Tikrit Journal of Pure Science Vol. 30 (3) 2025

DOI: ***https://doi.org/10.25130/tjps.v30i3.1760***

IRAQI Academic Scientific Journals

Tikrit Journal of Pure Science

ISSN: 1813 – 1662 (Print) --- E-ISSN: 2415 – 1726 (Online)

Journal Homepage: https://tjpsj.org/

Tikrit Journal of Pure Science

Inhibitory Effect of Olive (*Olea europaea*) Leaves Extract on *Candida* Yeast Isolated from the Respiratory System of Human

Mohammed Sami Farhan , Rand Salwan Numan

Department of Biology, College of Sciences, Tikrit University, Tikrit, Iraq

Received: 11 Sep. 2024 **Received in Revised Form:** 14 Oct. 2024 **Accepted:** 22 Oct. 2024

Final Proof Reading: 13 Apr. 2025 **Available Online:** 25 Jun. 2025

ABSTRACT

The laboratories of the Biology Department were used to conduct this investigation. at the College of Science - Tikrit University for the period from February 2024 to April 2024 and included the work of an aqueous and alcoholic extract of olive leaves. The study was conducted in two aspects, the first aspects is to show the effect of extracts (aqueous and alcoholic) of olive leaves using the diffusion method in the drilling, and the relationship was positive, i.e. whenever the higher the concentration of the extract, effect of its inhibitory becomes greater. The second aspects is to show the effect of antifungals on the yeasts used in the study. The results showed the effect of both the aqueous and alcoholic extracts at all concentrations. *Candida albicanis* yeast was more affected by the aqueous extract at a rate of inhibition diameter (16 mm), while *Candida kefyr* yeast was less affected by the aqueous extract at a rate of (12.2 mm). *Candida glabrata* yeast was more affected by the acetonic extract at a rate of (11.3 mm), while *C.kyfer* yeast was less affected by this extract at a rate of (6.5 mm). As for antifungals, a number of antifungals were used: Nystatin, Amphotericin, Clotrimazole, and Fluconazole. The antifungal Clotrimazole was the most effective, as it inhibited all the yeasts used in the study. The yeast *C. glabrata* was the most affected by this antifungal, with an inhibition diameter of (14 mm).

Keywords: Antifungals, *Candida*, Extract, Olive leaves, Respiratory system.

Name: Mohammed Sami Farhan **E-mail:** mohammed.s.farhan@tu.edu.iq

التأثير التثبيطي لمستخلصات اوراق نبات الزيتون على خميرة المبيضات المعزولة من الجهاز التنفسي للإنسان

محمد سامي فرحان، رند سلوان نعمان

قسم علوم الحياة، كلية العلوم، جامعة تكريت، تكريت، العراق

الملخص

انجز هذا البحث في مختبرات قسم علوم الحياة _كلية العلوم _ جامعة تكريت للفترة من شباط 2024 الى نيسان 2024 وتضمنت عمل مستخلص مائي وكحولي لأوراق نبات الزيتون. أجريت الدراسة في محورين الأول بيان تأثير المستخلصات (المائي والكحولي) لأوراق نبات الزيتون باستخدام طريقة الانتشار في الحفر وكانت العلاقة طردية أي كلما زاد تركيز المستخلص زاد التأثير المثبط له. والمحور الثاني لبيان تأثير المضادات الفطرية على الخمائر المستخدمة في الدراسة. وقد أظهرت النتائج تأثير كل من المستخلص المائي والكحولي وبجميع التراكيز. وكانت خميرة ***C. albicans*** اكثر تأثراً بالمستخلص المائي وبمعدل (16 mm) اما خميرة ***C. kefyr*** فكانت اقل تأثرا بالمستخلص المائي وبمعدل (12.2 mm). بينما خميرة ***C. glabrata*** *فكانت اكثر تأثراً* بالمستخلص الكحولي وبمعدل (11.3 mm) اما خميرة ***C. kyfer*** فكانت اقل تأثرا بهذا المستخلص وبمعدل (6.5 mm) وفيما يخص المضادات الفطرية حيث تم استخدام عدد من المضادات الفطرية Nystatin, Amphotericin, Clotrimazole, Flluconazole فكان المضاد الفطري **Clotrimazole** الأكثر فعالية حيث قام بتثبيط جميع الخمائر المستخدمة في الدراسة وكانت خميرة ***C. glabrata*** اكثر تأثرا بهذا المضاد وبقطر تثبيط (14 mm).

INTRODUCTION

The number of *Candida* spp species is over 150. However, just 20 species of them are known to be harmful to humans, with Candida albicans being one of the most prevalent kinds [(1)]. The proved Airborne borne pathogens such as bacteria, viruses and fungi can lead to respiratory tract infections and expose the respiratory system to a variety of diseases [(2)]. Mold-related fungi are a major cause of death for persons with impaired immune systems [(3)]. There are notable differences in the geographical distribution of the *Candida* species, especially between different hospital settings. Previous antifungal therapy and the patient's underlying condition both affect the location and frequency of *Candida* spp. [(4)]. The dimorphic yeast *Candida albicans* is regarded as an opportunistic human pathogen. The flora of the human reproductive, digestive and respiratory systems contain *Candida albicans*, which is present in (40%) to (60%) of healthy adults. Typically, it is a commensal organism [(5)]. There is variation in the ability of *Candida* species to form colonies on mucosal surfaces or inert materials [(6-8)] There is a chance that Candida albicans will cohabit and become pathogenic. This fungus is known as a polymorphic fungus, which is a significant pathogenic factor, and it can produce biofilm, true teliospore, false teliospore, and yeast development. In addition, the pathogenic potential of *Candida albicans* is demonstrated by its capacity to bind and penetrate host body cells, release hydrolyzing enzymes, sense touch, exhibit thigmotropism, and exhibit phenotypic flipping[(9)]. *Candida* species have developed strategies to evade the immune system. The immune system has the capacity to quickly recognize β-glucan, a particular polysaccharide found in the cell wall. Nevertheless, β-glucan is hidden inside the cell wall's outer mannoprotein layer makes it easier for the host's identifying systems to avoid the structural integrity of the cell

wall [10]. Due to its opportunistic nature and presence in natural flora, *Candida albicans* is thought to be one of the most important types of the fungus that cause disease infections. It is followed by *Candida glabrata*, which is accountable for (16 %) of infections of the circulatory system, along with other yeasts like *Candida parapsilosis*, *Candida krusei* and *Candida kefyr* [11]. It is observed that in addition to the variations in protozoa factors among species, such as the formation of germ tubes, mycelium, protein secretion, and variations in the additive pH, the actual differences amongst *Candida* spp. species stem from differences in their environmental naturalization [12]. *Oleaeuropaea* L. is a medicinal plant that has been a significant and effective therapeutic option for wise men and women because of its extensive role in human life, growing range of applications, and rising demand for it globally as a result of the rise in drug side effects. Additionally, it serves as the primary supply of active ingredients needed to prepare a variety of medications as well as the primary source for the creation of therapeutic treatments[13]. From this point on, it became necessary to identify alternatives to antifungals by employing a variety of medicinal plants that are used to treat infections brought on by pathogenic bacteria, as well as skin and systemic illnesses, including fungal diseases [14]. Plants are a rich source of both simple and complex secondary metabolic chemicals, many of which, including: phenols, alkaloids, tannins and others, have broad antibacterial and antifungal activity [15]. Apart from the previously mentioned health advantages, *Olea europaea* (OLE) could help treat a variety of infectious disorders. Very few papers explain the action of the whole extract, despite the fact that the antibacterial activity of the component oleuropein has been studied previously. Thus, the purpose of this study was to investigate OLE's activity against a variety of *Candida* in order to ascertain the extract's activity spectrum and to produce information that would either corroborate or refute the product's claims.

MATERIALS AND METHODS

Sample collection

A number of samples were obtained. The total number of yeast isolates was 100 samples from the Medical City Hospital and Al-Yarmouk Hospital in Baghdad Governorate, isolated from people suffering from respiratory diseases for the period from February 2024 to April 2024, by taking sputum samples. The age of the patients was between (15-50 years).

Diagnosis the isolates of yeast

Several biochemical tests were conducted on the samples, as stated in [16], to test the isolates and confirm their diagnosis:

Activation of isolates on culture media

Sabouraud dextrose ager (SDA) medium was used to cultivate the isolates, and the cultivated plates were incubated for two to four days at (37 °C). For each isolate, three duplicates were made in order to revive it, and the dorsal colony characteristics were recorded, as shown by (form, color, diameter and height) [17].

Dyeing of isolates

By the Loop transporter, a portion of the colony growing on SDA medium was taken, placed on a combined with a drop of lactophenol blue on a glass slide dye to observe the cells, covered with a cover slide, and tested.

Developing isolates on chromo agar medium

All isolates were incubated on SDA culture medium at a temperature of (37 °C) for (48 hours), after which following their transfer to chromo agar medium, these isolates were cultured for a duration of (37 °C). (48 - 72 hours). Chromo agar medium is a selective medium for isolating yeast, growing it, and identifying the dependent colony for the *Candida* isolates, such as; *C.krusei*, *C. albicans*, *C. glabrata* and *C. kefyer*, by the shape of the colonies and the color produced by the isolate [5] the colonies were diagnosed according to the manufacture company which identifies Candida by the color and external shape of the colony.

Chlamydospores test

This test was conducted using the methodology. A portion of a colony was taken with a transporter and inoculated with it in the medium of corn meal agar, making two parallel lines on the surface. Then the transporter was sterilized and two lines were made perpendicular to the two parallel lines for the purpose of attenuation. It was covered with a sterile slide cover, and the dishes were incubated at (25 °C) for (48 hours). After the conclusion of the incubation time, the cover of the slide was lifted with sterile forceps and placed on a glass slide containing a drop of cotton blue dye - lactophenol, and it was examined under a microscope with (10X) and (40X) power to observe chlamydial spores at the ends of the pseudohyphae.

Germ tube forming test

(0.5 mm) of human blood serum was placed in sterile test tubes, then each tube was inoculated with a portion active colony aged (24 hours) and incubated for tow-three hours, after which a drop of the suspension was taken, placed on a glass slide, covered with the slide cover, and then examined under a microscope under magnification power (40X) and (100X) to note the presence of the germ tube, this analysis is unique to the *C. albicans* species., as we can observe the formation of the cell with the germ tube to protrude from one side of the cell in the form of a bud three-four times longer than the cell itself [(18)].

Diagnosis of isolates by the Vitek2 Compact device

This test was conducted using the Vitek2 Compact diagnostic system based on the manufacture's industrial instructions. It is a fully automated diagnostic system used in diagnosing bacteria and medical yeasts. It relies mainly on a set of biochemical reactions and gives accurate results within hours [(19)].

Collect plant samples

Olive plant leaves were obtained from farms and homes in the city of Baghdad during the month of February 2024. The leaves were washed well with distilled water to remove the dust stuck on them and then dried in a room temperature environment. The dried leaves were then ground using an electric grinder. The plant samples, after turning them into powder, were kept inside plastic bags to prevent moisture from reaching them. They were kept away from sunlight. The information for each sample was recorded on a tape pasted on the outside of the bag.

Preparation of aqueous extract

(40 g) of dried and grinded olive leaves were shaped as a powder, then (160 mm) of distilled water was added to it. The mixture was then placed on the shaker for an hour and a half in order to mix well. After mixing, the mixture was placed in the refrigerator for (24 hours) for the purpose of soaking. After this process, the mixture was filtered through several layers of gauze to reduce undissolved suspended particles in the extract, and then the extract was filtered again through Millipore 0.45 to prevent the passage of germs through the filtrate. The filtrate is then placed in an electric oven at a temperature of (40-45 °C) until the filtrate dries and turns into a semi-gelatinous or solid substance. After drying, the dried material is placed inside tight glass bottles and placed in the refrigerator until use according to the method mentioned before [(20)].

Preparation of acetone extract

(40 g) of dried and grinded olive leaves were shaped as a powder, then (160 mm) of aceton was added to it. The mixture was then placed on the shaker for an hour and a half in order to mix well. After mixing, the mixture was placed in the refrigerator for (24 hours) for the purpose of soaking. After this process, the mixture was filtered through several layers of gauze to reduce undissolved suspended particles in the extract, and then the extract was filtered again through Millipore 0.45 to prevent the passage of germs through the filtrate. The filtrate is then placed in an electric oven at a temperature of (40-45 °C) until the filtrate dries and turns into a semi-gelatinous or solid substance. After drying, the dried material is placed inside tight glass bottles and placed in the refrigerator until use according to the method mentioned before [(20)].

DOI: https://doi.org/10.25130/tjps.v30i3.1760

Antifungal Susceptibility Test

The yeast susceptibility test was performed on Muller-Hinton Agar medium, where the colonies were transferred using a sterile cotton swab to the surface of the medium and left for (15 minutes) to dry. Then the antibiotic tablets were transferred with forceps to the surface of the agar and incubated at a temperature of (37 °C) for (24-48 hours). Then the results were read with a ruler by observing the diameters of the inhibition zone around the antibiotic tablets [21].

Determining the inhibitory activity of olive leaf extracts against some types of yeast

The method of propagation by drilling was used, according to what was mentioned by [22]. The solid Muller Hinton Agar medium was inoculated with a sterile cotton swab from bacterial and fungal suspension and made holes on the surface of the culture medium using a cork drill with a diameter of (6 mm), and the prepared concentrations of the extract were set at (0.15. mm) per hole using an accurate pipette. Use the sterile extraction solution as a model for comparison. The plates were left at room temperature for (20 minutes), then the plates were incubated at (37 °C) for (24 hours) for bacteria and (48 hours) for yeasts. The effectiveness of the isolated extracts and phenolic compounds was determined by measuring the diameter of the inhibition zone (halo) around each hole using a numbered ruler.

Testing the sensitivity of fungal samples to antifungals

The fungal samples were cultured using sterile cotton on solid Muller-Hinton medium. After that, the prepared antibiotics were placed on the culture medium and left for (15 minutes), and then the samples were incubated for (24-48 hour) before measuring the diameter of inhibition of each tablet and using the method used to measure the effectiveness of the extracts and active compounds themselves.

RESULTS AND DISCUSSIONS

Cultural characteristics for diagnosing yeast isolates

The identification of 100 yeast isolates was confirmed, distributed as follows: 50 *C. albicans*, 28 *C. glabrata*, 14 *C. krusei*, 8 *C. kefyr*. And diagnosed by the Vitek device according to the method [23], as the *Candida* was determined using the morphological features. and the rest of the cultural characteristics and biochemical tests, and the individuals of this genus appeared in the form of a white to milky colony, smooth and convex when grown in the medium of Sabouraud Dextrose Agar chloramphenicol (SDAC) for Tow-t days at a temperature of (37 °C), and the colony was examined microscopically after dyeing with the blue lactophenol dye, and it was noted that the cells appeared spherical to oval or long- single and budding, and the presence of false fungal spindles and it is noted that there is Pseudohyphae, and this is sometimes agreed with [24].

Microscopic Characteristics

At dyeing with cotton-lactophenol blue dye, *C.albicans* and *C.glabrata* appeared as spherical to semi-spherical budding cells, *C.krusei* as elongated to oval-shaped budding cells, and *C.kefyr* as short, oval budding cells, which is consistent with [25]. It was stained with Gram dye (crystal violet) as in Figure (1) and gave positive results as it was colored violet because, its wall contained the thick layer of peptidoglycan [26].

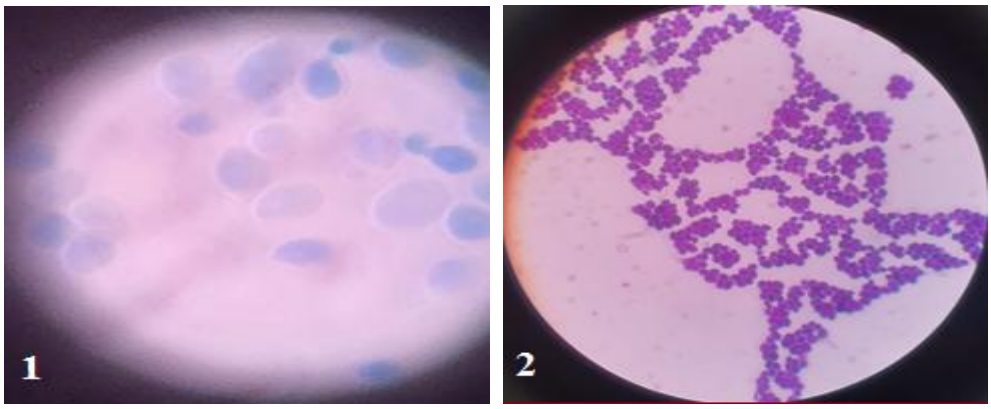


Fig. 1: C.*albicans* yeast: 1) dyed with cotton blue – lactophenol, 2) dyed with crystal violet (100X).

Identification of yeasts using the culture medium (CMA) Corn Meal Agar

This medium is used to detect the ability of this candida to form chlamydial spores, as the results obtained (Table 1) and Figure 2 showed that all of the studied *candida* does not have the ability to form chlamydial spores when grown in this medium, except the type *C. albicans*, which was identical to the results [26]. In addition, the colors were identical to the company that manufactured the medium, as thick-walled, circular-shaped chlamydial spores were formed at the end of the fungal hyphae, which were either single or in the form of clusters when these ovaries were cultivate in corn meal Agar (CMA) medium, and the reason behind the formation of these spores was the occurrence of starvation of these ovaries. Yeasts and a shortage of food sources, as these spores are formed when conditions are not appropriate, as this environment is described as a starvation medium for yeasts [22].

Table 1: The ability of yeasts to form chlamydial spores in CMA medium.

Yeasts	*C.albicans*	*C.glabrata*	*C.krusei*	*C.kefyr*
Results	+	-	-	-

+Indicates the capacity of yeasts to form chlamydial spores.
- Indicates the capacity of yeasts to form chlamydial spores.

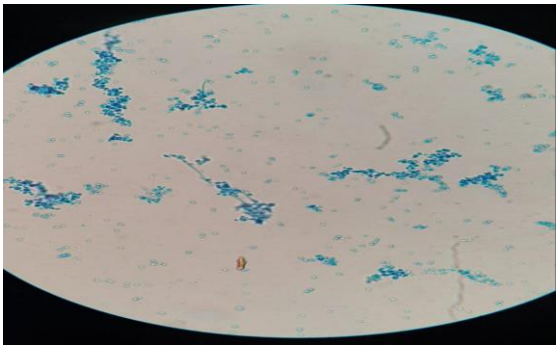
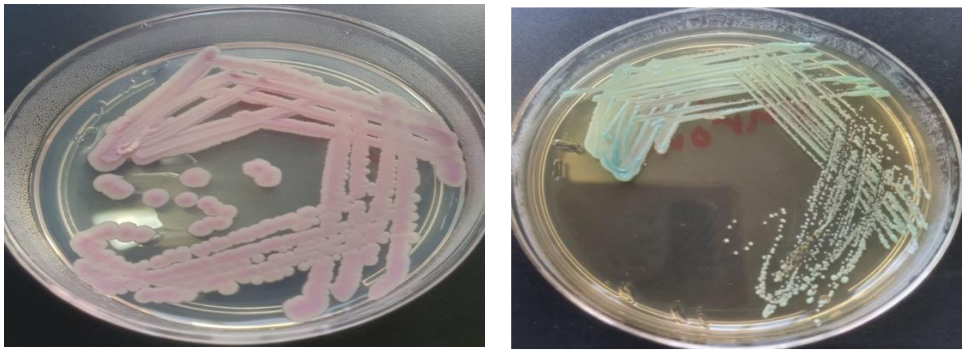


Fig. 2: Formation of chlamydial spores of C. albicans after 24 hour incubation at 25 °C at a power of 40X.

Diagnosis of yeast using Hi Chrom Agar

The growth test on the Chrom Agar medium is a quick and efficient biochemical test method. in diagnosing *Candida* species at the level of color after insemination and incubation compared to other tests, as the test results showed colonies with different colors on the Chrom Agar medium after incubation for (48 hours), and the *C. albicans* colony showed a light green color. As for the *C. glabrata* type, its colonies appeared in pink, and the rest of the isolates appeared in different colors, red and brown mixed as shown in Figure (3) and Table (2), and this is identical with both [27, 28].



C. glabrata *C. albicans*

C. kefyr *C. krusei*

Fig. 3: *Candida* yeast isolates on HiCrome *Candida* differential agar.

Table 2: The species of *Candida* spp. that isolated according to color on HiCrome *Candida* differential agar.

The species of *Candida spp*	medium color on chrome HiChrome Candida Differential Agar
C. albicans	Lig ht green
C. krusei	pale purple
C. kefyr	Creamy to white
C. glabrta	Creamy to pink

Germination tube formation test

The results of the germination tube formation test Table (3) showed that, all *C. albicans* yeast isolates were able to form a germination tube at a rate of 100%, as shown in Figure (4), while the other species did not form it, and these results were consistent with what was reached by [26, 29].

The ability to form a germ tube is an important virulence factor that is closely related to the pathogenicity of *C. albicans*. The importance of the germ tube lies in enabling the yeast to adhere to the surfaces of the host epithelial cells to cause infection. The formation of the germ tube is observed when *C. albicans* yeast changes from the yeast form to the hyphae form [18].

Table 3: The ability of isolated *Candida* SPP to form germ tube.

***Candida* spp.**	*C.albicans*	*C.glabrata*	*C.krusei*	*C.kefyr*
Germ tube	+	-	-	-

+ formed germ tube.

- Not formed germ tube.

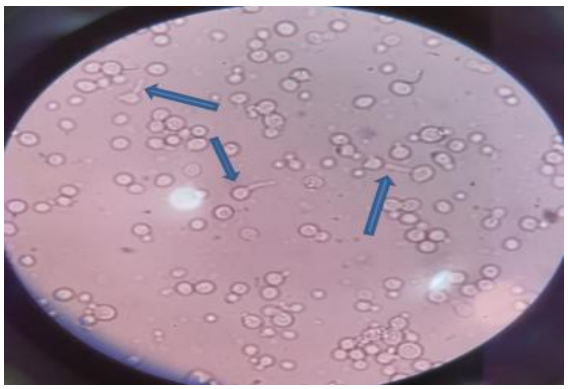


Fig. 4: Formation of the germ tube of a *C. albicans* yeast cell growing in human blood serum after two hours of incubation at 37 °C (40X).

The Candida species isolated and diagnosed in the study

Table (4) shows the numbers and percentages of the isolated *Candida* species in the study, which amounted to 100 samples, after conducting all the tests that were previously presented. The number of *C. albicans* isolates reached 50 isolates, representing (50 %), compared to Non-albicans *Candida*, which represented (50 %), and was distributed among the tested species as follows: 28 isolates, representing (28 %) of the *C. glabrata* species, 14 isolates, representing (14 %) of the *C. Krusei* species, and eight isolates, representing (8%) of the *C. kyfer* species. The results were close to what was reached by [30]. The reason for the increase of *C. albicans* yeast over other *Candida* species may be attributed to its great ability to adhere to the host surfaces causing candidiasis, its ability to form a large number of colonies and inhibit defense mechanisms in different parts of the host's body [31]. In addition to other virulence factors such as: its ability to form a germination tube, morphological transformation and biofilm formation and its ability to secrete enzymes such as; aspartic Proteinase responsible for protein analysis as well as phospholipase enzyme responsible for the analysis of phospholipids which are the main component of the yeast cell membrane [26]. In addition to being a normal flora in the body, it turns into a pathogenic one when the appropriate conditions are available and the host's immunity is weak. The increase in fungal infection in general is due to the use of medical devices (catheters), intravenous feeding and the random use of antibiotics [32].

Table 4: The Numbers and percentages of species of isolated *Candida* spp.

Candida spp.	Number	Percentage
C. albicans	50	50 %
C. glabrata	28	28 %
C. krusei	14	14 %
C. kefyr	8	8 %
Total	100	100 %

Study of the effect of plant extracts of olive leaves on some pathogenic *Candida* yeasts

The effect of the acetonic extract of olive leaves was studied against the yeasts of the Candida genus *C.albicans*, *C.glabrata*, *C.krusei* and *C.kefyr*, which are pathogenic to humans, using the method of diffusion in holes according to [22].

Table (5) shows the effect of the acetonic extract of olive leaves on the yeasts that were studied, and Figure (5) shows the effect of plant extracts of olive leaves on some pathogenic Candida yeasts.

Table 5: The effect of plant extracts (acetonic and aqueous) of olive plant leaves on some pathogenic Candida yeasts.

Types of Yeasts	Types of extracts	Concentration in mg/mm Inhibition halo diameter in mm				Average effect of extract
		25	50	75	100	
C.albicans	acetonic	2	10	12	15	7 BC 9.
	aqueous	8	16	20	20	A 16
C.glabrata	acetonic	8	10	13	14	B 11.3
	aqueous	12	13	15	16	A 14.0
C.krusei	acetonic	8	10	12	15	BC 11.2
	aqueous	8	11	14	19	B 13
C.kefyr	acetonic	2	4	8	12	CD 6.5
	aqueous	2	13	16	18	B 12.2

*Capital letters indicate significant differences inside the column. *All significant differences at the probability level P≤ 0.05.

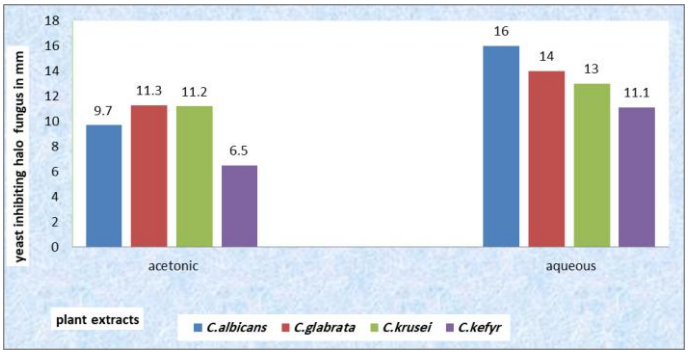


Fig. 5: The effect of plant extracts of olive leaves on some pathogenic *Candida* yeasts.

We note in Table (5) and Figure (5) that, the aqueous and acetonic extract of olive leaves was the most effective against all the yeasts studied and the highest level of effect of the aqueous extract was against *C. albicans* yeast, as the average effect was 16 mm, followed by *C. glabrata* and *C. krusei* yeast, on which the average effect of the aqueous extract was (14-13 mm), respectively. The lowest effect of the aqueous extract was recorded against *C. kefyr* yeast, at a rate of (11.1 mm). These results were close to what was reached by [22], who found that, the aqueous extract was more effective against all isolated yeasts than the alcoholic extract. This effectiveness of the aqueous extract is due to the high ability of water to dissolve the active compounds, as water is a good solvent for most of these compounds, especially phenols, alkaloid salts, some tannins and saponins[33]. mentioned in a study conducted using water and alcohol to extract the active compounds from garlic that water had a high ability to dissolve phenols and alkaloid salts such as tartaric and malic acids, which are known for their antibacterial effectiveness. The antifungal activity of these extracts is attributed to the active compounds in the plant, especially phenolic compounds, saponins, alkaloids and tannins. Phenols have a high activity against fungal cells according to [34, 35]. since the aromatic ring in these compounds contains a hydroxyl group (-OH) which tends to form a hydrogen bond with the active part of the enzymes inside the body of the germs, thus changing the sizes and properties of these enzymes. Consequently, these enzymes are no longer effective in the fungal or bacterial cell, leading to the cessation of certain vital pathways in the cell, which results in a cessation of the growth of these cells or their direct death. Phenolic compounds also, affect the balance of the cellular content by causing damage to some proteins on the cell membrane. In addition, they work to denature the protein, and these compounds are absorbed by the surfaces of proteins and are complex with them, changing the shape and size of the proteins, changing their properties, and thus changing the nature of their work, stopping one of the vital pathways in the cell, stopping the growth of the cell and killing it [36]. As for saponins, their work is primarily on ergosterol in the fungal cell membrane, as they work to destroy it, which leads to the losing the vitality of membrane, and thus the fungal cell dies [37]. Tannins work to precipitate proteins associated with the cell membranes of fungi, and thus they affect the process of entering and exiting materials from and to the fungal cell [38]. As for alkaloids, they do a similar job to what saponins do, and they also work to create a difference in the osmotic pressure of the fungal cell and play an important role in interfering with the construction of cell proteins, and thus negatively affect the process of building DNA [39]. Our results were similar to [40].

Candida sensitivity test to some antifungals: The results of Table (6) and Figure 6 indicate the sensitivity of the studied isolates to some antifungals.

Table 6: The effect of antifungals on yeasts.

	Nystatin (NY)	Amphotericin (AmB)	Clotrimazole (CTM)	Fluconazole (FCN)
C .albicans	0	0	10	12
c. glabrata	0	8	14	0
c. kefyr	5	0	12	15
c. krusei	0	0	21	16

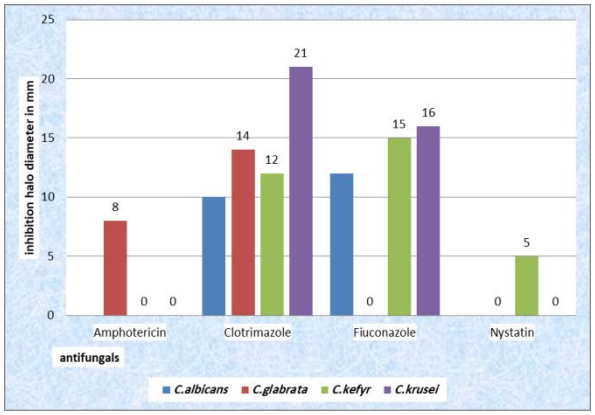


Fig. 6: The effect of antifungal on *candida*.

These results are consistent with those of [5, 41]. They found an effect of the antifungal Fluconazole on *C. albicans* yeast, and consistent with [41]. who found no effects of the antifungal Amphotericin on *C. albicans* yeast. They are consistent with [42]. They found no effect of the antifungal Fluconazole on *C. glabrata* yeast. The random use of antifungals by many people without taking into account the harm caused by the antifungals and the decrease in their medical effectiveness at times when the patient actually needs them, has caused these antifungals to lose their effectiveness if they are used randomly and irregularly, due to the increase in bacterial resistance to them and their adaptation to bear the effect of the amount of antifungals, so the antifungals become useless, in addition to other effects caused by antifungals in the body. Studies have confirmed that the use of antifungals increases the probability of infection with candida from (30-10 %) [43, 44].

These changes in drug sensitivity over time can be explained by the ability of yeasts to tolerate the toxic effects of the antibiotic and their ability to develop some resistance mechanisms. Such as; the discrepancy in the results of the current study with the outcomes of additional research in developed countries can be explained on the basis of the environmental location and excessive use of antifungals. Cases of genetic resistance transfer between resistant isolates to sensitive isolates may be explained by healthy practices and the negative personality, including the discharge of sewage into drinking water, which leads to the transfer of resistance genes [45].

CONCLUSIONS

The study's findings demonstrated that the aqueous extracts had greater inhibitory action than the alcoholic extracts at all doses. Additionally, the extract's inhibitory impact increases with its concentration.

Conflict of interests: The authors declared no conflicting interests.

Sources of funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contribution: Authors contributed equally in the study.

REFERENCES

1. Musleh TMM, Al-Saadi HAM. Diagnosis of yeasts isolated from the oral cavity and groin area in children of Kirkuk city/Iraq. Tikrit Journal of Pure Science. 2022;27(4):7-16. https://doi.org/10.25130/tjps.v27i4.27

2. Dheeb BI, Abdulla SN, Shanter ALQaysi SA, Al-Sarraj BM, Farhan MS. Extraction of Klebsiella pneumoniae and Candida albicans Biofilm and Studying their Cytotoxic Effects on Human Lymphocytes. Jordan Journal of Biological Sciences. 2023;16(4). https://doi.org/10.54319/jjbs/160418

3. Baddley JW, Pappas PG, Smith AC, Moser SA. Epidemiology of Aspergillus terreus at a university hospital. Journal of clinical Microbiology. 2003;41(12):5525-9.

https://doi.org/10.1128%2FJCM.41.12.5525-5529.2003

4. Guinea J. Global trends in the distribution of C andida species causing candidemia. Clinical Microbiology and Infection. 2014;20:5-10. https://doi.org/10.1111/1469-0691.12539

5. Abdulla SN, Dheeb BI, AL-Qaysi SA, Farhan MSS, Massadeh M, Hamid AF. Isolation and Diagnosis of Biofilms of Klebsiella pneumoniae Bacteria and Candida albicans Yeast, and Studying the Sensitivity of The Pathogens to Antibiotics and Antifungals. Egyptian Academic Journal of Biological Sciences, G Microbiology. 2023;15(2):73-88. https://doi.org/10.21608/eajbsg.2023.320271

6. Cavalheiro M, Teixeira MC. Candida biofilms: threats, challenges, and promising strategies. Frontiers in medicine. 2018;5:28. https://doi.org/10.3389/fmed.2018.00028

7. Németh T, Tóth A, Szenzenstein J, Horváth P, Nosanchuk JD, Grózer Z, et al. Characterization of virulence properties in the C. parapsilosis sensu lato species. PloS one. 2013;8(7):e68704. https://doi.org/10.1371%2Fjournal.pone.0068704

8. Silva-Dias A, Miranda IM, Branco J, Monteiro-Soares M, Pina-Vaz C, Rodrigues AG. Adhesion, biofilm formation, cell surface hydrophobicity, and antifungal planktonic susceptibility: relationship among Candida spp. Frontiers in microbiology. 2015;6:205. https://doi.org/10.3389/fmicb.2015.00205

9. Al-abide NM, Shugran AHM, Mezher MA. Antifungal effects of alcoholic extracts for plant belong to Brassicaceae family against Candida albicans isolated from patients. Tik J of Pure Sci. 2022;27(2):1-6. https://doi.org/10.25130/tjps.v27i2.59

10. Farhan MS, Abdullah BA, Mamdwooh AE, Numan RS. Review of Virulence Factors in Candida. Journal for Research in Applied Sciences and Biotechnology. 2024;3(2):75-82. https://doi.org/10.55544/jrasb.3.2.15

11. Vazquez JA, editor Invasive fungal infections in the intensive care unit. Seminars in respiratory and critical care medicine; 2010: © Thieme Medical Publishers. https://doi.org/10.1055/s-0029-1246289

12. Maza JL, Elguezabal N, Prado C, Ellacuría J, Soler I, Pontón J. Candida albicans adherence to resin-composite restorative dental material: influence of whole human saliva. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2002;94(5):589-92. https://doi.org/10.1067/moe.2002.126024

13. Al-Obeide AA, Al-Assie AH. ANALYSIS OF GENETIC DIVERSITY OF SOME OLIVEGENOTYPES USING RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKERS. Tikrit Journal of Pure Science. 2019;24(1):1-6. https://doi.org/10.25130/tjps.v24i1.323

14. da Silva-Rocha WP, Lemos VLdB, Svidizisnki TIE, Milan EP, Chaves GM. Candida species distribution, genotyping and virulence factors of Candida albicans isolated from the oral cavity of kidney transplant recipients of two geographic regions of Brazil. BMC oral health. 2014;14:1-9. http://www.biomedcentral.com/1472-6831/14/20

15. Jørgensen KJ, Gøtzsche PC, Johansen HK. Voriconazole versus amphotericin B in cancer patients with neutropenia. Cochrane Database of Systematic Reviews. 2006(1). https://doi.org/10.1002/14651858.CD004707.pub2

16. Arulmozhi S, Mazumder PM, Purnima Ashok PA, Narayanan LS. Pharmacological activities of Alstonia scholaris Linn. (Apocynaceae)-A review. 2007.

17. Hussein HS. Isolating and Diagnosing Some Types of Candida spp Yeast and Studying Their Sensitivity to Some Antifungals. South Asian Res J Pharm Sci. 2024;6(4):124-30. https://doi.org/10.36346/sarjps.2024.v06i04.002

18. Hidayati Y, Asnaily A, Jumaisal A, Simanjuntak JP. Comparison of Several Types of Plasma as

Media in the Germ Tube Test for Identification of Candida Albicans. Jurnal Teknokes. 2023;16(2). http://dx.doi.org/10.35882/teknokes.v16i2.505

19. Kaur R, Dhakad MS, Goyal R, Haque A, Mukhopadhyay G. Identification and antifungal susceptibility testing of Candida species: a comparison of Vitek-2 system with conventional and molecular methods. Journal of global infectious diseases. 2016;8(4):139-46. . https://doi.org/10.4103%2F0974-777X.192969

20. Al- Ahbabi Akthem KIB, Milad A Mezher. . Determination the inhibitory effect of olive leaf extracts on some pathogenic yeasts & bacteria. 2017.

21. AL-Bajilan A. Study of the inhibitory of Snake venom Macrovipra lantana against the Virulence factor of vaginal Candida spp: Doctoral thesis. Tikrit University Iraq, 133pp; 2016.

22. Al-Jaber GTN. Antibacterial activity of extracts of two phenolic compounds of sumac plant Rhus sp. Basra Research Journal (Scientific). . Basra Research Journal (Scientific). 2008(Issue Thirty-four. Part Two):p. 22-32.

23. Siopi M, Pachoulis I, Leventaki S, Spruijtenburg B, Meis JF, Pournaras S, et al. Evaluation of the Vitek 2 system for antifungal susceptibility testing of Candida auris using a representative international panel of clinical isolates: overestimation of amphotericin B resistance and underestimation of fluconazole resistance. Journal of Clinical Microbiology. 2024;62(4):e01528-23. https://doi.org/10.1128/jcm.01528-23

24. Bhavan PS, Rajkumar R, Radhakrishnan S, Seenivasan C, Kannan S. Culture and Identification of Candida albicans from Vaginal Ulcer and Separation of Enolase on SDS-PAGE. International Journal of Biology. 2010;2(1):84-93.

25. Haw B, Asma I, Eugene O, Sasidharan S. Phenotyping identification of candida albicans for the production of in house helicase for nucleic acid-based detections for fast diagnosis. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2013;4(2):576-83.

26. Hameed RM, Abed HA. Isolating and identifying yeasts in women with vaginal candidiasis and studying their ability to form biofilms and haemolysin. Samarra Journal of Pure and Applied Science. 2024;6(2 (2)):149-61. https://doi.org/10.54153/sjpas.2024.v6i2/2.726

27. Bayona JVM, García CS, Palop NT, Cardona CG. Evaluation of a novel chromogenic medium for Candida spp. identification and comparison with CHROMagar™ Candida for the detection of Candida auris in surveillance samples. Diagnostic Microbiology and Infectious Disease. 2020;98(4):115168.

28. Bentz ML, Le N, Min B, Nunnally NS, Sullivan V, Tran M, et al. Evaluation of CHROMagar Candida Plus for the detection of C. auris with a panel of 206 fungal isolates and 83 colonization screening skin-swabs. Microbiology Spectrum. 2024;12(4):e03564-23. https://doi.org/10.1128/spectrum.03564-23

29. Kumar R, Shukla P. Amphotericin B resistance leads to enhanced proteinase and phospholipase activity and reduced germ tube formation in Candida albicans. Fungal Biology. 2010;114(2-3):189-97. https://doi.org/10.1016/j.funbio.2009.12.003

30. Arora S, Dhuria N, Jindal N, Galhotra S. Speciation, biofilm formation and antifungal susceptibility of Candida isolates. International Journal of Research and Development in Pharmacy & Life Sciences. 2017;6(2):2517-21. http://dx.doi.org/10.21276/IJRDPL

31. Dhanasekaran D, Vinothini K, Latha S, Thajuddin N, Panneerselvam A. Human dental biofilm: Screening, characterization, in vitro biofilm formation and antifungal resistance of Candida spp. The Saudi Journal for Dental Research. 2014;5(1):55-70. https://doi.org/10.1016/j.ksujds.2013.10.001

32. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. Cell. 2017;168(4):707-23. https://doi.org/10.1016/j.cell.2017.01.017

33. Al-Saadi HARA. Study of the effect of some plant extracts and antifungals on some skin fungal infections of disease samples in Kirkuk city: Tikrit University; 2012.

34. Alimosazadeh P AKM, Sanei SJ. . Phytochemical and anticandidal efficacy of Olea europaea leaf extract from different cultivars and seasonal variation. . J Herbmed Pharmacol. 2023;12(4):505-11. https://doi.org/10.34172/jhp.2023.45000

35. Yahia M MS. An overview of chemical composition and fungicidal activity of Olive (Olea europaea L.) Leaf Extract. Egypt J Chem 2023;66(1):303-13. https://doi.org/10.21608/ejchem.2022.120206.5397

36. A y-ZJ, Vega-Vega V, Rosas-Domínguez C, Palafox-Carlos H, Villa-Rodriguez JA, Siddiqui MW, et al. Antioxidant and antifungal potential of methanol extracts of Phellinus spp. from Sonora. 2012;29:132-8. https://doi.org/10.1016/j.riam.2011.09.004

37. Yendo AC, de Costa F, Gosmann G, Fett-Neto AG. Production of plant bioactive triterpenoid saponins: elicitation strategies and target genes to improve yields. Molecular biotechnology. 2010;46:94-104. https://doi.org/10.1007/s12033-010-9257-6

38. Noman SJ, Suliman AK. Effect of tannin fruit extracts on Candida albicans yeast: Tannin nanoparticles. International Journal of Medical Sciences. 2023;6(2):10-8. https://doi.org/10.32441/ijms.6.2.2

39. Essghaier B, Ben Khedher G, Hannachi H, Dridi R, Zid MF, Chaffei C. Green synthesis of silver nanoparticles using mixed leaves aqueous extract of wild olive and pistachio: characterization, antioxidant, antimicrobial and effect on virulence factors of Candida. Archives of Microbiology. 2022;204(4):203. mailto:https://doi.org/10.1007/s00203-022-02810-3

40. Kinkela Devčić M, Pasković I, Kovač Z, Knežević PT, Morelato L, Glažar I, et al. Antimicrobial Activity of Olive Leaf Extract to Oral Candida Isolates. Microorganisms. 2024;12(8):1726. https://doi.org/10.3390/microorganisms12081726

41. Nikoomanesh F, Falahatinejad M, Černáková L, Dos Santos ALS, Mohammadi SR, Rafiee M, et al. Combination of farnesol with common antifungal drugs: Inhibitory effect against Candida species isolated from women with RVVC. Medicina. 2023;59(4):743. https://doi.org/10.3390/medicina59040743

42. de Barros DB, e Lima LdO, da Silva LA, Fonseca MC, Ferreira RC, Neto HD, et al. α-Pinene: docking study, cytotoxicity, mechanism of action, and anti-biofilm effect against Candida albicans. Antibiotics. 2023;12(3). https://doi.org/10.3390/antibiotics12030480

43. Czajka KM, Venkataraman K, Brabant-Kirwan D, Santi SA, Verschoor C, Appanna VD, et al. Molecular Mechanisms Associated with Antifungal Resistance in Pathogenic Candida Species. Cells. 2023;12(22):2655. https://doi.org/10.3390/cells12222655

44. Maubon D, Garnaud C, Calandra T, Sanglard D, Cornet M. Resistance of Candida spp. to antifungal drugs in the ICU: where are we now? Intensive care medicine. 2014;40:1241-55. https://doi.org/10.1007/s00134-014-3404-7

45. Argüelles A, Sánchez-Fresneda R, Guirao-Abad JP, Lozano JA, Solano F, Argüelles J-C. Insight into the Antifungal Effects of Propolis and Carnosic Acid—Extension to the Pathogenic Yeast Candida glabrata: New Propolis Fractionation and Potential Synergistic Applications. Journal of Fungi. 2023;9(4):442. https://doi.org/10.3390/jof9040442