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The inhibitory effect of the cloves *Syzygium aromaticum* on the growth of *Candida albicans*

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DOI:10.21608/jbaar.2024.335177.1106

Abstract

Samples were taken from people wearing dentures to investigate the presence of fungal isolates belonging to the genus *Candida*, which were diagnosed through a direct examination using crystal violet stain and the germ tube formation test was then conducted to identify the isolates belonging to *Candida albicans*. The Dalma technique was used to confirm the diagnosis. The effect of the antibiotic Nystatin on *Candida albicans* isolates was tested, and the minimum inhibitory concentration was determined. The IU5000 concentration achieved the highest percentage of inhibition. The effect of some washes used by denture wearers, such as water and salt water, was also tested, and the sodium hypochlorite solution at 1% was the best in inhibiting *Candida albicans*, with 90-100% percentage inhibition. Subsequently, the minimum inhibitory effect of the aqueous and alcoholic extracts of cloves on *Candida albicans* isolates was studied, which showed that the aqueous and alcoholic extracts of cloves at 15 and 50 mg/ml, respectively, achieved the best percentage of inhibition with 80-90%.

Keywords: *candida albicans*, *Syzygium aromaticum*, MIC.

Introduction

Yeasts are widespread eukaryotic organisms. *Candida albicans* is an opportunistic commensal of warm-blooded organisms, including humans. They colonize the mucosal surfaces of the oral cavity, vagina, and gastrointestinal tract [1]. Therefore, *Candida albicans* infection is infrequent in healthy individuals. Candidiasis can be categorized into superficial infections (e.g. oral and vaginal thrush), chronic infections (e.g. mucocutaneous candidiasis), and deep infections (e.g. myocarditis and acute septicemia) that mainly represent clinical problems due to several reasons, including immunosuppressive therapies [2], long-term

catheterization, use of broad-spectrum antibiotics, and immunocompromized individuals [3-6]. Diseases caused by this genus can be avoided by using plant extracts that limit their growth and biological activity [7], and these substances are described as antifungals and are safer to use. One such substance is cloves, which are used for obtaining healthy and shiny teeth due to the mechanism of action of its various chemical components. Research indicates that it contains many medically useful properties, including antiseptics, detergents, and fluoride [8-10]. Cloves are used in some therapeutic applications, such as toothpaste and mouthwashes [11,12] Cloves and

their derivatives act as fungicidal agents by increasing cell permeability and changing the shape of cells of various types of fungi such as *Aspergillus niger*, *Candida albicans*, and types of dermatophytes [13]. Therefore, the current study aimed to collect oral swabs from people wearing dentures at different ages and investigate the presence of *Candida*. Diagnosis of *C. albicans* isolates was conducted using approved diagnostic methods, after which the effect of the antibiotic

Nystatin on *C. albicans* isolates was determined, the minimum inhibitory concentration (MIC) was determined, the effect of some mouthwashes used by denture wearers on *C. albicans* was studied and compared with the antifungal Nystatin. The study also included the detection of the ability of using the aqueous and alcoholic extracts of cloves as antifungals to inhibit *C. albicans*, to determine their MIC, and to compare them with the antifungal Nystatin.

Materials and methods

Sample collection

Samples were collected by taking an oral swab from 15 denture wearers of different ages ranging from 50 to 85 years old from a dental consulting clinic in Mosul. The following information was obtained from the respondents who took the samples.

☒ Gender	Male	Female
☒ Age		
☒ Having diabetes	Yes	No
☒ Is using any treatment regimen (specific to diabetes)?	Yes	No
☒ Does the sample suffer from chronic diseases?	Yes	No
☒ Does the sample suffer from other diseases?	Yes	No
☒ Does the sample suffer from gum infections?	Yes	No
☒ Is the individual a smoker?	Yes	No
☒ Dose the sample use antifungal or antibacterial agents?	Fungal	Bacterial
☒ Dose the sample use antibiotics for more than 2 weeks?	Yes	No
☒ What dose the sample use to clean his teeth?	Siwak	Water
☒ Type of denture	Upper	Lower

Yeast isolation

All swabs were cultured on Sabrouaud's glucose agar (SGA) medium supplemented with 100 mg/L chloramphenicol. The plates were incubated at 37 °C for 48 h. The growth of smooth, white, creamy, waxy, shiny, and convex yeast colonies was observed.

Diagnosis of *C. albicans*

Isolates belonging to the *Candida* genus were diagnosed using the following diagnostic methods.

Direct examination: This was performed by using the loop carrier from the yeast colonies growing on the (SGA) medium and spreading them on a glass slide, then staining with Gram stain, and examining them

microscopically to confirm diagnosis according to their shape and arrangement under an oil lens [14,15]

Germ Tube Test: This test was performed on 48-hour-old yeast colonies to identify *C. albicans* isolates by taking a load from a *C. albicans* colony using a loop vector to a glass vial containing 0.5 ml of human blood serum. The vials were then incubated at 37°C for 2-3 hours, then a drop of the sample was transferred to a glass slide, and a cover was placed. The sample was then examined under a microscope to observe the germ tube [16,17].

Dalmau plate technique:

This technique was used to diagnose *C. albicans* by taking a part of the fungal colony without touching

the agar using a culture needle, and two parallel lines were drawn on the surface of the CMTA medium. After sterilizing the culture needle, perpendicular lines were made on the previous two lines to dilute the number without scratching the culture medium, and finally, a cover slide was placed on it. The plates were incubated at 28°C for 24 h. The contact cover slide to the growing yeast colonies was lifted and placed on a glass slide, and a drop of lactophenol stain was placed on it for microscopic examination to observe the squamous spores [12,18,19].

Antibiotic sensitivity test:

The Disc Diffusion Method was used to conduct this test using the antibiotic Nystatin at a concentration of 500,000 IU. About (3-5) pure colonies were transferred to tubes containing normal saline solution, and their density was adjusted against that of a standard McFarland tube, which is equivalent to (1×10^8) cells/cm³. A sterile cotton swab was soaked in this suspension, and the excess was removed by rotating the swab on the inner walls of the tube. Then, the swab was lifted from the tube, the fungal suspension was spread on the glucose-Sabouraud medium, and the plates were left for (3-5) minutes to absorb and dry, then the antibiotic discs were fixed using sterile forceps, then the plates were incubated at (37°C) for 24 h. The inhibition zones forming at different concentrations of Nystatin, including (500000, 250000, 125000, 62500, 31250, 15625, 7812.5, and 3906.25) (IU), were measured to determine the (MIC) for use as an antifungal to *C. albicans* [2].

Sensitivity of *C. albicans* to mouthwashes

Several types of mouthwashes used by denture users were used including (water, water and salt, water and bleach together, Zak solution, Biofresh, Genstein's follicle stain, and sterile tablets). The drilling method involved making holes with a diameter of 6 mm on the surface of the SGA medium using a Coor power cork drill. A pure yeast colony was transferred to tubes containing nutrient broth medium NB and incubated for 24 h at 37°C. The density of the

colonies was compared to that of a standard McFarland tube (1×10^8 cells/cm³). A sterile cotton swab was soaked in this suspension, and the excess was removed by rotating the swab on the inner walls of the tube. The swab was then lifted from the tube. The yeast suspension was spread on the SGA medium, and the plates were left for (3-5) minutes to absorb and dry. Then, 0.1 ml of the wash was placed in the hole. The plates were then incubated at 37°C for 24 h. The diameter of the inhibition zone around the hole was measured to determine the effect of washes on the growth of yeast, and the results were compared with those of the antifungal Nystatin [20,21].

Preparation of clove extracts

Preparation of the aqueous extract

The aqueous extract of clove buds was prepared according to the [21], method by mixing (40) g of clove plant with a sufficient amount of distilled water, crushing the sample with a blender inside an ice bath, stirring the mixture using a stirrer magnatisium for (60) min, and leaving it at (5) ° C for 72 h for soaking. The sample was then filtered through several layers of gauze and centrifuged at 3000 rpm for 5 min. Another filtration was conducted using a Buechner funnel with filter papers (Whatman No. 1). The raw plant extract was lyophilized. The samples were kept in plastic bottles with tightly capped lids until use.

Testing the inhibitory activity of the aqueous extract

(1) gm of the dry plant extract previously prepared was taken and dissolved in (5) ml of distilled water to obtain a plant extract with a concentration of (200) mg/ml. This standard concentration is the basis for preparing the subsequent concentrations used in this study. The sterilization of the extract was done using (0.45 μm) Membrane filters. To obtain the minimum inhibitory concentration (MIC), the dilution law ($N_1 V_1 = N_2 V_2$) was used to prepare different concentrations of aqueous extract, including (5, 10, 15, 20, 25, 30) mg/ml) to be tested. The plates were

cultured at 37°C for 24 h. Triplicate plates were used for each treatment, and the results were calculated by taking the average of the diameters of the inhibition zones for each treatment, and then the percentages of inhibition were calculated [22].

Preparation of the alcoholic extract: The alcoholic extract of cloves was prepared based on [23] and modified from the method described in [24] by mixing (20) g of cloves in (200) ml of (95%) ethanol, crushing by a blender in an ice bath, stirring the mixture using an electric motor Magnetic steroid for (60) min, and leaving it at (5) °C for 72 h to soak. Filtration was performed using several layers of gauze, centrifuged at 3000 rpm for 5 min, and filtered again using a Buechner funnel with filter papers (Whatman No. 1). The resulting extract was dried using a rotary evaporator and stored in a freezer until use.

Sterilization of the alcoholic extract of cloves was sterilized by taking (1) g of the extract prepared previously and dissolving in (5) ml of Ethelin glycol. Then, the mixture was sterilized by pasteurization at (63-66) °C for 20 minutes [25,26].

Test for the inhibitory activity of the alcoholic extract

To estimate the inhibitory activity of the alcoholic extract of cloves on *C. albicans*, the disc diffusion method was used as described in (3-4). The disks were immersed in the prepared alcoholic extract of cloves at the following concentrations: (100, 50, 25,

12.5, 6.25, 3.125, 1.56). The disks were fixed using sterile forceps on the surface of the inoculated plates and incubated at 37°C for 24 h. After incubation, the diameters of inhibition were measured, and the minimum inhibitory concentration of the extract was determined [27].

Results and Discussion

Collection of samples and data from individuals wearing dentures

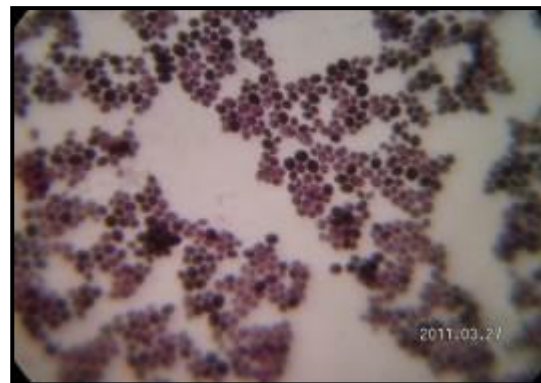
The presence of *C. albicans* was investigated using oral swabs of 15 people wearing dentures from the dental consulting clinic in Mosul, and a questionnaire was administered to the participants regarding the private information mentioned in (3-1). Ten isolates belonging to the genus *Candida* were obtained, which are (3, 5, 7, 8, 9, 11, 12, 13, 14, 15). Six of these isolates belonged to *C. albicans*, which are (3, 5, 8, 12, 14, 15), 4 from females and 2 from males, and their ages ranged between 50 and 80. The initial investigation of isolates belonging to the genus *Candida* was carried out as follows:

Direct morphological examination of colonies growing on an SGA medium was observed. They were smooth, creamy white, waxy, shiny, and convex colonies, as in (Figure 4-1) [28].

Microscopic examination using Gram stain showed an oval-to-spherical shape with a violet color, which confirmed that they belong to the genus *Candida* as in (Figure 4-2), The result was identical to the study [29].



(Figure 4-1) Image showing *Candida* colonies growing on SGA medium.



(Figure 4-2) Micrograph showing cells of the *Candida* isolates at 100X magnification

Diagnosis of *C. albicans* isolates

Germ tube formation test: This method showed elongated growth of the germ tube of the cell in preparation for the formation of a new cell, as shown in Figure (4-3). This confirms that this isolate belongs to *C. albicans*. The results were identical to those of a previous study [30].

Dalma technique:

The diagnosis of *C. albicans* yeast was confirmed using the Dalma technique, due to its ability to form pseudohyphae and chlamydo spores at a temperature of 28°C for 24 h, because the nature of the formation of chlamydo spores depends on the change in temperature and the low-ventilation conditions (microaerophilic), which were provided by placed slide cover over the colony before incubation, as shown in Figure (4-4), The result was identical to the study [12,17,18].

Data about people wearing dentures

By observing the data of people shown in Table (1), people wearing dentures with numbers (1, 2, 4, 6, 10) did not show any fungal infection because some of them used minor, sterile pills, water, and salt. Despite the fact, that the first patient suffered from

general weakness, the second patient suffered from gingivitis, the fourth patient dose not suffer from any disease, the sixth patient was a smoker, and the tenth patient suffered from toxic thyroid disease. The people wearing dentures with numbers (7, 9, 11, and 13) showed infection with *Candida* spp., and people (7, and 9) did not suffer from any disease but showed infection due to their use of water, salt, and toothpaste for the cleaning. Patient (11) suffered from gingivitis and used water for cleaning, and patient (13) did not suffer from anything and used sterile pills for cleaning. Those wearing dentures with numbers (3, 5, 8, 12, 14, 15) showed infection with *C. albicans*. Patient (3) suffered from toxic thyroid gland infection and gingivitis, patient (5) suffered from general weakness and gingivitis and used bacterial antibiotics for more than two weeks, whereas patient (8) did not suffer from any disease but used water, salt, and toothpaste for cleaning. Patient (12) did not suffer from any disease but used bacterial antibiotics for more than 2 weeks, whereas patient (14) suffered from high blood pressure and used toothpaste, water, and salt for cleaning. The patient (15) had arthritis and used bacterial antibiotics for more than 2 weeks. He also used water, salt, and bleach for cleaning.

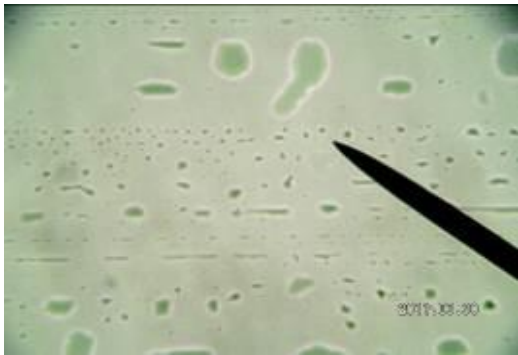
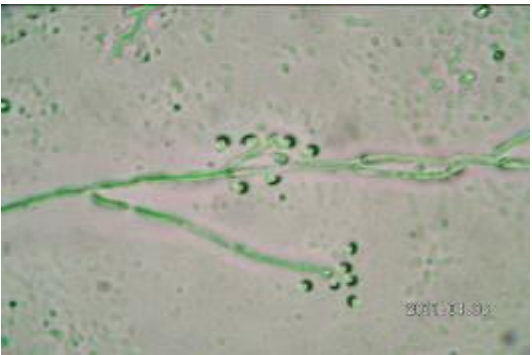
	
<p>(Figure 4-3) Image showing germ tube formation by isolates of the species. albicans at 100X magnification.</p>	<p>(Figure 4-4) Image illustrating the formation of chlamydo spores of <i>C. albicans</i> at 40X magnification</p>

Table (1) Questionnaire of people wearing dentures

The cleaning	Is the person a smoker?	Should I take antibiotics for more than two weeks?	Should I use antifungal or antibacterial agents?	Does he/she suffer from gum infections?	Does he suffer from other diseases?	Do you have chronic diseases?	Diabetic	Are you over 50 years old?	Kit location	Isolate Candida genus	the age	Sex	Samples
Water, vinegar, and salt	No	no	No	No	general weakness	no	no	Yes	Alawi	Nothing	74	♀	1
Sterile pills	No	no	No	Yes	no	no	no	Yes	Lower and upper extremities	Nothing	56	♂	2
Water and salt content	No	no	No	Yes	no	toxic thyroid gland	no	Yes	Lower	<i>C. albicans</i>	80	♀	3
Water and salt content	No	no	No	no	no	no	no	Yes	Alawi	Nothing	52	♂	4
Sterile pills	No	Yes	Tetracyclin	Yes	general weakness	no	no	Yes	Lower and upper extremities	<i>C. albicans</i>	65	♀	5
Water and Qasir	Yes	No	No	no	no	no	no	Yes	Alawi	Nothing	58	♂	6
Water and salt content	No	No	No	no	no	no	no	Yes	Lower	Candida ssp.	64	♂	7
Toothpaste, water, and salt	No	No	No	no	no	no	no	Yes	Lower	<i>C. albicans</i>	70	♀	8
Toothpaste	No	No	No	no	no	no	no	Yes	Lower and upper extremities	Candida ssp.	80	♂	9
Water and Qasir	No	No	No	no	no	toxic thyroid gland	no	no	Alawi	Nothing	50	♂	10
Water	No	No	No	no	no	no	no	no	Alawi	Candida ssp.	50	♀	11
Water and salt content	No	yes	Ampicillin	no	no	no	no	Yes	Upper	<i>C. albicans</i>	58	♂	12
Sterile pills	No	No	No	no	no	no	no	Yes	Upper	Candida ssp.	65	♀	13
Toothpaste, water, and salt	No	No	No	no	no	pressure	no	Yes	Lower and upper extremities	<i>C. albicans</i>	71	♂	14
Minors, water, and salt	No	Yes	Ampicillin+ Ampoxilin	no	no	no	no	yes	Lower and upper extremities	<i>C. albicans</i>	68	♀	15

According to information in Table (1), the majority of infections were in women, which is attributed to women being exposed to pressures in their lives that are completely different from those in men, such as pregnancy and childbirth. In 2004, Jones mentioned that pregnancy increases the incidence of Candida because of hormonal changes during pregnancy, which stimulates the accumulation of carbohydrates in the vaginal wall. In addition, there are cases of a lack of health awareness in some people, which has caused an increase in the incidence of diseases in men and women.

Effect of the antibiotic Nystatin on *C. albicans*: The results obtained from the sensitivity test are illustrated in Figure (4-5), and the average diameter of the inhibition zone results are shown in Table (2). Nystatin is the best antibiotic against *C. albicans*, as indicated by Ellepola (2005), and 500,000 IU is the best concentration used to treat oral candidiasis.

Depending on the above results, the antibiotic Nystatin at different concentrations (500,000, 250,000, 125,000, 62,500, 31,250, 15,625, 7812.5,

3906.25) (IU) gave increasingly effective results. The lowest inhibitory concentration for the yeast was (3906.25) IU with an average inhibition zone of 6.2-6.8 mm, while the concentration (62,500) IU gave an average inhibition zone between 7 and 7.5 mm. The highest inhibition rate was observed at a concentration (500,000) IU, with an average diameter of the inhibition zone of 8-11 mm. Therefore, a Nystatin concentration of 500,000 IU was used because it is the best concentration to inhibit *C. albicans*. The diameter of the inhibition zone with (10) mm was used for the antibiotic Nystatin for comparison with other treatments [31,32].

Effects of mouthwashes on *C. albicans*

To determine the effect of some mouthwashes used by people wearing dentures on *C. albicans*, the inhibitory effects of the following mouthwashes: (water, water and salt, water and sodium hypochlorite solution, Zak gargle, Biofresh gargle, Gensten violet dye, sterile pills) were studied and the results appeared in Figure (4-6) and Table (3).

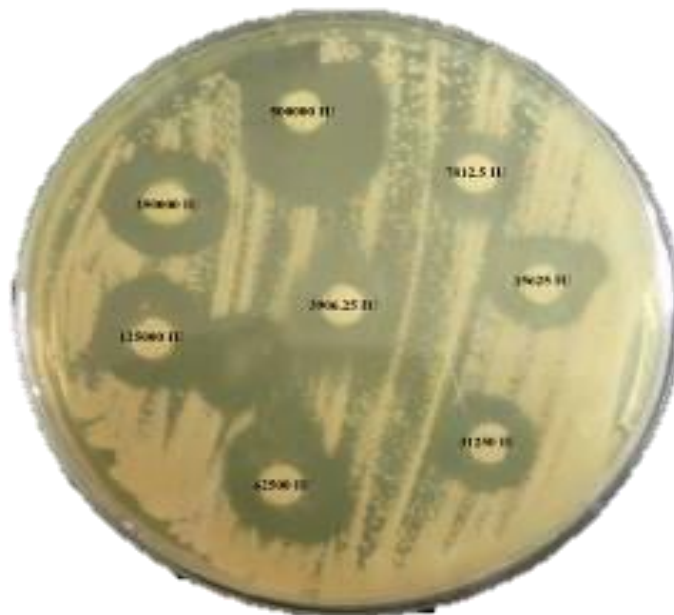


Figure (4-5): A picture showing the average diameter of the inhibition zone of the antibiotic Nystatin.

Table (2): Effect of the antibiotic Nystatin on the inhibition zone rate of *C. albicans* yeast colonies and the minimum inhibitory concentration (MIC) (mM)

500000	250000	125000	62500	31250	15625	7812.5	3906.25	Concentrations Isolation Type
10	8	8	7.4	7	7	6.8	6.8	<i>C. albicans</i> 3
8	8	7.5	7.5	7.2	7	6.6	6.8	<i>C. albicans</i> 5
10.5	8	7.5	7	7	6.8	6.5	6.4	<i>C. albicans</i> 8
11	8	7.5	7.4	7	7	6.8	6.2	<i>C. albicans</i> 12
11	8	7.4	7	7	6.8	6.5	6.4	<i>C. albicans</i> 14
10.8	8	7.5	7	7	6.8	6.5	6.5	<i>C. albicans</i> 15

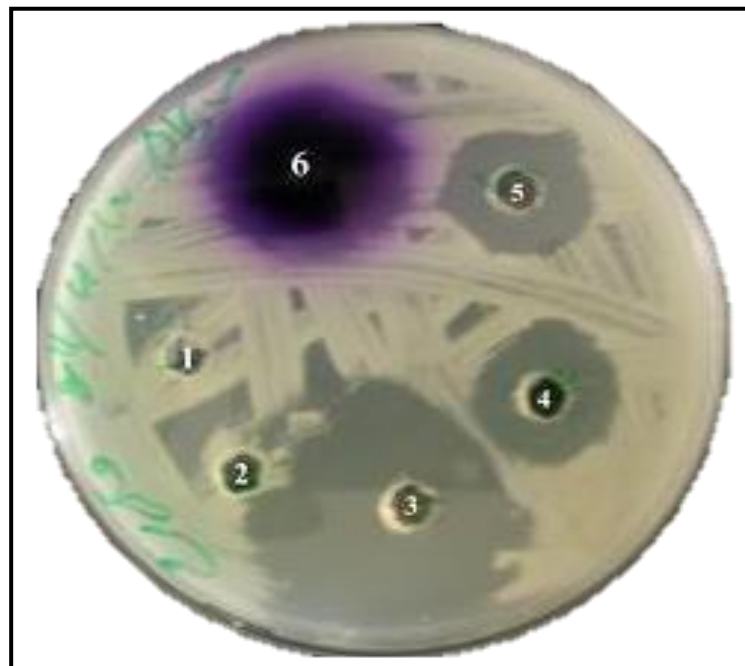


Figure (4-6): A picture showing the effect of mouthwashes on the diameter of the inhibition zone of *C. albicans* yeast

- 1: Water; 2: Water and salt; 3: Bleach
 4: Zak solution, 5: Biofresh solution, 6: Gensten stain

Table (3): The inhibitory effect of mouthwashes used by denture wearers on the average diameter of the inhibition zone of *C. albicans* yeast isolates (mm)

Water and bleach	Genistein dye	Sterile tablets	Biofresh solution	Zak solution	Water and salt content	Water	The type of lotion	
							Isolation Number	
(100)++	(85)+	(80)+	(75)+	(75)+	-	-	<i>C. albicans</i>	1
(90)++	(80)+	(80)+	(70)+	(70)+	-	-	<i>C. albicans</i>	2
(95)++	(80)+	(80)+	(80)+	(75)+	-	-	<i>C. albicans</i>	3
(100)++	(90)++	(90)++	(70)+	(73)+	-	-	<i>C. albicans</i>	4
(90)++	(80)+	(80)+	(70)+	(75)+	-	-	<i>C. albicans</i>	5
(90)++	(80)+	(75)+	(75)+	(70)+	-	-	<i>C. albicans</i>	6

%Inhibition percentage-No inhibition + Inhibition diameter between 6 – 9 mm + inhibition diameter between 9 – 12mm

It was noted that water, water, and salt did not give any inhibition rate, which confirms that there was no growth of the genus *Candida* in people with numbers (3, 7, 8, 12, 14) When Zak solution and the biofresh solution were used in the test, they gave a good inhibition rate, so it is recommended to use them as mouthwashes for all people. The inhibition rate for the Zak solution was 70-75%, whereas the Biofresh solution had an inhibition rate of 70-80%. The Gensten dye and sterile pills had a good inhibition rate, which confirms that they are good antifungals, as noted in the patient (2) who did not show any fungal infection when using these pills. The inhibition rates for Gensten dye and sterile pills were 80-90% and 70-90%, respectively. Therefore, Gensten dye is used in children to treat oral infections with *Candida*, but because its color remains on the surface of the mouth, adults do not want to use it, so sterile pills were used instead, which gave a similar inhibition rate. Using water and bleach gave an inhibition rate of 90-100%, noting

that people with numbers (1, 6, 10) used this solution for cleaning, so they did not show any infection with *Candida*. On the other hand, a patient (15) who used bleach showed an infection with *C. albicans* due to arthritis and the use of bacterial antibiotics for more than 2 weeks. It is noted that the results were ascending, despite the high inhibition rate of bleach, it is not preferable to use it for a long period due to its side effects on the mouth and gums. Figure (4-6) shows the average diameter of the inhibition zone for the mouthwashes used in this study.

Effects of aqueous clove extract on *C. albicans*

The effect of aqueous clove extract on *C. albicans* was studied by conducting a sensitivity test at the following concentrations (30, 25, 20, 15, 10, 5) and determining the minimum inhibitory concentration (MIC). The results were shown in Table (4), and Figure (4-7) showed the average diameters of the inhibition zone for *C. albicans* colonies using aqueous clove extract.

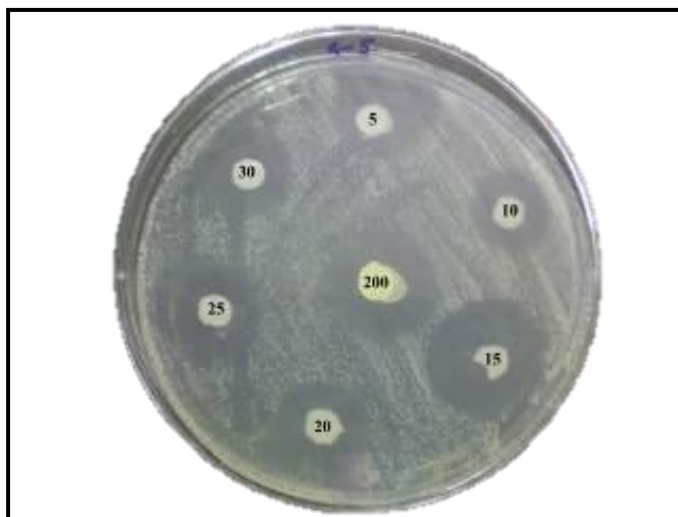


Figure (4-7): A picture showing the effect of the aqueous extract of cloves on the average diameter of the inhibition zone of *C. albicans*.

Table (4) Effect of the aqueous extract of cloves on the average diameter of the inhibition zone of *C. albicans* isolates (mm)

30	25	20	15	10	5	Nystatin 500000 IU	Extract concentration Isolation number
(65) MS	(70) MS	(75) MS	(80)+	(65) MS	(68) MS	10	<i>C. albicans</i>
(65) MS	(64) MS	(70) MS	(83)+	(65) MS	(65) MS	10	<i>C. albicans</i>
(64) MS	(70) MS	(80)+	(85)+	(80)+	(65) MS	10	<i>C. albicans</i>
(68) MS	(72) MS	(80)+	(87)+	(80)+	(70) MS	10	<i>C. albicans</i>
(64) MS	(75) MS	(80)+	(90)+	(72) MS	(64) MS	10	<i>C. albicans</i>
(68) MS	(65) MS	(82)+	(85)+	(70) MS	(64) MS	10	<i>C. albicans</i>

Percent inhibition

MS diameter of water clove inhibition is less than the diameter of inhibition of antibiotic Nystatin

+ The diameter of inhibition by water clove is equal to the diameter of inhibition by the antibiotic Nystatin.

The results indicated that the aqueous clove extract inhibited *C. albicans*. The lowest inhibitory concentration was 5 mg/ml, and the highest inhibitory concentration was 15 mg/ml. Then, a gradual decrease in the inhibitory rate

was observed at a concentration of 30 mg/ml, and this result was close to the result at a concentration of 5 mg/ml. It was noticed that the aqueous clove concentration at 15 mg/ml is the best concentration with an inhibition rate between 80% and 90%; therefore, it is preferable to use because it is safe for humans and is a good antifungal. It is characterized by containing chloride, fluoride, and sulfur compounds, which have different physiological effects and play protective roles against tooth

decay. It has also been proven that cloves have anti-inflammatory and antibacterial properties [33-36].

4-5 Effects of the clove alcoholic extract on *C. albicans*

The effect of clove alcoholic extract on *C.*

albicans was studied by performing a sensitivity test at the following concentrations: (100, 50, 25, 12.5, 6.25, 3.125, 1.56) and determining the minimum inhibitory concentration (MIC). The results as shown in Figure (4-8) and Table (5) appeared.

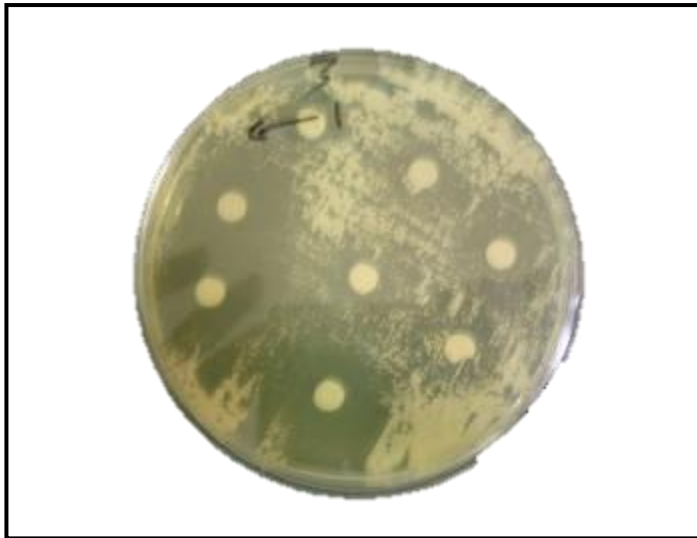


Figure (4-8): A picture showing the effect of the alcoholic extract of cloves on the diameter of the inhibition zone of *C. albicans*.

Table (5) Effect of clove alcoholic extract on the average diameter of the inhibition zone of *C. albicans* isolates (mm)

100	50	25	12.5	6.25	3.125	1.56	Nystatin 500000 IU	Extract concentration Isolation number
MS (70)	(80)+	MS (70)	(80)+	(80)+	-	-	10	<i>C. albicans</i>
MS (68)	(82)+	MS (68)	MS (64)	-	-	-	10	<i>C. albicans</i>
MS (75)	(85)+	MS (75)	MS (70)	(82)+	MS (65)	-	10	<i>C. albicans</i>
(82)+	(90)+	(80)+	(80)+	MS (75)	MS (70)	-	10	<i>C. albicans</i>
MS (70)	(90)+	MS (70)	MS (70)	MS (65)	-	-	10	<i>C. albicans</i>
(80)+	(84)+	(80)+	MS (70)	-	-	-	10	<i>C. albicans</i>

Percent inhibition

MS Water clove inhibition diameter was less than the antibiotic Nystatin inhibition diameter

- No inhibition

+ The water clove inhibition diameter is equal to the antibiotic Nystatin inhibition diameter.

The results indicated an increasing inhibitory rate of 50 mg/ml, with the highest inhibition percentage of 80-90%, and was similar to that obtained by the aqueous clove extract at a concentration of 15 mg/ml. The alcoholic extract exerted an inhibitory effect because it contains cloves of phenolic compounds that have antibacterial activity. Their effectiveness in inhibiting the formation of amino acids and proteins may be mediated by interference with amino acid molecules and proteins through hydrogen bonds, thereby blocking this process, The results of this study are consistent with those of other studies that indicated the effect of clove on microorganisms in general. siak contains a number of chemical compounds that are beneficial to the body generally and the mouth particularly [37-39] Nystatin is one of the most effective antibiotics with the highest inhibition zones (this is because nystatin works to inhibit the production of ergosterol, which is important in building the cell membrane of *Candida*[40,41]This study showed that the inhibitory effect of alcoholic and aqueous extracts is due to the chemical components present in them, such as alkaloids, flavonoids, phenols, saponins, tannins, resins, and terpenes, as these components interfere with metabolic processes and fungal growth, thus destroying these fungi In addition, it contains Eugenol, a type of phenolic compound that has anti-microbiological effect and works to inhibit the mechanism of the cell membrane of microorganisms, thus inhibiting the growth of the microorganism , It is one of the phenolic compounds that have the ability to disrupt the adhesion of bacteria and enzymes and the transfer of cell membrane proteins, while alkaloids can inhibit the growth of bacteria by binding to DNA [42,43].

Conclusion

Conducted to isolate and diagnose samples taken from people's apostate dentures to investigate *Candida* isolates were carried out, which were identified by direct examination using crystal violet dye, and then a test germ tube formation to identify

isolates belonging to the species *Candida albicans* and confirmed diagnosis using the Dalma technique. Nystatin inhibition against *Candida albicans* isolates, determines the minimum inhibitory dose, 5000 IU dose resulted in the highest inhibition percentage. In addition to that the effect of some mouthwashes, such as water, and salty water, was tested, and the test result showed that 1% sodium hypochlorite was the best in the inhibition for *Candida albicans* with 90-100% inhibition percent ranging. The inhibitory effect of plant, aqueous, and alcoholic extract were also studied, the result of this test showed that aqueous extract at the concentration of 15 mg/ml and alcoholic extract concentration of 50 mg/ml achieved a better inhibition percentage, which ranged between 80-90%.

Acknowledgments

The author thanks the University of Mosul/College of Science/Biology department.

Conflict of interest: NIL

Funding: NIL

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