). It is also clear from the findings presented in this study the prevalence of oral Candidiasis between smoking patients was in higher frequency (68%) than

in the non-smoking patients (16%). Statistically, our data indicated that there was a highly significant difference between the incidence of oral candidiasis and smoking (P- value = 0.010).

Muhsin *et al.*, (2014), showed that ethanolic extract of ginger and garlic (each alone) exhibit variable level of inhibition (ranging from 9 to 20 mm) against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumonia*, *Streptococcus pyogenes* *Pseudomonas aeruginosa* and *Proteus mirabilis* and this indicated that the ethanolic extraction gives better extraction than that of aqueous one. On the other hand our results indicated that ginger exhibit variable level of inhibition against *Candida albicans*, *C. glabrata*, *C. krusei*, *C. guilliermondii*, *C. parapsilosis* and *C. tropicalis* (ranging from 11.8 to 14.1 mm) at plant extract concentration 25 $\mu\text{g/ml}$. Generally, our results showed a highly inhibitory activity to all tested ethanolic plant extracts against *Candida albicans*, *C. glabrata*, *C. krusei*, *C. guilliermondii*, *C. parapsilosis* and *C. tropicalis* and the diameter of inhibition zones were directly proportional to the increase in the concentration of plant extracts. Statistically, our results showed a significant difference between all the different concentrations of all tested plant extracts (P-value < 0.05). Alsaidy (2014), indicated that oil extracts of thyme, peppermint, basil, cinnamon gave lowest growth rates in the concentration 100 mg/ml diameters growth of 3, 2, 2 and 2 mm and the highest inhibition percentages 90.0, 93.9, 92.8 and 93.3%, and the highest growth rates in concentration 20 mg/ml growth diameters 25, 20, 20 and 23 mm and minimum inhibition percentages of 16.6, 39.3, 28.5 and 23.3%, respectively, with significant differences between the concentrations (p value < 0.05). Similarly, our data indicated that ethanolic plant extract of cinnamon and clove was the most effective against all tested isolates (*Candida albicans*, *C. glabrata*, *C. krusei*, *C. guilliermondii*, *C. parapsilosis* and *C. tropicalis*), the inhibition zone diameter recorded 34.6, 31.5; 31.5, 24.3; 35.8, 26.0; 27.8, 28.5; 45.0, 31.0, 45.5, 29.5 mm. respectively.

Our study showed that cinnamon plant extract at 100 µg/ml concentration, had higher inhibitory effect on the growth of all isolated *Candida* species. These results were consistent with Douidi *et al.* (2014) study in Iran that was performed via disk diffusion method on *Candida* fungus, Ibtisam (2011) study in KSA via well diffusion assay technique on Gram-positive *Staphylococcus aureus* and *Bacillus subtilis*, gram-negative *Pseudomonas aeruginosa* and *Escherichia coli* and yeast represented by *Candida albicans*, Rukayadi *et al.* (2006) study in Korea using cinnamon extract on six species of *Candida* with micro dilution method and Lopez *et al.* (2005) study in Spain that was performed via disk diffusion method on *Candida* fungus.

The hypothesis of no difference or no relationship between the size of inhibition zone of antifungal drugs and all tested ethanolic plant extracts paired t-test was performed. For nine pairs, 30% of pairs rejected null hypothesis in favor of alternate hypothesis, there is no significant difference between the mean values of Amphotericin B, Black cumin and Chamomile inhibition zone (P-value > 0.05). Itraconazole was significantly related to all tested ethanolic plant extracts (P-value < 0.05), while Voriconazole was showed no significant difference to all tested ethanolic plant extract except with cinnamon and clove. Similar results obtained by Pathak (2012), he found that there is no significant difference between the mean values of the size of zone of inhibition of Amphotericin B and herbal extracts; Clotrimazole was significantly related with *Azadirachta indica* and *Murraya koenigii*, but not with *Allium sativum*, and Voriconazole shown no-significant relation with all the herbal extracts tested.

Our result showed that the MIC for all plant extracts was ranged from 15-28 µg/ml. The MIC of *Candida albicans* to cinnamon plant extract recorded 15 µl/ml. This finding is in agreement with Douidi *et al.* (2014) study, he showed that Cinnamon essential oil had higher inhibitory effect on the growth of different strains of *Candida albicans* at 15 µg/ml. In conclusion, the present study has demonstrated that *Candida albicans* was the most common isolates among oral candida infection, also the incidence of oral candida infection between poor control diabetic patients and smokers was higher than in a good control diabetic patients and non-smokers. Ethanolic plant extract of Cinnamon and Clove were recorded higher inhibitory activity to all tested *Candida* species.

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12/1/2014