مجلـــــة السعيد للعلـــــوم الإنســـانيـــة والتطبيقية
Al - Saeed Journal of Humanities and Applied Sciences
journal@alsaeeduni.net
Vol (6), No(1), January, 2023 المجلد(6)، العدد(1)، 2023م
ISSN: 2616 – 6305 (Print) ISSN: 2790-7554 (Online)

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تاريخ تسليم البحث 2022/12/25م تاريخ قبوله للنشر 2023/1/22م

https://alsaeeduni.net/colleges/research-and-strategic/2017-03-10-08-03-59

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Abstract:

Background: Oral candidiasis is one of the most common oral disease of human caused by *Candida albicans*, and can cause superficial infection. **Objective:** The present study was done to evaluate the prevalence of *Candida albicans* infection in the oral cavity among dental clinic patients in Taiz city, Yemen. And determining the sensitivity of the isolated *Candida albicans* to some antifungal drugs and plant extracts. **Methods:** A total (200) specimens with oral candidiasis have been collected from dental patients by using the oral rinse technique, from October 2021 to January 2022. Diagnosed by dentist of clinics and hospitals, and identified by microbiological procedures. **Results:** Most of the isolates were *Candida albicans* representing in 101(50.5%) of total oral sample isolates. The non-Albicans species are the second most common representing in 51(25.5%). The remaining oral sample isolates are of non-yeast growth representing in 48(24%). Fluconazole and Itraconazole have been tested against 20 isolates of *C. albicans*. All the drugs, examined in *vitro*, show antifungal sensitivity that shows (80%). Plant extracts of *Salvadora persica* and *Commiphora myrrha* have been tested against 20 isolates of *C.albicans*. Four concentration of alcoholic extracts for investigated plants show antifungal activity in *vitro* better than aqueous extracts. **Conclusions:** *Candida albicans* were the most prevalent isolate from oral cavity of dental patients with oral candidiasis. Plant extracts of *Salvadora persica* and *Commiphora myrrha* show antifungal activity in *vitro*.

Keywords: *C. albicans*, non-Albicans, Oral Candidiasis.

انتشار عدوى المبيضات البيضاء في التجويف الفموي بين مرضى عيادات الاسنان في مدينة تعز، اليمن

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الملخص

داء المبيضات الفموي هو أحد أكثر أمراض الفم شيوعًا في الانسان والتي تسببها المبيضات البيضاء، ويمكن لهذه الفطريات أن تسبب التهابات ابتدأ من الالتهابات السطحية للجلد إلى الالتهابات الجهازية التي تهدد الحياة. **هدف البحث:** أجريت الدراسة لتقييم مدى انتشار عدوى المبيضات البيضاء في تجويف الفم بين مرضى عيادات الأسنان في مدينة تعز، اليمن. وتحديد حساسية المبيضات البيضاء المعزولة لبعض الأدوية المضادة للفطريات وبعض المستخلصات النباتية. **الطريقة:** تم جمع (200) عينة من مرضى الاسنان المصابين بداء المبيضات الفموي باستخدام تقنية غسول الفم (المضمضة)، خلال الفترة من شهر أكتوبر 2021 إلى شهر يناير 2022. تم تشخيصها من قبل أطباء الاسنان في العيادات والمستشفيات، وتم تعريفها ميكروبيولوجيا في المختبر من خلال مجموعة من الفحوصات. **النتائج:** معظم العزلات كانت فطريات المبيضات البيضاء ممثلة في 101 (50.5٪) من مجموع العينات الفموية. الأنواع غير المبيضات البيضاء كانت ثاني أكثر الأنواع شيوعًا بنسبة 51 (25.5٪). أما بقية عزلات العينات الفموية فأظهرت عدم نمو للفطريات (الخمائر) بنسبة 48 (24٪). تم اختبار مضادين للفطريات هما الفلوكونازول والإيتراكونازول على20 عزلة من فطريات المبيضات البيضاء. أظهرت جميع المضادات الفطرية التي تم فحصها في المختبر حساسية ضد الفطريات بنسبة (80٪). تم اختبار اثنين من المستخلصات النباتية لنباتي السواك والمر على20 عزلة من المبيضات البيضاء. أظهرت أربعة تراكيز من المستخلصات الكحولية للنباتات التي تم فحصها نشاطًا مضادًا للفطريات في المختبر أفضل من المستخلصات المائية. **الاستنتاجات:** فطريات المبيضات البيضاء كانت الأكثر انتشارا في التجويف الفموي بين مرضى الأسنان المصابين بداء المبيضات الفموي. اظهرت المستخلصات النباتية للسواك والمر نشاطًا مضادًا للفطريات في المختبر.

الكلمات المفتاحية: المبيضات البيضاء، الأنواع غير البيضاء، داء المبيضات الفموي.

1. INTRODUCTION

The oral cavity is a suitable place for the presence of many oral flora, which may be natural inhabitants and coexist with some of them. When there is any imbalance in this relationship or the presence of other predisposing factors, they turn into the pathogen (Leung *et al.*, 2013).

Candida is a fungus and was first isolated in 1844 from the sputum of a tuberculosis patient (Mandell *et al*., 1994). *Candida sp* belong the Eumycota kingdome, family of Ascomycota, according recent classification by Kidd *et al*. (2016). The *Candida* species are Gram positive, budding yeast cell that harvestqs pseudo hyphae both in culture and in tissues and excretions that they exhibit filamentous morphology in the saprophytic phase, grow at 37 °C. Mono-cell yeasts with small (4-8μm) ovoid cells, polymorphic. *Candida* species produce well on most culture media and usually do not need special media to growth (Ogba *et al.*, 2013).

Oral candidiasis is a superficial opportunistic infection of the oral cavity by *Candida albicans*, first described in 1838 by pediatrician Francois Veilleux. Local and systemic factors facilitating the development of the disease (Scully *et al*. 1994). It is one of the common fungal infection, affecting the oral mucosa. These lesions are caused by the yeast *Candida albicans* which is the most common *Candida species* isolated from the oral cavity in both healthy and diseased states (Vila *et al*., 2020).

The most prevalent *Candida* species isolated from the oral cavity is *Candida albicans* (Cannon *et al*., 1995; and Coronado-Castellote, & Jiménez-Soriano, 2013). *C. albicans* is a polymorphic organism because it can grow in yeast, hyphal and pseudo-hyphal modes and may produce chlamydospores in certain growth conditions (Odds. 1988 reviewed by Cannon and Chaffin., 1999).

Many researchers referred to prevalence of oral candidiasis in the mouth both in normal and pathological state. However, there is no available information about the prevalence of oral candidiasis in Yemen, to the best of our knowledge.

There are two main groups of antifungal drugs; the polyene macrolides, and azole derivatives. In order to successfully treat oral candidiasis, however, if the yeast responsible for the candidiasis develops antifungal drug resistance. Resistance to imidazoles and triazoles, particularly

fluconazole, is more common than to polyenes, allylamines and echinocandins (Cannon *et al*., 2009 and Jorgensen, 1980).

Many studies noted the effect of plant extract on *Candida albicans* as *Salvadora persica* and *Commiphora myrrha*. Result of a variety of chemical components which have been identified in plants extracts (Bruneton, 1993; Al Bagieh, 1994; Almas *et al*., 1997 and Abdelrahman *et al*., 2003). This may be considered to be used as an alternative treatment.

The limited studies that deal with prevalence of *Candida albicans* among dental patients in Yemen in general and the city of Taiz in particular, was one of the most important reasons for carrying out the present study. In addition, there is a problem in diagnosing oral infections by dentists in Yemen. Confusion between oral candidiasis with oral bacterial infection, can cause a deficiency in the treatment process. There are global reports indicating the increasing phenomenon of *Candida albicans* resistance to antibiotics (Cannon *et al*., 2009 and Jorgensen, 1980), and the possibility of using some plant extracts as an alternative treatment to antibiotics.

The aim of this study is to evaluate the prevalence of *Candida albicans* infection in the oral cavity among dental clinic patients in Taiz city, Yemen. So, the specific objectives of the present investigations have been designed to study estimating the prevalence of *Candida albicans* that cause oral disease of human regarding sex and age of patients in Taiz city, Yemen, and determining the sensitivity of the isolated *Candida albicans* to some antifungal drugs and plant extracts, in *vitro*.

2. MATERIALS & METHODS

2.1. Clinical Diagnosis and Sampling

2.1.1. Ethical Approval

The study proposal was evaluated and approved by the Ethics Committee for Scientific Research of Faculty of Medicine and Health Sciences, Taiz University, reference number: ECTU2022/2-4.

2.1.2. Patients

This study has been carried out within 4 months period starting from October 2021 to January 2022, including 200 outpatient cases with oral candidiasis infection. Diagnosed by dentist of clinics and hospitals, admitted to Hospital and several dental clinics in Taiz city, Yemen.

2.1.3. Samples Collection

A total (200) specimens of oral candidiasis have been collected from dental patients by oral rinse technique. Each case was asked to rinse the mouth for 60 seconds with 10 ml of sterile phosphate-buffered saline (PBS; 0.01M phosphate-buffered saline solution, pH 7.2) and expectorate the wash into a 50 ml sterile container. The samples were immediately transported to the microbiology laboratory (Coulter *et al*. 1993).

2.2. Identification Process

The mycological identification was based on macroscopic and microscopic examination of the culture isolates. The macroscopic examination of isolated species was characterized by duration of growth, surface morphology and pigment production on the reverse (Emmons *et al*. 1977; Kidd *et al*. 2016; & Campbell *et al*. 2013).

2.2.1. Direct Examination

All isolates were grown on Sabouraud dextrose agar plates, exanimated microscopically, were placed on clean slide mounted with cover-slip and then the slide wormed mildly and then examined under the microscope looking for Candida budding cells. Moreover the isolates were stained with Gram stain to detect their reply to stain (Saranya *et al*. 2014 & Yan *et al*. 2013).

2.2.2. Morphological Tests:

All isolates were grown Sabouraud dextrose agar plates (SDA) and incubated at 37 °C for 24- 48 hrs. to isolate the *Candida* colonies are pure to study their shape, size, and color and texture. This is according to (Saranya *et al*. 2014; Kidd *et al*. 2016 & Campbell *et al*. 2013).

2.2.3. Germ Tube Production:

C. albicans produce true germ tubes (long tube-like projection extending from the yeast cells) when incubated 2 hrs. in serum at 37 °C (Ahmad *et al*. 2010 & Reiss *et al*. 2012).

2.2.4. Hypertonic SDA

Microbiological tests to confirm identification as growth on Hypertonic Sabouraud Agar was produced as described by Alves *et al*. (2002). Isolates from a 48 hrs. culture on Sabouraud Dextrose agar plates were inoculated on SDA supplemented with 6.5% NaCl and then statically incubated for 96 hrs. at 37 °C.

2.2.5. Growth at 45 ºC:

Isolates of *Candida sp* tested for abilities to growth on SDA at 45 °C and monitoring daily for 10 dayes. Growth at 45 °C was considered a useful test for differentiation *C. albicans* from *C. dubliniensis*, because *C. dubliniensis* (no growth) from *C. albicans* (growth) as described by Pinjon *et al*. (1998).

2.3. Antifungal drugs:

A total (20) isolates of *Candida albicans* from the oral infection were tested for sensitivity against tow antifungal agents (HiMedia, India), Fluconazole 10 mcg and itraconazole 30 mcg, using agar disc diffusion method. Overnight grown *Candida albicans* was first adjusted to 0.5 McFarland turbidity standards (1.0×10^8 colony forming units/ml) and seeded onto Sabouraud Dextrose agar plates with a sterile cotton swab. Plates were then left at room temperature for 15 min (Chaudhary *et al*. 2022).

2.4. Plant Extracts:

A total (20) isolates of *Candida albicans* from the oral infection were tested for sensitivity against tow of plant extracts using Agar Well Diffusion Technique as described by Cui *et al.* (2021). Aqueous extracts and alcoholic extracts of *Salvadora persica* and *Commiphora myrrha*, were collected from traditional Yemeni marketing at Taiz city. All the plant extracts prepared from Aqueous extracts and alcoholic extracts. Plant samples were dried in the oven at 50 °C for 48 hrs. and then powdered. Twenty grams of this powder were soaked in 200 ml of each of the solvents namely distilled water, ethanol or methanol for 24 hrs. Media was used to evaluate the antifungal activity as Sabouraud Dextrose Agar was used for yeast as described by Koleva *et al.* (2002).

2.5. Data Analysis:

Data were statistically analyzed using the SPSS program version 21 The difference in distribution of the *Candida albicans* between groups was based on comparison of frequency distributions by a chi-square test. p value < 0.05 was considered significant.

3. RESULTS & DISCUSSION

3.1. Microbiological Analysis

3.1.1. Identification of *C. albicans*

A total of 200 specimens was identified from cases of oral infection among dental patients, during the period from October 2021 to January 2022, contingent on the morphological features on culture medium (Sabourauds dextrose agar and hypertonic sabourauds dextrose agar), germ tube formation, growth at 45 ºC and gram stain. Most of the isolates were *Candida albicans* representing in 101(50.5%) of total oral sample isolates. The non-Albicans species are the second most common representing in 51(25.5%). The remaining oral sample isolates are of non-yeast growth (negative cases), representing in 48(24%) of the total oral sample isolates, so the results of the study show prevalence of *Candida albicans* in the oral cavity compared to non-Albicans species (Table 1; Fig.1,2).

Table (1): Microbiological analysis of 200 cases investigated during the period from October 2021- January 2022.

Result of Identification	Identification Process					
	Direct Ex.		Growth on culture media		Germ tube production	Growth at 45 ºC
	Wet preparation (yeast cells)	Gram stain (G+)	SDA	Hypertonic SDA		
C. albicans	101	101	101	101	101	101
Non-Albicans	51	51	51	0	4	0
No-yeast growth	0	0	0	NA	NA	NA
Total	152	152	152	101	105	101

NA: no analysis **SDA**: Sabouraud Dextrose Agar.

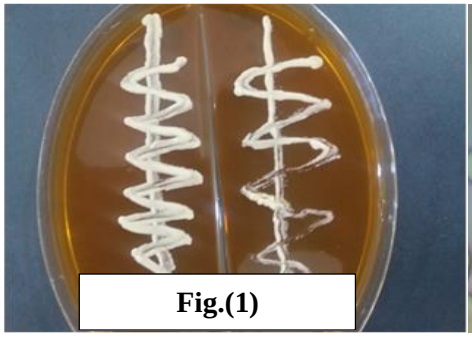

Fig.(1)

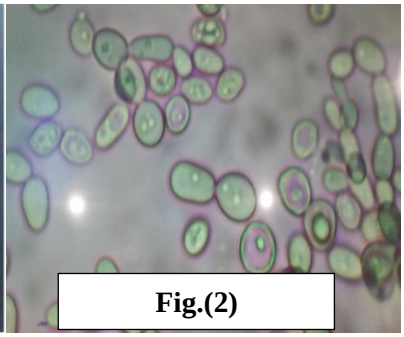

Fig.(2)

Fig. (1*): Candida albicans* colonies growth on (SDA)
Fig. (2): Wet preparation for *Candida albicans*

Our results about microbiological identification are similar to those obtained by Odds (1991) who reported that the most frequently used primary isolation medium for *Candida* is SDA which, although permitting growth of *Candida*, suppresses the growth of many species of oral bacteria due to its low pH. Akgül & Çerikçioğlu (2009) found that none of the phenotypic methods, except for the modified salt tolerance test, revealed 100% successful results in discrimination of *C. albicans* and *C. dubliniensis* species. It is suggest that in the case of absence of PCR and other identification systems, these two phenotypic tests can be used in routine laboratories to obtain a presumptive result. The germ-tube test is the standard laboratory method for identifying *C. albicans*. The test involves the induction of hyphal outgrowths (germ tubes) when subcultured in horse serum at 37 ºC for 2–4 hrs. Approximately, 95% of *C. albicans* isolates produce germ tubes, a property also shared by *C. stellatoidea* and *C. dubliniensis* (Williams and Lewis, 2000).

About detection of growth at 45 ºC our results are similar to those obtained by Sullivan *et al*.(1995) that reported that *C. dubliniensis* can grow well at 30 ºC and 37 ºC, producing creamy white colonies on solid media similar to *C. albicans*. However, differing from *C. albicans*, it grew poorly or was unable to grow at 42 ºC on SDA or potato dextrose agar. In contrast to this, Pinjon *et al*. (1998) found that 9.2% of the *C. dubliniensis* isolates showed partial growth at 42 ºC after 48 hrs. but that none of the 120 *C. dubliniensis* isolates tested grew at 45 ºC after 48 hrs. In the case of *C. albicans*, all 98 isolates grew at 42 ºC and 97 grew at 45 ºC. Similar results regarding the inability of *C. dubliniensis* isolates to grow at 45 C were obtained by other researchers (Fotedar & Al Hedaithy, 2003; Musleh & Al-Saadi, 2022).

However, our result is different from that of Kurzai *et al*. (2000) that reported that 15.9% of *C. albicans* isolates also failed to grow at 45 ºC. In addition, Gales *et al*. (1999) reported that 23% of the tested *C. albicans* isolates failed to grow at 45ºC. Kirkpatrick *et al*. (1998) reported an even higher percentage of *C. albicans* isolates (36%) that failed to grow at 45 ºC. Pincus *et al*. (1999) stated that these variable results may be explained by poor temperature control in incubators or differences in media composition.

3.2. General Analysis of Oral Infection:

3.2.1. Prevalence of *Candida albicans* in the Samples Population:

A total of 200 dental patients infected by oral infection has been collected from October 2021 to January 2022. Among the examined specimens 152 samples (76% of total specimens), have oral candidiasis by *Candida albicans* and non-Albicans species. Among the positive oral candidiasis, 101 (50.5% of total specimens) are infected by *Candida albicans*. Whereas 51(25.5% of total specimens) are infected by non-Albicans species. While the remaining specimens 48(24%) have shown no yeast growth - negative cases (Fig.3). These results are similar to those obtained by Rindum *et al.* (1994). This study comprised 100 healthy dentate adults and 53 patients with oral candidiasis. The isolated yeasts were identified to species level. Strain phenotypes of 147 *Candida albicans* isolates.

Martins *et al*. (2010), studied oral *Candida* carriage of patients attending a dental clinic in Braga, Portugal, a total of 97 patients were analyzed. It showed that, *C. albicans* was identified in 79% of the samples, being the predominant *Candida* species. Muadcheingka & Tantivitayakl (2015) investigated the prevalence of *Candida albicans* and non-albicans *Candida* (NAC) species from oral candidiasis patients in 207 oral candidiasis patients. The results are: *C. albicans* (61.6%) was still the predominant species in oral candidiasis patients with and without denture wearer, respectively, followed by non-albicans *Candida* (NAC) species. The proportion of mixed colonization with more than one *Candida* species was 18% from total cases.

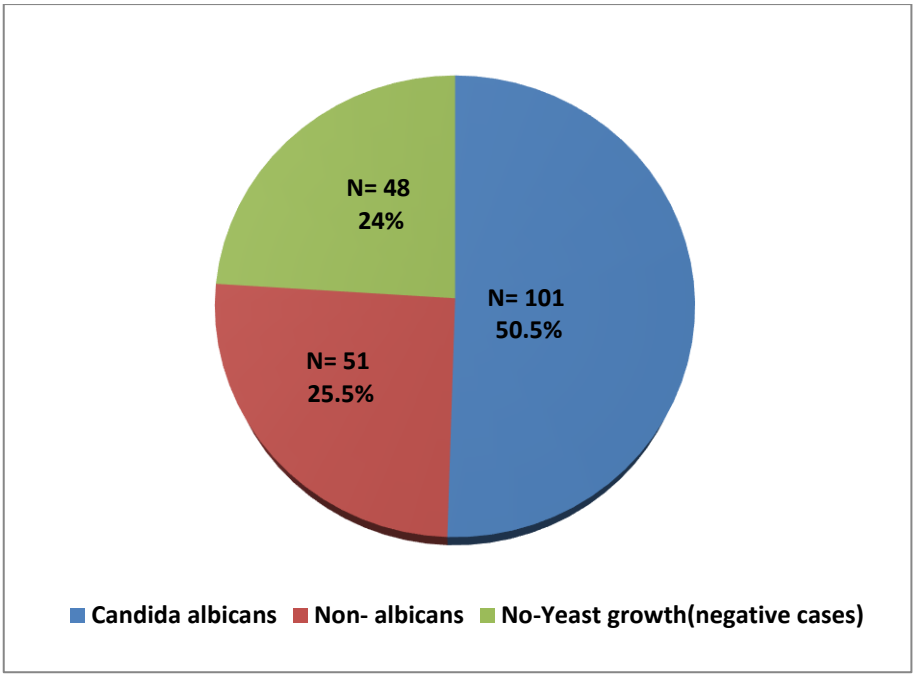

Figure (3): Distribution of *Candida albicans* among Sample of Dental Patients.

Similar observation about the prevalence of *Candida albicans* on oral cavity reported by Alrayyes *et al*. (2019) who observed that out of the 104 subjects examined, 45 (43.4%) were found to be colonized with *Candida spp*. *C. albicans* was the most frequently encountered being isolated from 23 (21.9%) individuals and constituting (55.64%) of the total isolates. Other *Candida* species were also isolated though less frequently as *C. glabrata* and *C. krusei* both being found in 5 (11%), 4 *C. dubliniensis* (8.9%), 3 *C. parapsilosis* (6.7), 2 *C. tropicalis* (4.4%), and 1 (2.2%) *C. famata*.

3.2.2. Incidence of Oral Candidiasis In Relation to the Sex of Patients:

The microbiological analysis of oral infection patients in relation to sex of dental patients is illustrated in fig. (4). The results of *Candida albicans* infection were indicated that the female patients are highly than of male patients. This has occurrence in 56.4% of female, and 43.6% of male patients. Results of chi-square test show that there is no statistically significant association between sex of dental patients and *Candida albicans* infections P=0.679 (P>0.05) (Fig. 4). Similar observations have been reported by Martins *et al*. (2010) who indicated that from the 97 patients evaluated, 53 were identified as oral *Candida* carriers: 81.1% were females

(n=43), and 18.9% males (n=10). There was no association between *C. albicans* or NCAC species carriage within gender (P=0.7). Muadcheingk & Tantivitayakl (2015) found that the distribution of *C. albicans* and non-albicans *Candida* species isolated from oral candidiasis patients relation to gender was in females 108 isolates *Candida albicans* species and 66 isolates Non-albicans *Candida* species, while in males, it was 46 isolates *Candida albicans* species and 30 isolates Non-albicans *Candida* species.

However, our result is different from that reported by Sasikumar (2016) who noted that among 69 males, 14 were positive for *C. albicans* (20.30%) and 55 were negative for *C. albicans* (79.7%). Among 39 females, 6 were positive for *C. albicans* (15.4%) and 33 were negative for *C albicans* (84.6%). The results showed that the presence of *C. albicans* among males and females was not significant with (P= 0.528).

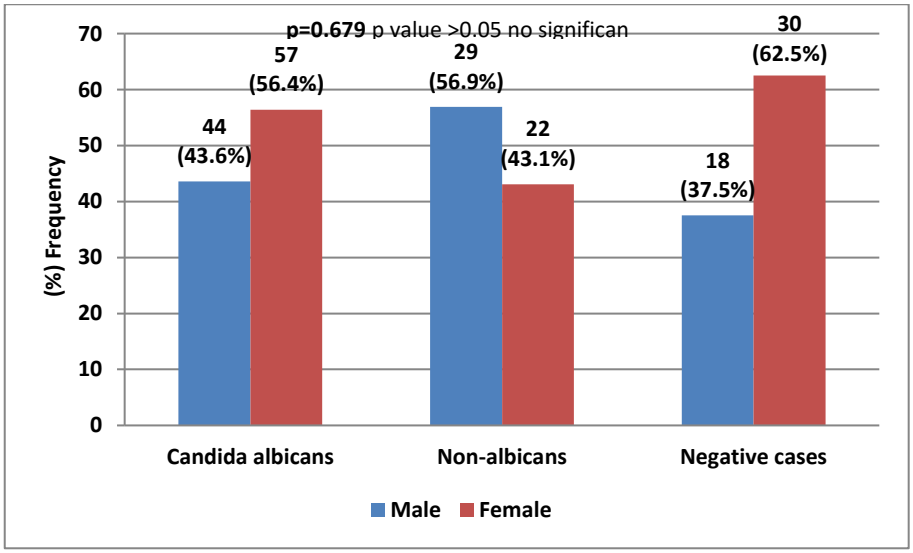

Figure (4): Incidence of Oral Infection in Relation to Sex of Patients.

3.2.3. Incidence of Oral Candidiasis in Relation to Age Patients:

The results of *Candida albicans* infection have indicated that the age group between 20-26 years is more susceptible and occurs in 17.8% of total *Candida albicans* infection cases, followed by age group of 55-61 years that appears in each 15.8%, and the rest ages between 34-40 and 48-54 years appear in each 14.9%, whereas age group 27-33 years appears in 10.9%, and age groups between 13-19 and 41-47 years are represented by 7.9%, and age group 62-68 years appear 6.9%, respectively. On the other hand, the age

group less than 12 years and more than 69 years are less susceptible (Fig. 5). Results of chi-square test from figure (5) have shown that there is a statistically significant association between different age groups of dental patients and *Candida albicans* infections (P<0.01) The current results are basically similar to those obtained by Al-Kebsi *et al.* (2017) who noticed that the results of *Candida albicans* indicate that the age was ranged from 20 – 27 years, with mean age ± SD equal to 22.1±2.1 years for female samples, and for male samples the mean age ± SD was 23.4±2.3 years. Most of the samples were in age group 20-22 years (43%) and in age groups 22-25 years were 32.8%, and samples in age group ≥ 26 years count only 24.2% of the total.

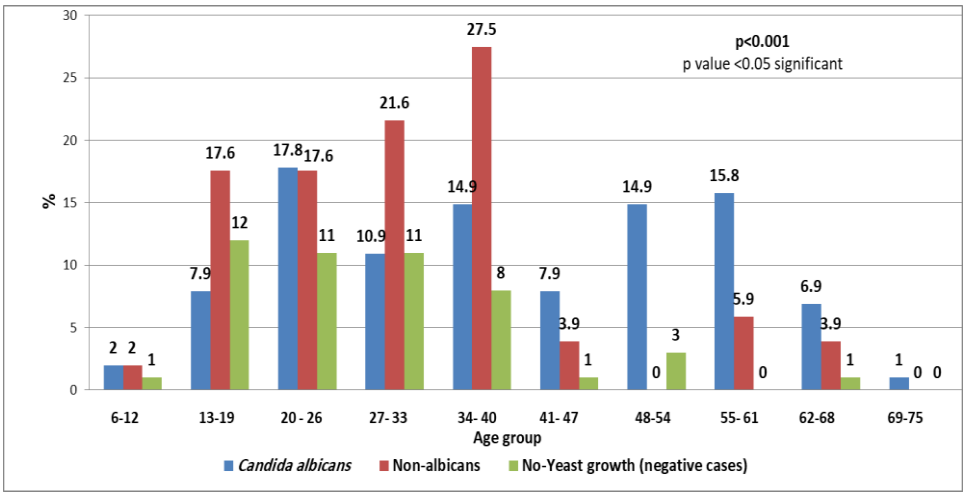

Fig. (5): Incidence of Oral Candidosis in Relation to Age of Patients.

Our result is different from that reported from Philadelphia by Bouquot *et al*. (2002) in which no difference in the rate of mouth colonization by *Candida albicans* occurred with age, but similar to that reported from Yemen by Al-Kebsi *et al.* (2017) in which the highest rate occurred in 20-22 age groups.

3.2.4. Incidence of Patients' Education Levels in Relation to Oral Candidiasis:

Candida albicans infection is highly appeared in illiterate level patients represented in 87.5% of the tested number, followed by university or above education and secondary education (48.5% and 46.8%) respectively, whereas 35% of basic education patients, on the other hand, patients who

hardly can read and write are in 16.7% Table (2). Results of chi-square test from table (1) shown that there is a statistically significant association between education levels of dental patients and *Candida albicans* infections (P<0.05). Our result is different from that reported by Lyon *et al*. (2006) who reported that in terms of education, the percentage of *Candida* spp. carriers with fewer than 4 years of study was high among oral candidiasis patients and was statistically significant. Thus, education can be an important factor for the carrier state, as it may determine the degree of comprehension of instructions on hygiene, especially among dental patients.

Table (2): Incidence of Patients' Education Level in Relation to Oral Candidiasis.

Level of education	*C. albicans*		Non-albicans		Negative cases		Total cases		p-value
	F	%	F	%	F	%	F	%	
Illiterate	21	87.5	2	8.3	1	4.2	24	12	0.001
Read & write	1	16.7	2	33.3	3	50	6	3	
Basic ed.	7	35	6	30	7	35	20	10	
Secondary ed.	22	46.8	16	34.1	9	19.1	47	23.5	
University or above	50	48.5	25	24.3	28	27.2	103	51.5	
Total	101		51		48		200	100	

F: frequency p value <0.05 significant.

3.3. Antifungal activity:

Tow antifungal drugs commonly used in oral candidiasis have been tested against the 20 isolates of *Candida albicans* in the present work. The results indicate that the susceptibility of *Candida albicans* towards fluconazole (10mcg) has been recorded to have mean inhibition zone diameter 35.6 mm, among 20 isolates, 16 (80%) isolates shown as sensitive, whereas, 4(20%) isolates appeared as resistant. Itraconazole (30mcg) was recorded to have inhibition zone mean diameter 22.4 mm, 16(80%) isolates shown as sensitive, whereas 4(20%) isolates appeared as resistant Table (3). The current results are different to those obtained by Mahmoud *et al*. (2021) who found that 76.19 % (32/42) were Fluconazole resistant. Ahmed (2019) showed that a total of 83 isolates of *Candida albicans* were analyzed for their susceptibility to fluconazole. MIC assessment showed that the isolates were susceptible to antifungal compound. Lyon *et al*. (2006) reported that, 90% of the *C. albicans* isolates were inhibited by a concentration of 2.0 µg/mL of fluconazole. Among *C. albicans* isolates obtained from dental

wearers and among the total samples, MIC 90 (concentration that inhibits 90% of the isolates tested) reached 8.0 µg/mL. In *C. albicans* isolates, resistance to fluconazole was observed. Itraconazole showed strong inhibition towards *C. albicans* isolates (MIC90 = 0.06 µg/mL).

Table (3): The Mean of Inhibition Zone of 20 Isolates of *Candida albicans* to Antifungal Drugs.

Name of Drug	Disc Content	No. of Sensitive Isolates, %	No. of Resistant Isolates, %	The Mean of Inhibition Zoon (mm)
		N=20		Mean ± SD
Itraconazole	30 mcg	16(80%)	4(20%)	22.4± 3.8
Fluconazole	10 mcg	16(80%)	4(20%)	35.6± 5.5

SD.: Stander Deviation N: number of samples

Al-Shamahy *et al*. (2020) reported that all C*andida* species isolates were resistance to fluconazole, was found in 40% of *C. tropicalis* and 20% in *C. glabrata* and 13.8% in *C. albicans*, who found that fluconazole showed a good performance against the *C. albicans* isolates studied. Similarly, results obtained by Lyon *et al*. (2006) who demonstrated that 100% of *C. albicans* strains were susceptible to fluconazole. In a study performed by Resende and Resende (1999) itraconazole was the most efficient antifungal drugs for most species of *Candida* and fluconazole was the least efficient. In our study, itraconazole was effective against the *Candida albicans*.

3.4. Plant Extracts Activity:

The antifungal activities of plant extracts (*Salvadora persica* and *Commiphora myrrha*) investigated against 20 isolates of *Candida albicans* in the present work, the results indicated that the susceptibility of *Candida albicans* towards aqueous and alcoholic plant extracts. The mean of inhibition zone of aqueous extracts of *Salvadora persica* toward concentration of extract (50, 100, 150, 200) mg/ml is (5, 9.4, 17, 23.1) mm in diameter, whereas the mean of inhibition zone of alcoholic extracts toward the same concentrations appeared (15, 19.9, 24.7, 30.1) mm in diameter (Fig. 6).

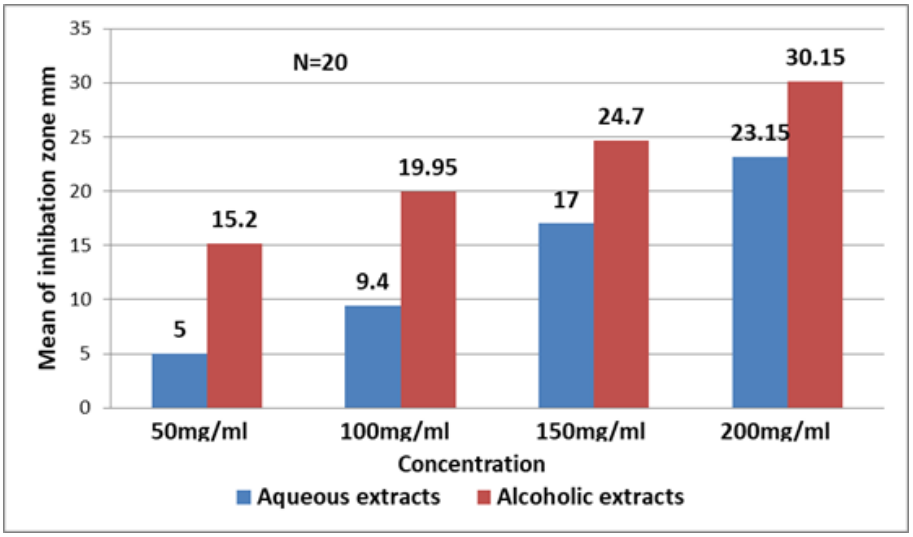

Figure (6): Response of 20 *Candida albicans* Isolates against *Salvadora persica* Extracts.

The current results are similar to those obtained by Noumi *et al*. (2010) & Aljarbou *et al*. (2022) who conducted to investigate the anti-candida activities of *S. persica* extracts. Antifungal effects are reported as inhibition zones and '*in vitro'*. The results obtained by using the disc diffusion method recorded in SDA, fresh *S. persica* extracts show significant antifungal activity against almost all of the tested *Candida albicans* strains. Overall, the best antifungal activity has been against *C. albicans* for dry *S.persica* diluted alcohol extract.

In our results the mean of inhibition zone of aqueous extracts of *Commiphora myrrha* toward concentration of extract (50, 100, 150, 200) mg/ml is (1, 6.05, 14.8, 20.5) mm in diameter, whereas the mean of inhibition zone of alcoholic extracts toward the same concentration appeared (15.5 21, 26.2, 31.4) mm in diameter (Fig. 7). Similer observation by Al-Abdalall (2016) found that the results showed that two types of the Myrrha (*Commiphora myrrha* and *C. molmol*) aqueous extracts inhibited all the tested microbes including *C. albicans*. Also, the alcoholic extract had inhibitory effect on the growth of three pathogenic tested isolates including *C. albicans*. By performing the chemical analysis for the Myrrha, it was noted that it contains three components known for their antimicrobial effect. Abdallah *et al*. (2009) & Al Ahmadi (2006) stated that the *Commiphora* resins are rich in furanosesquiterpenoids compounds in a total number of 20

different compounds from such type. The separated compounds extracts of *Commiphora* resin have showed activity that resists the fungi.

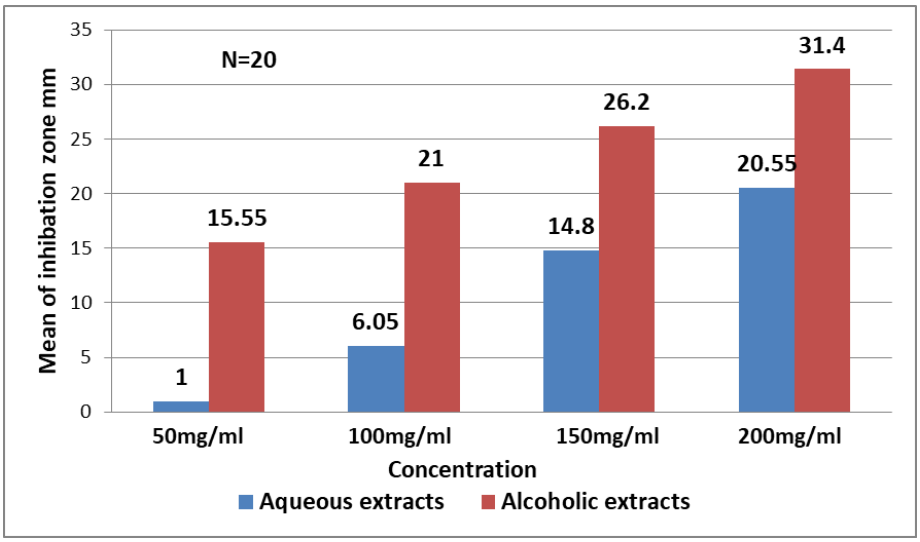

Figure (7): Response of 20 *Candida albicans* Isolates against *Commiphora myrrha* Extracts.

CONCLUSION

The prevalence of *Candida albicans* in the oral cavity among dental patients was evaluated in 200 patients. Among the examined specimens 152 sample (76% of total specimens), have oral candidiasis by *Candida albicans* and non-Albicans species. Among the positive oral candidiasis, 101 (50.5% of total specimens) are infected by *Candida albicans*. Whereas 51(25.5% of total specimens) are infected by non-Albicans species. While the remaining specimens 48(24%) have shown no yeast growth (negative cases). *Candida albicans* infection have indicated that the female patients are highly than of male patients. This has occurred in 56.4% of female, and 43.6% of male patients. The highest incidence of *C. albicans* was in the age group (20-26) years, while less than 12 years (children) and more than 69 years are less susceptible were least infected. *Candida albicans* infection are highly appeared in illiterate level patients, on the other hand, patients who hardly can read and write were least infected. The antifungal drugs (Fluconazole and Itraconazole), examined *in vitro*, show antifungal sensitivity that shows (80%). All the concentration of alcoholic extracts for investigated plants (*Salvadora persica* and *Commiphora myrrha*) show antifungal activity in vitro better than aqueous extracts.

REFERENCES

Abdallah EM., Khalid AS., & Ibrahim N. (2009). Antibacterial activity of oleo-gum resins of *Commiphora molmol* against methicillin resistant *Staphylococcus aureus*. *Sci. Res. Essay*; 4:351-356.

Abdelrahman HF., Skaug N. & Francis GW. (2002). *In vitro* antimicrobial effects of crude Miswak extracts on oral pathogens. *Saudi Dental Journal*; 14: 26-32.

Ahmad I., M. Owais., M. Shahid., & F. Aqil. (2010). Combating Fungal Infections: Problems and Remedy. *Springer, New York*, USA.

Ahmed, L. T. (2019). Genotyping and Antifungal Susceptibility of *C. albicans* Isolated from Infected women. *Research Journal of Pharmacy and Technology*, 12(11), 5171-5176.

Akgül Ö., & Çerikçioğlu N. (2009). Hypertonic sabouraud dextrose agar as asubstrate for differentiation of *Candida dubliniensis*. *Mycopathologia*, 167(6), 357-359.

Al Ahmadi SM. (2006). Chemical analysis and study the biological activity of gums resin oil of *Commiphora myrrh*. M.Sc. Thesis in King Abdulaziz University Jeddah.

Al-Abdalall A. H. A. (2016). Effect of plants extracts on the growth of *Candida albicans* and *Staphylococcus aureus*. *African Journal of Pharmacology*, 10(16), 337-345.

Al-Bagieh NH., Idowu A., & Salako NO. (1994). Effect of aqueous extract of miswak on the *in vitro* growth of *Candida albicans*. *Microbios*; 80: 107–113.

Aljarbou F., Almobarak A., Binrayes A., & Alamri H. M. (2022). *Salvadora persica*'s Biological Properties and Applications in Different Dental Specialties: A Narrative Review. *Evidence-Based Complementary and Alternative Medicine*.

Al-Kebsi AM., Othman MO., & Al-Shamahy HA. (2017). Oral *C. albicans* colonization and non-*candida albicans candida* colonization among university students, Yemen. *Universal J Pharm Res*. 2(5):5-11.

Almas K., Al-Bagieh N. H., & Akpata E. S. (1997). In vitro antimicrobial effects of extracts of freshly cut and 1-month-old miswak chewing stick. *Biomedical Letters* (United Kingdom).

Alrayyes S. F., Alruwaili H. M., Taher I. A., ElrahawyK. M., Almaeen A. H., Ashekhi A. O., & Alam M. K. (2019). Oral Candidal carriage and associated risk indicators among adults in Sakaka, SaudiArabia. *BMC Oral Health*, 19(1), 1-7.

Al-Shamahy H. A., Al-labani M. A., & Al-akwa A. A. (2020). Biofilm formation & antifungal susceptibility of *Candida* isolates from oral cavity of denture wearer and free denture individuals. *EC Dental Sci*, 19(10), 58-66.

Alves SH., Milan EP., de Laet Sant'Ana P., Oliveira LO., Santurio JM., & Colombo AL. (2002). Hypertonic sabouraud broth as a simple and powerful test for *Candida dubliniensis* screening Diagn *Microbiol Infect Dis*. 2002; 43:85–6. doi:10.1016/ S0732-8893(02)00368-1.

Bouquot Brad W. Neville., Douglas D. Damm., & Carl M. Allen Jerry E. (2002). Oral and maxillofacial pathology (2nd. ed.). Philadelphia:*W.B.Saunders*. 189–197.

Bruneton J. (1993). Pharmacogosie, phytochimie, plantes médicinales. Technique & Documentation, *Lavoisier, Paris*, p 348.

Calderone R. A., & Gow N. A. R. (2002). Host recognition by *Candida* species. In Candida and Candidiasis, pp. 67-86. Edited by R. A. Calderone. Washington DC, USA: *American Society for Microbiology (ASM) Press*.

Campbell C. K., & Johnson E. M. (2013). Identification of pathogenic fungi. *John Wiley & Sons*.

Cannon R. D., & Chaffin W. L. (1999). Oral colonization by *Candida albicans*. *Critical Reviews in Oral Biology & Medicine*, 10(3), 359-383.

Cannon R. D., Holmes A. R., Mason A. B., & Monk. B. C. (1995). Oral *Candida*: clearance, colonization, or candidiasis?. *Journal of dental research*, 74(5), 1152-1161.

Cannon R. D., Lamping E., Holmes A. R., Niimi K., Baret P. V., Keniya M. V., & Monk B. C. (2009). Efflux-mediated antifungal drug resistance. *Clinical Microbiology* Reviews, 22(2), 291–321. doi:10.1128/ CMR.00051-08.

Chaudhary P., Basnet, S., Chaulagain M., Khadgi A., & BK, S. (2022). P350 Oropharyngeal candidiasis in HIV infected patients: associated risk factors and antifungal susceptibility testing of *candida* species by disc diffusion method. *Medical Mycology*, 60(Supplement_1),

myac072P350. patients with denture stomatitis. J. Contemp. Dent. Pract. 13, 456–459.

Chauhan N., Dongmei L. I., Singh P., Calderone R. A., & Kruppa M. (2002). The cell wall of Candida spp. In *Candida* and Candidiasis, pp. 159-175. Edited by R. A. Calderone. Washington, DC: *American Society for Microbiology (ASM) Press.*

Coronado-Castellote L., & Jiménez-Soriano Y. (2013). Clinical and microbiological diagnosis of oral candidiasis. *Journal of clinical and experimental dentistry*, 5(5), e279.

Coulter WA., Kinirons MJ., & Murray SD. (1993). The use of a concentrated oral rinse culture technique to sample oral candida and *lactobacilli* in children and the relationship between *Candida* and *Lactobacilli* levels and dental caries experience: A pilot study. *Int J Paediatr Dent* 3(1): 17- 21.

Cui, Z. H., He, H. L., Wu, S. B., Dong, C. L., Lu, S. Y., Shan, T. J., & Sun, J. (2021). Rapid screening of essential oils as substances which enhance antibiotic activity using a modified well diffusion method. *Antibiotics*, 10(4), 463.

Dangi YS., Soni MS., & Namdeo KP. (2010). Oral candidiasis: A review. *Int J Pharm Pharm Sci*. 2:36-41.

Douglas L. J. (2003). *Candida* biofilms and their role in infection. *Trends Microbiol* II,30-36.

E. Noumi., M. Snoussi., H. Hajlaoui., E. Valentin., A. Bakhrouf. (2010). Antifungal properties of *Salvadora persica* and *Juglans regia L*. extracts against oral *Candida* strains. *Eur J Clin Microbiol Infect Dis*. 29:81–88, DOI 10.1007/s10096-009-0824-3.

Emmons C. W., C. H. BinfordJ. P. Utz., & K. J. Kwon-Chung. (1977). Medical Mycology. 3rd ed. *Lea and Febiger*. Philadelphia, PA.

Fotedar R., & Al Hedaithy S. S. A. (2003). *Candida dubliniensis* at a university hospital in Saudi Arabia. *Journal of clinical microbiology*, 41(5), 1907-1911.

Gales AC., Pfaller MA., & Houston AK. (1999). Identification of *Candida dubliniensis* based on temperature and utilization of xylose and a-methyl-D-glucoside as determined with the API 20C AUX and Vitek YBC systems. *J Clin Microbiol*. 37: 3804–8.

Hoyer L. L. (2001). The ALS gene family of *Candida albicans*. *Trends Microbiol* 9, 176-180.

Jorgensen A. (1980). Pharmacokinetic studies in volunteers of intravenous and oral cis (Z)-flupentixol and intramuscular cis (Z)-flupentixol decanoate in viscoleo®. *European journal of clinical pharmacology*, 18(4), 355-360.

Kidd S., Halliday C. L., Alexiou H., & Ellis D. (2016). Descriptions of medical fungi. Vol. 3, pp. 34-50.

Kirkpatrick WR., Revankar SG., & McAtee RK. (1998). Detection of *Candida dubliniensis* in oropharyngeal samples from human immunodeficiency virus-infected patients in North America by primary CHROMagar *Candida* screening and susceptibility testing of isolates. J Clin Microbiol. 36: 3007–12.

Koleva I. I., Van Beek T. A., Linssen J. P., Groot A. D., & Evstatieva L. N. (2002). Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochemical Analysis: *An International Journal of Plant Chemical and Biochemical Techniques*, 13(1), 8-17.

Kurzai O., Korting H-C., & Harmsen D. (2000). Molecular and phenotypic identification of the yeast pathogen *Candida dubliniensis*. *J Mol Med*. 78: 521–9.

Leung W. K., Dassanayake R. S., Yau J. Y., Jin L. J., Yam W. C., Manfredi M Polonelli L., Aguirre- Urizar JM., Carozzo M., & McCullough MJ. (2013). Urban legends series: oral candidosis. *Oral Dis*.19: 245−261.

Lyon J. P., da Costa S. C., Totti V. M. G., Munhoz M. F. V., & de Resende M. A. (2006). Predisposing conditions for *Candida spp*. carriage in the oral cavity of denture wearers and individuals with natural teeth. *Canadian journal of microbiology*, 52(5), 462-467.

Mahmoud D. E., Faraag A. H. I., & Abu El-Wafa W. M. (2021). In vitro study on the potential fungicidal effects of atorvastatin in combination with some azole drugs against multidrug resistant *Candida albicans. World Journal of Microbiology and Biotechnology*, 37(11), 1-13.

Mandell GL., Bennett JE., & Dolin R. (1994). Anti-fungal agents. Principles and practice of infectious diseases. 4th ed. New York: *Churchill Livingstone*; p. 401-10.

Martins M., Henriques M., Ribeiro A. P., Fernandes R., Gonçalves V., SeabraÁ., & Oliveira R. (2010). Oral *Candida* carriage of patients attending a dental clinic in Braga, Portugal. Revista *iberoamericana de micologia*, 27(3), 119-124.

Mayer FL., Wilson D., & Hube B. (2013). *Candida albicans* pathogenicity mechanisms. *Virulence*. 4(2): 119–128.

Muadcheingka T., & Tantivitayakul P. (2015). Distribution of *Candida albicans* and non-albicans *Candida* species in oral candidiasis patients: Correlation between cell surface hydrophobicity and biofilm forming activities. *Archives of oral biology*, 60(6), 894-901.

Musleh T. M. M., & Al-Saadi H. A. M. (2022). Diagnosis of yeasts isolated from the oral cavity and groin area in children of Kirkuk city/Iraq. *Tikrit Journal of Pure Science*, 27(4), 7-16.

Naglik J. R., Challacombe S. J., & Hube B. (2003). *Candida albicans* secreted aspartyl proteinases in virulence and pathogenesis. *Microbiol Mol Biol* Rev 67, 400-428.

Odds F.C. (1988). *Candida* and candidosis. 2nd ed. *Bailliere Tindall*, London, UK.

Odds. (1991). Sabouraud(’s) agar. Journal of Medical and Veterinary Mycology, vol. 29, pp. 355–359.

Ogba O. M., L.N. Abia-Bassey., J. Epoke., B.I. Mandor., & G.D. Iwatt. (2013). Characterization of *Candida* species isolated from cases of lower respiratory tract infection among HIV/ AIDS patients in Calabar, Nigeria. *World. J. AIDS*, 3(03):201.

Pincus DH., Coleman DC., & Pruitt WR. (1999). Rapid identification *of Candida dubliniensis* with commercial yeast identification systems. *J Clin Microbiol*. 37: 3533–39.

Pinjon E., Sullivan D., Salkin I., Shanley D., & Coleman D. (1998). Simple, inexpensive, reliable method for differentiation of *Candida dubliniensis* from *Candida albicans*. *J Clin Microbiol*. 36: 2093–5.

Prasanna KR. (2012). Oral candidiasis – A review. *Scholarly J Med*. 2:6-30.

Reiss E., H. J. Shadomy., & G. M. Lyon. (2012). Fundamental Medical Mycology. *Wiley-Blackwel*, Canada.

Resende J.C.P., & Resende M.A. (1999). In vitro antifungal susceptibility of clinical isolates of *Candida spp*. from hospitalized patients. *Mycoses* 42, 641–644.

Rindum J. L., Stenderup A., & Holmstrup P. (1994). Identification of *Candida albicans* types related to healthy and pathological oral mucosa. *Journal of oral pathology & medicine*, 23(9), 406-412.

Saranya, K. Moorthy., S. Malar., T. Punitha., R. Vinodini., M. Bhuvaneshwari., & C. Kanimozhi. (2014). Prevalence and Antifungal susceptibility pattern of *Candida albicanis* from Low Socio- Ecomic Group. *International Journal of Pharmacy and Pharmaceutical Sciences*, Vol 6 Issue 2:0975-1491.

Sasikumar P. L. (2016). Prevalence of *Candida albicans* in chronic periodontitis patients. Doctoral dissertation, Sri Ramakrishna Dental College and Hospital, Coimbatore.

Scully C., Ei-Kabir M., & Samaranayake L. P. (1994). *Candida* and oral candidosis: a review. *Critical Reviews in Oral Biology & Medicine*, 5(2), 125-157.

Sullivan DJ., Westerneng TJ., Haynes KA., Bennett DE., & Coleman DC. (1995). *Candida dubliniensis sp*. nov.: phenotypic and molecular characterisation of a novel species associated with oral candidosis in HIV infected individuals. *Microbiology*. 141: 1507–21.

Terezhalmy GT., & Huber MA. (2011). Oropharyngeal candidiasis: Etiology, epidemiology, clinical manifestations, diagnosis, and treatment. Crest Oral-B at dentalcare.com Contin Educ Course. 1-16.

Vila T., Sultan, A. S., Montelongo-Jauregui D., & Jabra-Rizk M. A. (2020). Oral candidiasis: a disease of opportunity. *Journal of Fungi*, 6(1), 15.

Williams., & M. A. O. Lewis. (2000). Isolation and identification of *Candida* from the oral cavity, *Oral Diseases*, vol. 6, no. 1, pp. 3–11.

Yan L.J., N. Thangthaeng., N. Sumien., & M.J. Forster (2013). Serum dihydrolipoamide dehydrogenase is a labile enzyme. *J. biochem. Pharmacol. Res*., 1(1): 30**.**