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Impact the alcoholic and aqueous extract, clove *(Syzygium aromaticum)* clove oil and sodium chloride on growth of *Candida albicans*

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ABSTRACT

Candidiasis is an acute or chronic infection that is always caused by Candida species, normal commensals of the oral mucous membranes of healthy individuals. Between October 2024 and March 2025, we collected a number of specimens from oral thrush patients visiting dental clinics in Al-Najaf Governorate (n = 50). These specimens were then transferred to the Advanced Mycology Laboratory, Faculty of Science University of Al-Kufa for diagnostic and experimental purposes. The objective of the present study was to isolate and identify Candida albicans from various clinical cases by means of different diagnostic methods including direct microscopy and microbiological culture. Moreover, Inhibitory activities of alcoholic extract of clove oil in different concentrations (1%, 2% and 3%), Clove at four levels (0.25g,0.5g,50g and l g) and sodium chloride were examined over Candida albicans growth In the present study, we evaluated antifungal activity of these agents (alcoholic and aqueous extracts of clove oil, clove and sodium chloride) against Candida albicans using well diffusion method and interpreted findings according to Statistical analysis. In the Study the effect of antifungal of alternative material in concentration (2.5,5,10)mg\ml from clove the Inhibition zone(mm) of *candida albicans* for 7 isolate are (3,4,6),(7,6,8),(3.5,3,4),(5.5,7,9),(4,3.5,4), (4,6,9),(4.5,8,10) the effect of antifungal of alternative material in concentration(2.5,5,10)mg\ml from clove oil Inhibition zone(mm) of *candida albicans* for 7 isolate (4,3.5,5),(3.5,5,6), (4,6,7),(4.5,5.5,7),(3.5,4,5),(3,6,7), (4,5,6) the effect of antifungal of alternative material in concentration (2.5,5,10)mg\ml from sodium chloride Inhibition zone(mm) of *candida albicans* for 7 isolate(2,4,5),(3,4,4.5),(3.5,3,4),(4,6,6.5), (3,3.5,4.5), (3,5,7), (3,4,6) the effect of antifungal of alternative material in concentration (2.5,5,10)mg\ml from alcoholic extract clove Inhibition zone(mm) of *candida albicans* for 7 isolate (16,17,18),(14,15,20), (16,17,18),(10,14,20),(12,15,20), (6,9,17),(6,7.5,13) the effect of antifungal of alternative material in concentration(2.5,5,10)mg\ml from aqueous extract clove Inhibition zone(mm) of *candida albicans* for 7 isolate (13,14,16),(17,19,24),(11,12,15),(4.5,6,10), (10,14,20),(12,15,20), (13,14,25)

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1. INTRODUCTION

Oral candidiasis (thrush) is a common opportunistic fungal infection of the oral mucous membranes. Candida albicans, the main causative agent, is an exceptionally flexible commensal microbe that has co-evolved with its human host; however local

modifications in the host are able to trigger this organism to switch from being a harmless commensal to a pathogen. This transition is predominantly driven by various virulence factors, especially cell surface adhesion molecules, proteolytic enzymes, morphological switches and antifungal resistance mechanisms. The co-adhesion of C. albicans with other bacterial species is crucial to survive within the oral cavity, and many synergistic relationships with various other oral microorganisms have already been reported assisting in host colonization. Because the oral mucosa is frequented by many pathogens, the host immune response and, therefore, local innate immune defenses play a central role in maintaining *Candida* in its commensal state. In particular, in addition to its role in preventing Candida adhesion onto epithelial surfaces, saliva harbors an extensive panel of anti-Candida components that form part of the host innate immune arsenal [9]. In addition, the adaptive immune response, in particular mediated by T helper 17 (Th17) cells, is essential for mucosal immunity as it controls Candida early multiplication and also limits its subsequent invasive progression into deep tissue. Candida albicans is a distributed opportunistic microorganism present normally in commensal microbiota but it has the ability to switch to pathogenic devenia which causes infection on both immunocompetent and immunocompromised individuals.. Ideal identification and Diagnosis of fungl infection beased on phenotypic characteristics and biochemical test. virulence factors in *C. albicans* such as biofilm formation , germ tube ,adhesion, hydrolytic enzymes, such as lipases, proteases, phospholipases, and hemolysin and catalase enzyme facilitates effective colonization and enables the organism to establish infection within the host, particularly when favorable predisposing conditions are present. The colonizing oral population can also serve as a reservoir for dissemination leading to potentially lethal infections. resistant to current antifungal this study aimed to found alternative antifungal agents with better efficacy , safe agents with broad-spectrum activity [1].

1.2 Aim of study

The present study aimed to compare the inhibitory effect of alcoholic and aqueous extract clove oil and clove and sodium chloride on *Candida albicans* growth
throughout the incoming steps:-

1. Isolation and identification of *Candida* spp and *Candida albican* from oral Candidiasis using conventional methods (culture on Sabouraud dextrose agar and CHROMagar .
2. Study effects (alcoholic extract on growth of *C. albicans*
4. Study effects alcoholic and aqueous extract clove oil and clove and sodium chloride on *Candida albicans* growth

2.MATERIALS AND METHOD

2.1 Preparation of culture media and biochmical test

All culture media have been made depending on the instruction of manufactures manual and was the pH adjusted to 7.2 by pH meter after sterilization by Autoclave at 121°C for 15 minutes with exception CHROM agar media.

2.1.1.CHROM Agar *Candida* media

It has been prepared depending on the manufacturer's instructions by dissolving 42.72 gm powder of CHROM agar in 1000 ml distilled water ,and then the solution was heated until boiling degree to dissolve the medium completely [2]**.**

2.1.2. Sabouraud dextrose agar medium (SDA)

This agar medium was prepared in accordance with the manufacturer's guidelines by dissolving 65 g of SDA powder in 1000 mL of distilled water. The final pH was adjusted to 6.5, after which chloramphenicol was added at a concentration of 250 mg/L prior to autoclaving. This medium was utilized to cultivate pathogenic and commensal fungi and yeasts, Chloramphenicol is a broad-spectrum antibiotic inhibitory to a wide range of Gram-negative and Gram-positive bacteria, while streptomycin to prevent saprophytic fungi [3] .

2.1.3 Sabouraud dextrose broth medium (SDB)

This medium was prepared by dissolved dextrose 20gm and peptone 10 gm in 1000 ml distilled water, the final pH was set at 6.5 and then sterilized by autoclave. This medium was utilized to define the possibility of *Candida spp*. biofilm formation [4].

2.2 Preparation alternative material (olive oil and clove):1g of active
ingredient was prepared and dissolved in 2ml of (DMSO) solution.

2.3 Preparation of Stains and Solutions

2.3.1.Safranine

This stain is pink or purple in color prepared by using 2gm of safranine stain and 100ml of distilled water, to identification of biofilm formation (prepared manual).

2.3.2.DMSO 2%

Dimethyl sylfoxide this solution is colorless prepared by dilute 200µl of DMSO in 10 milliliters distill water and using to dissoluble the chemical substances within appropriate concentration.

2.3.3. Preparation suspension of *Candida* spp.

The *Candida* suspension was prepared by adding 10 mL of distilled water into a test tube, followed by inoculation with a colony using a sterile wire loop obtained from an actively growing culture in Sabouraud Dextrose Broth (SDB) or from growth on

Sabouraud Dextrose Agar (SDA). The resulting suspension was then standardized to McFarland standard No. 3, after which it was considered ready for use [5].

2.4 Identification of *Candida* albicans

C. albicans was determined according to the morphological characteristics on culture medium and other characteristic formation as the following :-

2.4.1. Colonial morphology

All isolates were culturing on SDA. Then plates were put at 37ºC for 24-48 hr to isolate pure *Candida* colonies to test their characteristics, color size and shape.

2.5 Antifungal susceptibility

In Vitro Antifungal Activity of Two Alternative Agents for the Growth of Candida spp. was tested using the well diffusion method on Mueller–Hinton agar. For each Petri dish, wells were created in the center and added with four different concentrations of each agent. Antifungal weighed out compounds were diluted in 10% DMSO and added to each well with a pipette, delivering 50 µL of antifungal solution. All tests were executed in triplicates for every concentration and incubated at 37 °C for the time period ranging from two to four days. After 4 days, the zones of inhibition were measured in millimeters.

2.6 Methods

2.6.1 Collection of samples

The study included 50 samples have been taken from oral candidiasis patients. Samples with various clinical cases were obtained from Dental clinics Center City in Al-najaf Governorate, from january 2024 to february 2025 these samples were conducted to the laboratory of advanced microbiology/Faculty of Science/UniversityAl-Kufa for diagnosis and study.

2.6.1.1 Oral cavity samples

Materials and MethodsDuring the duration between December 2024to March 2025, a total of 50clinical specimens were collected from patients with clinical diagnosis oral thrush visiting dental clinics in Al-Najaf Governorate. These samples were transported with caution to Advanced Mycology Laboratory at Faculty of Science, University of Kufa for identification and further studies. Candida spp. are part of normal commensal flora and can be recovered from the oral cavity of half of the general population without causing disease. Despite being members of the commensal flora, systemic predisposing factors may render them geographic opportunists and enable pathogenogenesis. Diabetes mellitus, inherited or acquired immunodeficiency states, malignancies (including oral cancers), and nutritional deficiencies are among a number of risk factors that can lead to their alteration to pathogenic forms[6].

3. RESULTS AND DISCUSSION

3.1 Morphological identification

3.1.1 Identification *Candida* on SDA medium

All specimens were cultured on Sabouraud Dextrose Agar (SDA), in which the Candida spp. generated colonies from cream to yellowish colored. Colony characteristics These colonies demonstrated rapid growth with maturity observed within 24–48 hours, and depending on the species eaten had a smooth and shiny or dry texture. These findings are consistent with previously reported [2]. This study collected 50 samples from patients with oral thrush who visited dental clinics in Al-Najaf Governorate during the period of October 2024 to March 2025. Subsequently, the samples were transferred to the Advanced Mycology Laboratory at the Faculty of Science, University of Kufa for identification and further analyses. No less than oral candidiasis is caused by genera of candida species in situations where the immune systems of hosts subjected to these effects beillide. Disruption of local mucosal immunity, e.g. by oral corticosteroids leading to fungal overgrowth and therefore a pseudomembrane [7]. The Candida species are part of the normal oral microbiota in almost half of the population, with no disease produced, but multiple systemic predisposing factors including diabetes mellitus (DM), immunodeficiency (acquired or congenital), malignancies, RXs head and neck cancers and malnutrition can make them change their non-pathogenic state to a pathogenic one[8].

3.1.2 Identification of *Candida* spp. on chromagar medium

Chromagar is a selective culture media for yeast isolation that allows the differentiation and presumptive identification of several species of Candida from other yeasts isolations [9]. Chromagar Candida, used as a differential media in the current study, displayed characteristic colony morphologies (Figure 3.1): Smooth and light green for C. Visit website to read more on this news. albicans colonies; dull greenish blue for C. tropicalis isolate.. Chromogenic media need to be more interpreted as used for rapid and reliable identification of Candida at the species level since color change is a result of enzyme-substrate interaction attachment between these two types. This method facilitates a more rapid and accurate identification than traditional culture methods, especially useful in cases of mixed yeast infections [10]. All isolated yeast species were shown to produce growth on Chromagar Candida, after an incubation period of 48 h at 37 °C (data not shown), with most strains exhibiting good and clear growth properties observed in accordance with the manufacturer's specifications.

3.2 Antifungal susceptibility (Alternative materials)

Table (3-1) Effects of clove on *Candida albicans* in con. (2.5 ,5,10)mg/ml

No. isolate	Inhibition zone(mm)	Inhibition zone(mm)	Inhibition zone(mm)
1.	3	4	6
2.	7	6	8
3.	3.5	3	4
4.	5.5	7	9
5.	4	3.5	4.5
6.	4	6	9
7.	4.5	8	10

Table (3-2) Effects of clove oil on *Candida albicans* in con. (2.5 ,5,10)mg/ml

No. isolate	Inhibition zone(mm)	Inhibition zone(mm)	Inhibition zone(mm)
1.	4	3.5	5
2.	3.5	5	6
3.	4	6	7
4.	4.5	5.5	7
5.	3.5	4	5
6.	3	6	7
7.	4	5	8

Table (3-3) Effects of sodium chloride on *Candida albicans* in con. (2.5 ,5,10)mg/ml

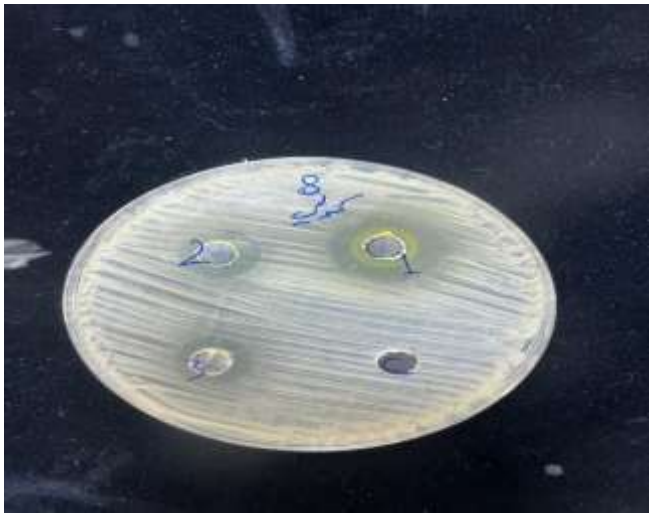
No. isolate	Inhibition zone(mm)	Inhibition zone(mm)	Inhibition zone(mm)
1.	**2**	**4**	**5**
2.	**3**	**4**	**4.5**
3.	**3.5**	**3**	**4**
4.	**4**	**6**	**6.5**
5.	**4**	**3.5**	**4.5**
6.	**3**	**5**	**7**
7.	**3**	**4**	**6**

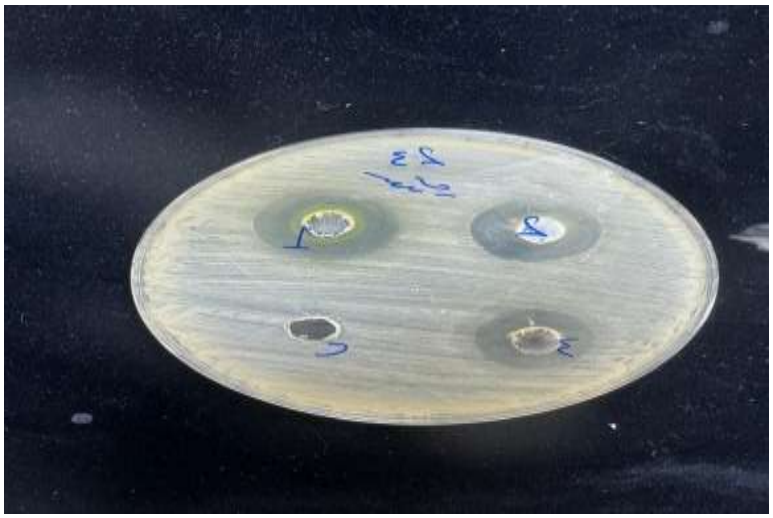
Table (3-4) Effects alcoholic extract Clove of clove on *Candida albicans* in con. in con. (2.5 ,5,10) mg/ml

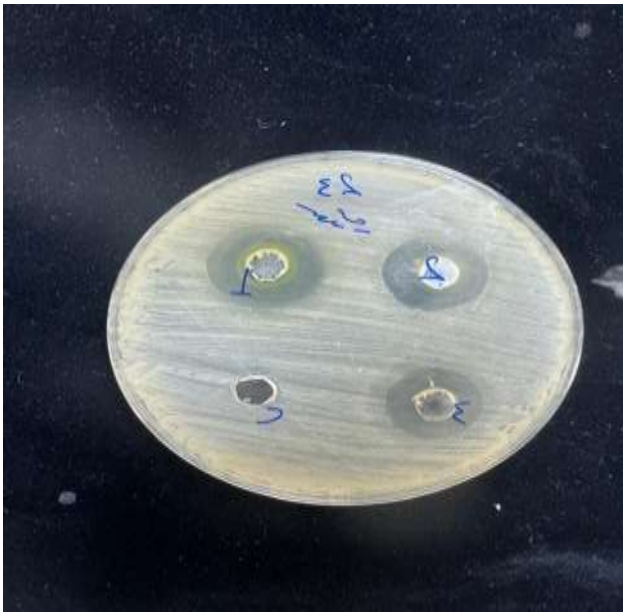
No. isolate	Inhibition zone(mm)	Inhibition zone(mm)	Inhibition zone(mm)
1.	**16**	**17**	**18**
2.	**14**	**15**	**20**
3.	**16**	**17**	**18**
4.	**10**	**14**	**20**
5.	**12**	**15**	**20**
6.	**6**	**9**	**12**
7.	**6**	**7.5**	**13**

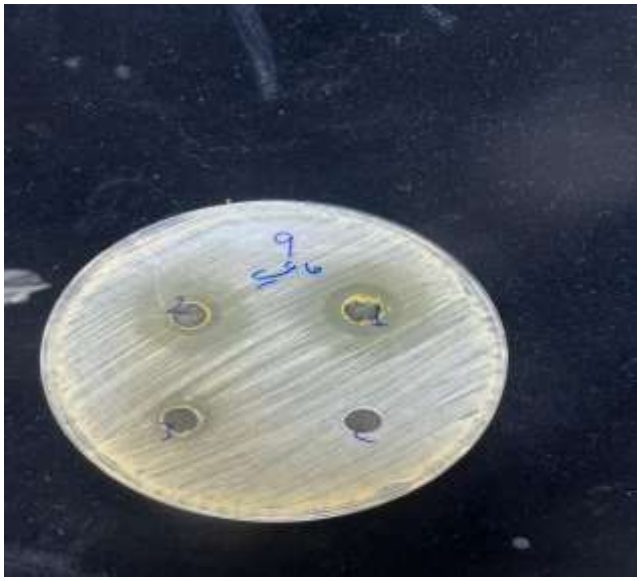
Table (3-5) Effects aqueous extract of Clove on *Candida albicans* in con. in con. (2.5 ,5,10) mg/ml

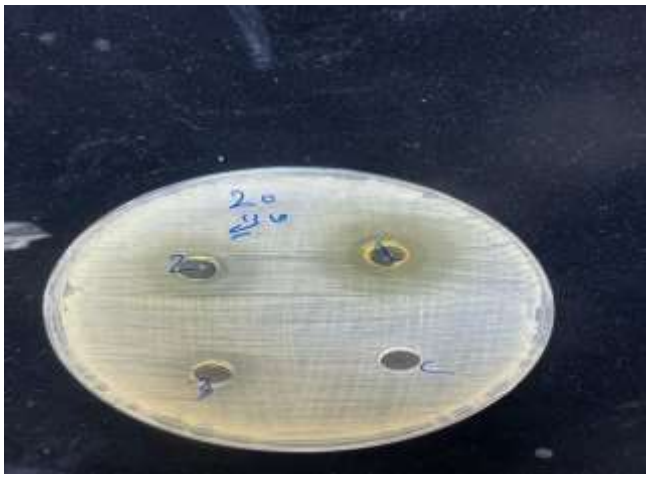
No. isolate	Inhibition zone(mm)	Inhibition zone(mm)	Inhibition zone(mm)
1.	**13**	**14**	**16**
2.	**17**	**19**	**24**
3.	**11**	**12**	**15**
4.	**4.5**	**6**	**10**
5.	**10**	**14**	**20**
6.	**12**	**15**	**20**
7.	**13**	**14**	**25**











Figure(3.1-7): showing Inhibition zone(mm) of *candida albicans*

In the Study the effect of antifungal of alternative material clove Inhibition zone(mm) of *candida albicans* for 7 isolate(3,4,6),(7,6,8),(3.5,3,4),(5.5,7,9),(4,3.5,4),(4,6,9),(4.5,8,10) the effect of antifungal of alternative material clove oil Inhibition zone(mm) of *candida albicans* for isolate(4,3.5,5),(3.5,5,6),(4,6,7),(4.5,5.5,7),(3.5,4,5),(3,6,7),(4,5,6) the effect of antifungal of alternative material sodium chloride Inhibition zone(mm) of *candida albicans* for 7 isolate(2,4,5),(3,4,4.5),(3.5,3,4),(4,6,6.5),(3,3.5,4.5),(3,5,7),(3,4,6) the effect of antifungal of alternative material alcoholic extract clove Inhibition zone(mm) of *candida albicans* for 7 isolate (16,17,18),(14,15,20),(16,17,18),(10,14,20),(12,15,20),(6,9,17),(6,7.5,13) the effect of antifungal of alternative material aqueous extract clove Inhibition zone(mm) of *candida albicans* for 7 isolate (13,14,16),(17,19,24),(11,12,15),(4.5,6,10),(10,14,20),(12,15,20),(13,14,25)

Another name of clove oil — is eugenol oil, because it comes from the clove plant (Syzygium aromaticum), which has been used for thousands of medicinal purposes. It is frequently used in aromatherapy and as a flavoring agent in food products like drinks (mostly tea) and many oral care products such as toothpaste. It has been used topically in alternative medicine for the relief of toothache[11], although there have been few clinical studies verifying its analgesic efficacy. Besides its widespread use as a fragrance [1] and food additive, clove (Syzygium aromaticum) has also been used in traditional medicine to treat asthma and allergic diseases such as atopy [2], especially in Korea. And also, it is used in dental and medical practice for the same but as a general antiseptic. The earlier nominated study by Ahmad et al. Finally, it has been shown that clove oil has antimicrobial activity against a wide range of fungal pathogens, including those linked to urogenital infections [2]. Clove oil in particular has displayed substantial fungicidal properties on opportunistic fungi including C. albicans, C. neoformans and A. fumigatus 2. Eugenol is recognized as the main bioactive substance that may be responsible for this antifungal activity and it represents >90% of the volatile clove bud oil (S. aromaticum L) [12]. Eugenol has long been recognized for its pharmaceutical properties, such as bactericidal, fungicidal and anesthetic effects. Fungicidal Activity Curiously, its antimicrobial function seems even stronger against fungi relative to bacteria. Clove oil is composed of other additional constituents in addition to eugenol (which usually comprises 49–87% of the oil), including β-caryophyllene (4–21%), eugenyl acetate (0.5–21%), α-humulene, and traces (<1%) of many minor compounds, all possibly influencing biological activity

[12-14, 20].

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