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Research Article

Study of The Effect of Combination of Some Medicinal Plant Extract Against Candida albicans Isolated From Clinical Sourse

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Abstract:

Background: *Candida albicans* are opportunistic pathogens that affect mucosal, dermal, urogenital, and gastrointestinal areas, necessitating the search for effective treatments against them. Recently, plant extracts such as Garlic (*Allium sativum*), Ginger (*Z. officinale*), myrrh (*Commiphora myrrha*), and propolis have been used to treat infections caused by these yeasts. **Methods**: The *Candida albicans* were isolated and identified from their different clinical sources, alcoholic extracts were prepared at different concentrations (10, 25, 50, 75, 100) mg $ml^{-1}$to study of inhibitory effect of alcoholic extracts of Garlic, Ginger, Myrrh and Propolis each alone and mixed with Ginger against *C. albicans*. **Results**: The results showed all alcoholic extracts had variable inhibitory effect against yeast growth, with the concentration of 100 mg ml^{-1} recording the highest inhibitory effect, while the alcoholic extract of propolis at a concentration of 50 mg ml^{-1} was the most effective. It was also shown, by study of the combination (mixing) between alcoholic Ginger extract and other extracts, that there different degrees of inhibition of yeast growth **Conclusions**: The study revealed the importance of plant extracts' effect when mixed to control resistant *C. albicans* that threaten human health.

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Introduction

Candida albicans, the white yeast, is one of the commensal yeasts that is widespread everywhere and is one of the most common pathogenic organisms found on the skin and mucous membranes such as the oral cavity, gastrointestinal tract, and vagina. It can grow in the form of yeast, pseudohyphae, or true hyphae depending on environmental conditions. **[1]**

They can be found on parts of normal human skin and are considered to be commensal microbes in the intestines, so they are typically present in approximately 60% of healthy individuals. *C. albicans* can also colonize many sites in the human body, such as the skin, pharynx, lower respiratory tract, gastrointestinal tract, and urogenital tract. Moreover, *C. albicans* is also present in hospitals and poses a particular concern, especially in intensive care units and catheterization, because bloodstream candidiasis associated with venous lines can serve as substrates for biofilm formation. **[2]**

White Candida is more pathogenic when it transitions from yeast form to filamentous form. According to data related to fungal infections in hospitals, the mortality rate among immunocompromised patients infected with *C. albicans* is estimated to be 40%, making *C. albicans* the most dangerous infectious fungal strain. **[1]**

There have been changes in the epidemiology of *C. albicans* over the many decades and in different geographical areas due to some host factors in patients, certain therapeutic practices such as chemotherapy, organ transplantation, and the use of intensive care. The indiscriminate use of antifungals had a clear impact during the recent COVID-19 pandemic, as the incidence of candidemia during the outbreak was five times higher than it was before the pandemic. **[3]**

The pathogenicity of *C. albicans* is supported by several virulence factors, such as the secretion of hydrolytic enzymes, the ability to adhere to medical devices or host cells, the formation of biofilms, and the transition from yeast form to filamentous form. **[2]**

C. albicans has the ability to form biofilms on both living and non-living surfaces, which are aggregates of cells that adhere to solid surfaces. This is the most common growth phase for many microbial species and often enhances their resistance to drugs. **[4]** Biofilms contribute to cell spread, protection from the immune system, and the development of resistance to antifungal agents such as Azoles and Polyenes. **[5]**

The pathogenic capability of *C. albicans* is attributed to several morphological properties and virulence factors that enable it to resist antifungal treatments, adapt to environmental stresses, and evade the host's immune system. The morphological properties include metabolic flexibility, rapid adaptation to fluctuations in environmental pH, and robust nutrient acquisition systems. The virulence factors encompass morphological transition between yeast forms and filamentous forms (phenotypic switching), biofilm formation, the expression of adhesive substances, and invasion. **[6]**

Candida species must be capable of effectively colonizing their host as well as adapting to a variety of determinants such as (nutrients, pH, temperature (37 - 40°C), carbon dioxide concentrations (5.5%), amino acids, and various biological conditions such as carbon source and nutrient availability. **[7]**

The repeated use of antifungals has led to the development of strong resistance in *C. albicans*. Several adaptation mechanisms for resistance to antifungal drugs and tolerance have been identified, such as: modifying the drug target or overexpressing it, regulating multidrug transporters, and activating cellular stress responses. **[8]**

There are four classes used to treat *C. albicans* infections: Azoles, Polyenes, Echinocandins, and Pyrimidine analogues. **[5]**

Antifungal drugs available are usually limited compared to antibacterial drugs. Patients undergoing treatment with antifungals show difficulty in recovery due to the emergence of antifungal resistance, and three types of antifungal resistance have been described: first, primary or intrinsic resistance, which appears before exposure to antifungal agents; second, secondary or acquired resistance, which develops after exposure to an antifungal agent; third, clinical resistance. **[9]**

Medicinal plants are commonly used as an alternative to antifungal agents to reduce resistance. Medicinal plants produce an unlimited number of bioactive compounds, and their use has been exploited as antimicrobial agents that can work alone or in conjunction with antibiotics to enhance antimicrobial activity against a wide range of microbes. **[10]**

The medicinal value of medicinal plants is linked to their therapeutic efficacy, which is governed by the possession of active biological compounds known as secondary metabolites, such as flavonoids, alkaloids, terpenes, glucosides, phenols, plant steroids, curcumines, in addition to pigments and antioxidants that add high value to the medicinal properties of these plants. Secondary metabolites are produced through specific biosynthetic pathways and are distributed in different parts of the plant, such as leaves, roots, stems, bark, flowers, fruit, and seeds. **[11]**

In this study, Garlic (*Allium sativum*) extract and Ginger (*Z. officinale*) extract were used to test their ability to inhibit the growth of *C. albicans* yeasts. Many studies have indicated the importance of Garlic (*Allium sativum*) in inhibiting the growth of *C. albicans* yeast. **Chinedu (2019)** mentioned that *C. albicans* yeast showed significant sensitivity to Garlic (*Allium sativum*) extract, attributing the effectiveness of Garlic (*Allium sativum*) extract to its chemical components such as sulfur, peroxidase, allicin, and others (main components) that are soluble in water, thus retaining most of their antimicrobial (fungicidal) properties during the extraction process. **Agustantina** (2021) treated *C. albicans* yeast with several concentrations of Garlic (*Allium sativum*) extract and noted that the higher concentrations showed effectiveness in inhibiting the yeast. **[12] [13]**

Studies have indicated that Ginger (*Z. officinale*) extract has antifungal effects. For example, **Khan *et al.* (2021)** noted that the use of methanolic Ginger (*Z. officinale*) extract (Meth-Gin) combined with antifungals effectively reduces pro-inflammatory cytokines and the rate of programmed cell death in tissues infected with *C. albicans.* **Khalaf *et al.* (2020)** reported on the effect of alcoholic Ginger (*Z. officinale*) extract on the inhibition of biofilm formation in *C. albicans* yeast. **[14] [15]**

Marjoram extract is used as an antifungal and is used in the treatment of *C. albicans*. **[16]** and athlete's foot, and subcutaneous ulcers. The British Herbal Pharmacopoeia recommends mouthwash with Myrrh (*Commiphora myrrha*) tincture for gingivitis and ulcers. **[17] Zaher et al. (2024)** indicated that the use of aqueous extract of marjoram had a clear effect against *C. albicans* due to its possession of many active compounds such as: (2-Pentanone, 4-hydroxy-4-methyl, Undecanal, Indan-1,3-diol monopropionate, and Triacetin). This was also indicated by the study conducted by **Abdulbary (2018)**, which confirmed the role of marjoram extract as an antifungal. **[18] [19]**

Propolis is a resinous substance collected by honey bees from different parts such as stems, leaves, and flowers of plants and trees, like *Populus spp*, Eucalyptus, *Betula alba*, *Alnus glutinosa Medik*, and conifers. **[20]** Propolis possesses anti-inflammatory, anti-cancer, antioxidant, anti-allergic, and antimicrobial activities and most of the biological activities found in propolis are attributed to its association with secondary metabolites of the plants, which include phenolics and terpenoids. **[21]** The alcoholic extract of propolis contains several biologically active substances that are usually variable depending on the type of plant and other conditions. Among the most important compounds are phenolics such as Chrysin, Aromadendrin-40-O-methyl-ether, and Galangin, terpenoids like Artepillin C, Baccharin, Drupanin, and coumaric acids. **[22]**

The study aimed to evaluate the inhibitory effect of Alcoholic Extracts of Ginger (Z. officinale), Garlic (Allium sativum), Myrrh (Commiphora myrrha) and Propolis against *C. albicans* on the one hand and on the other hand, a study of the synergistic effect of ginger extract (Z. officinale) with garlic extracts (Allium sativum), myrrh (Commiphora myrrha) and propolis against *C. albicans.*

Methods Sample Preparation

- **Isolation and Identification of Fungal Samples:**

Samples previously collected from hospitals in Karbala city were inoculated onto solid Sabouraud dextrose medium in ready-to-use and sterilized Petri dishes, and then the samples were incubated at a temperature of 37°C for 24 – 48 hour. After that, the isolates were morphologically diagnosed by observing the morphological characteristics of the yeast colonies, including the shape, color, and texture of the colony. They were then stored in the refrigerator until further use. **[23]**

Biochemical Diagnosis :

1. Morphological and Microscopic Diagnosis:

After cultivation on solid Sabouraud dextrose medium, fungal growth was observed, and the shape, color, texture, diameter and height of the colonies were determined morphologically. Later, a quantity of the growing yeast was taken and transferred to a glass slide, a drop of blue lactophenol stain was added, it was fixed by heat, and then covered with a cover slip. It was then placed under the microscope and examined at a magnification of 40x to observe the shape of the cells and the size of the buds. **[24]**

2. Diagnosis using Chrom Agar Medium:

Chrom agar medium was used after obtaining various species of the Candida genus to differentiate yeast types based on the yeast color. The isolates taken on the above medium were incubated for 48 hour at a temperature of 37°C, and then the results were recorded according to the color of the Candida spp. colonies. **[25]**

3. Germ Tube Test:

The germ tube test is used to distinguish between the types of Candida yeasts that can form the germ tube and other yeast types that cannot form it. The test is performed by taking a portion of the colony and adding it to a test tube containing 0.5 ml of serum. The tube is incubated at a temperature of 37°C for a period of 2-4 hour. After this, a drop of the suspension is taken and placed on a clean glass slide, covered with a coverslip, and examined under a microscope at a magnification of 40x. **[26] [27]**

4. API 20E Test:

The Candida API system contains ten tubes that can detect five types of carbohydrates through acidification tests and various enzymes (seven). The testing procedures are based on sugar acidification and enzyme activity. This is a very accurate and simple method that can be completed without the intervention of computers. It produces observable changes in color and morphological characteristics to identify Candida species. **[7]**

5. Biochemical Diagnosis using the Vitek 2 System:

It is a fully automated diagnostic system used to diagnose pathogenic yeasts using the Vitek 2 YST card. The diagnostic system is mainly based on biochemical reactions. The card contains 46 biochemical tests, some of which measure carbon consumption, others measure nitrogen consumption, in addition to some enzymatic activities. They are distributed over 46 holes and have specific weights and follow the instructions of the supplier company. The Vitek-2 system includes Vitek2 cards that allow species identification by comparing the biochemical profile with a large-scale database. **[28]**

6. Molecular Identification

Molecular diagnostic methods based on DNA sequencing are highly accurate and sensitive techniques for identifying and distinguishing *C. albicans* from other Candida species. Polymerase chain reaction (PCR) is a leading technology in molecular microbiology, and the molecular aspect uses the PCR technique to identify *C. albicans*. A specialized primer has been used for the purpose of diagnosing bacterial isolates.

7. Preparation of Plant Extracts

The extracts of medicinal plant extracts (garlic fruits (*Allium sativum*), ginger stems (*Z. officinale*), myrrh resin (*Commiphora myrrha*), raw propolis) used in this study were prepared according to (Rios *et al*., 1987) by weighing 10 grams of each plant extracts and

adding to 200 ml of 70% ethanol, placing it in a flask and incubating it in a shaker for 1 hour. It was then placed in a centrifuge 3600 rpm for 10 minutes, after which the extract was filtered first using layers of medical gauze and then using Whatman filter paper Whatman No. 1. The filtrate was placed in clean, sterile Petri dishes and dried in an oven at 45 ˚C. The dry extract was scraped using a clean, sterile knife and stored in the refrigerator at 4 ˚C after weighing until use. The process was repeated several times to obtain a sufficient amount of extracts.

8. Prepare the Alcoholic Extract Mixture

Five grams of Ginger (*Z. officinale*) were mixed with five grams of medicinal plants (Garlic (*Allium sativum*), Myrrh (*Commiphora myrrha*) and propolis) in a 1:1 weight ratio, and 200 ml of 70% ethanol was added. The mixture was left at 25 degrees Celsius in a shaking incubator for 24 hour, then filtered through several a piece of gauze. The mixture gauze was centrifuged at 3000 rmp for 10 minutes, and then the mixture was filtered again through filter paper Whatman No. 1 filter paper. The extract was dried at 45 ˚C and the plant material was stored in airtight plastic containers until use. **[29] [30]**

9. Testing the Inhibitory Effect of Alcoholic Extracts

The *C. albicans* inoculum was prepared . The well diffusion Method using to test the inhibitory effectiveness of alcoholic extracts alone and mixed with Ginger (*Z. officinale*) on *C. albicans*

With serial dilutions of plant extracts (10, 25, 50, 75 and 100 mg/ml). Each well contained 100 µL of each extract or combination of plant extract with Ginger (*Z. officinale*) extract the, plates were incubated for 24 -48 hour at 37 ˚C the diameter of inhibition zone recorded.

10. Statistical Analysis

To determine the significant differences between the inhibition rate of the plant extracts under study and the concentration of these extracts, the significant differences were determined, and the significant differences between the means were tested using L.S.D at the 5% level.

Results Population of the Study

This study was conducted on 119 patients suffering from clinical symptoms of ear, nose, and throat diseases, burns, wounds, eye injuries, urinary tract infections, vaginal swabs (in women), and oral ulcers in infants across several hospitals in Karbala. The ages varied between 1 and 70, and the age groups were distributed by gender with 80 samples (67.22%) from females and 39 samples (32.8%) from males. The age group (31–50 years) was the most exposed to injury with 57 samples (47.9%) of those included in the study, while the age group (1–2 years) was the least exposed with (10) samples (8.4%).

Identification of Isolates :

1. Morphological and Microscopic Diagnosis:

White or creamy-colored colonies were observed, with a texture somewhat resembling butter, slightly elevated from the medium, appearing convex and smooth, with colony diameters ranging from 1-3 mm, as shown in figure(1).

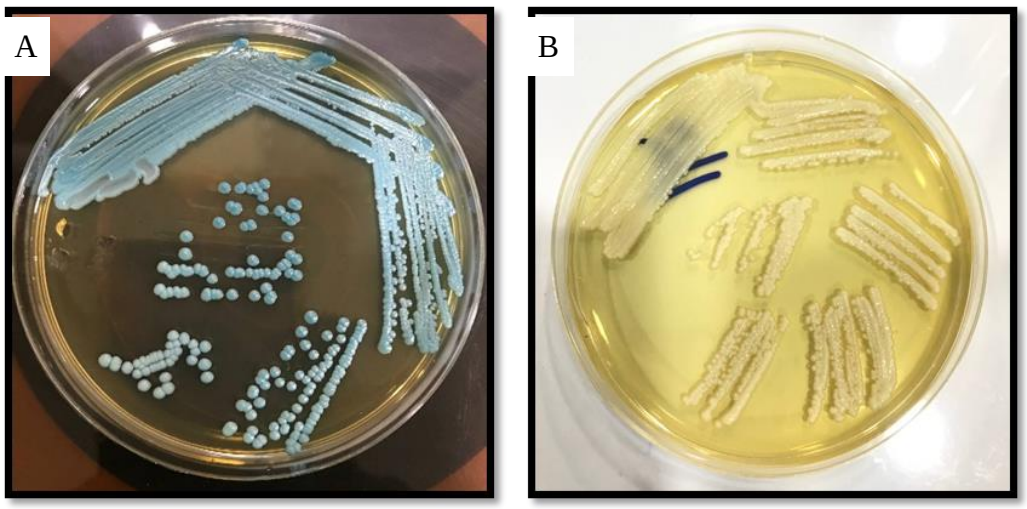

Figure (1): (A) *C. albicans* colony on chrome agar (B) *C. albicans* colony on Sabouraud dextrose agar

2. Diagnosis using API Candida System

The API Candida test was performed for the isolates, and all isolates were identified as *C. albicans*. It includes five sugar assimilation tests (glucose, galactose, sucrose, trehalose, and raffinose) and seven enzymatic tests (b-maltosidase, a-amylase, b-xylosidase, b-glucuronidase, urea hydrolysis, N-acetylb-glucosaminidase, and b-galactosidase). The results were read after incubation for 18 to 24 hour at 35 degrees Celsius. A four-digit profile was created for each isolate based on the reactions it produced. Identifications were made by referencing the list of digital profiles provided by the manufacturer. Figure (2)

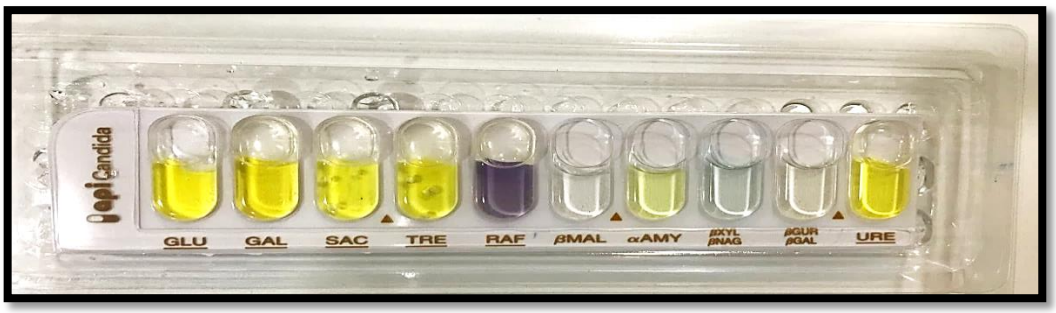

Figure (1): API Candida Yeast Test for *C. albicans*

3. Diagnosis using Vitek2 device

The diagnosis of the isolated species was confirmed using Vitek2 device and Vitek 2 YST card by comparing the results of the biochemical reactions with the information available in the system database. Vitek2 device has high accuracy and speed in diagnosing isolates at the species level and according to the card used for diagnosis.

4. Molecular Identification

Molecular diagnosis was performed using polymerase chain reaction technology to confirm the diagnosis of (31) samples of *Candida spp.*, which were previously diagnosed by the Vitek2 device and Vitek 2 YST card, based on specific primers for *C. albicans*, as shown in Figure (2). The samples were subjected to polymerase chain reaction (PCR) for molecular diagnosis, as well as to amplify the targeted region within the 25rRNA gene sequence. The results of the polymerase chain reaction technique

showed that the samples were positive for (25) isolates of *C. albicans* yeast isolates out of the (31) tested isolates, this results were consistent with. **[31] [32]**

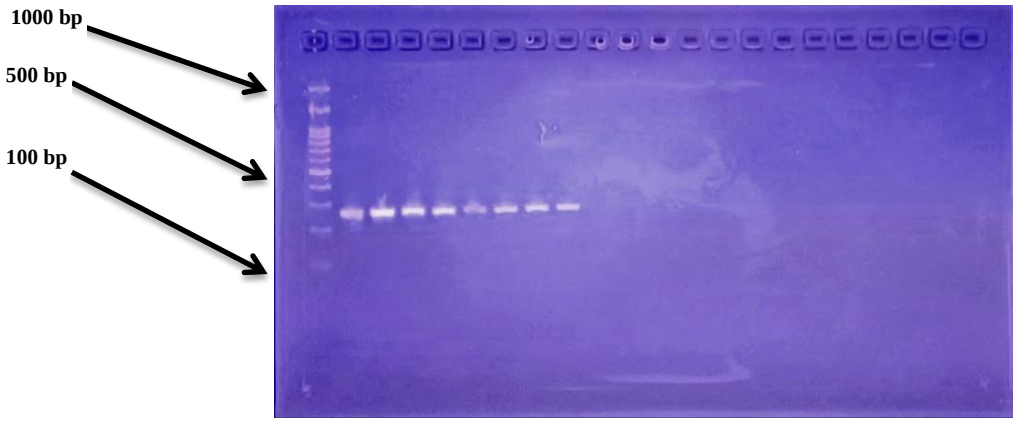

Figure (2): Agarose gel electrophoresis for PCR product of 25rRNA gene

5. The inhibitory Effect of Alcoholic Extracts of Ginger (*Z. officinale*), Garlic (*Allium sativum*), Myrrh (*Commiphora myrrha*) and Propolis against *C. albicans*:

Table (1) shows that most of the extracts of Ginger (*Z. officinale*), Garlic (*Allium sativum*), Myrrh (*Commiphora myrrha*) and propolis against *C. albicans* clearly showed yeast growth inhibitory activity, which was estimated by measuring the growth inhibition zone in mm. The results showed that the growth inhibitory activity increased with increasing concentration of the extracts and the concentration of 100 mg/ml showed the highest inhibitory effect on the growth of Candida and was statistically superior to the rest of the concentrations the propolis extract recorded highest rate inhibition zone at concentration 100mg/ml.

Table (1) : Effect of Alcoholic Extracts of Ginger (*Z. officinale*), Garlic (*Alliumsativum*), Myrrh (*Commiphora myrrha*) and Propolis against *C. albicans*

Plant extract / Concentration	Mean inhibition zone diameter ± SD (mm) for extracts plants			
	Ginger (*Z. officinale*)	**Garlic (*Allium sativum*)**	**Myrrh (*Commiphora myrrha*)**	**Propolis**
10 mg/ml	8.50	8.50	8.50	8.50
25 mg/ml	9.17	8.00	8.00	8.67
50 mg/ml	10.00	10.33	10.33	13.67
75 mg/ml	10.67	10.50	10.67	10.67
100 mg/ml	11.17	11.17	11.50	11.67
LSD	**0.407**	**0.332**	**0.525**	**2.519**

6. Synergistic Effect of Ginger (*Z. officinale*) Extract with Garlic (*Allium sativum*), Myrrh (*Commiphora myrrha*) and Propolis Extracts against *C. albicans*:

The table (2) shows that the mixture of Ginger (*Z. officinale*) and Garlic (*Allium sativum*) had an effect on *C. albicans* and showed a diameter higher 18 mm at the concentration of 100 mg/ml, which is higher than the diameters of inhibition that appeared in examining the inhibitory effect of each extract alone 11.17 mm for both Ginger (*Z. officinale*) and Garlic (*Allium sativum*) An increase of 7.4%. This is an indication of the occurrence of a synergistic effect between the alcoholic extract of Ginger (*Z. officinale*) and Garlic (*Allium sativum*).

Table (2): Synergistic Effect of Ginger (*Z. officinale*) and Garlic (*Allium sativum*) against *C. albicans*

Concentration mg/ml	Mean inhibition zone diameter ± SD (mm) Ginger (Z. officinale) and Garlic (Allium sativum) synergy
10	6.83
25	10.33
50	16.17
75	17.17
100	18.00
LSD	**0.621**

The results of the synergy between Ginger (*Z. officinale*) extract and Myrrh (*Commiphora myrrha*) extract showed statistically significant antifungal effects against *C. albicans* (Table 3). A decrease in the efficacy of the extracts was observed when mixed together compared to the effect of each extract individually. The concentrations used for this synergistic compound (10, 25, 50, 75, and 100) mg/ml recorded lower inhibition rates compared to using these concentrations for each extract alone. This is an indication of the occurrence of a antagonism effect between the alcoholic extract of Ginger (*Z. officinale*) and Garlic (*Allium sativum*).

Table (3): Synergistic Effect of Ginger (*Z. officinale*) and Myrrh (*Commiphora myrrha*) against *C. albicans*

Concentration mg/ml	**Mean inhibition zone diameter ± SD (mm) Ginger (Z. officinale) and Myrrh (Commiphora myrrha) synergy**
10	6.00
25	7.50
50	8.17
75	9.00
100	9.67
LSD	**0.332**

The results obtained from the synergy of Ginger (*Z. officinale*) extract and propolis indicated a statistically significant effect against *Candida albicans* (Table 4). An increase in the effectiveness of the extracts was observed when they were mixed together compared to the effect of each extract individually. The increase was notable in the inhibition results at the concentrations used in this study. This is an indication of the occurrence of a synergistic effect between the alcoholic extract of Ginger (*Z. officinale*) and Propolis.

Table (4): Synergistic effect of Ginger (*Z. officinale*) and Propolis against *C. albicans*

Concentration mg/ml	**Mean inhibition zone diameter ± SD (mm) Ginger (Z. officinale) and Propolis synergy**
10	8.33
25	9.50
50	10.83
75	11.17
100	14.00
LSD	**0.575**

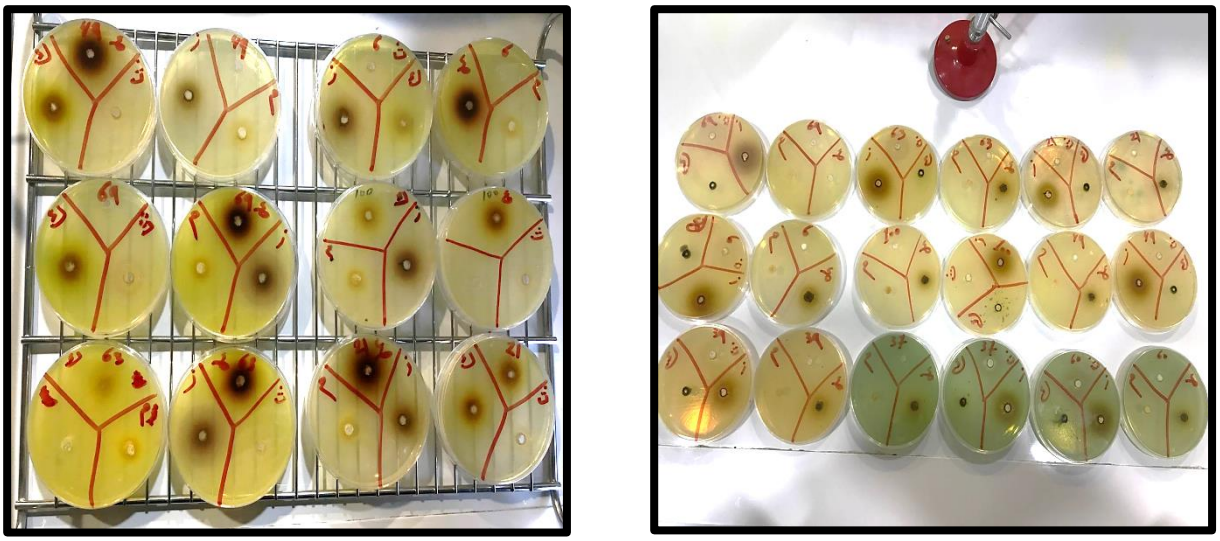

Figure (3): The inhibitory Effect of Alcoholic Extracts of medicinal plant

DISCUSSION

The resistance of microorganisms to antibiotics is a challenge in treating infections caused by these pathogens. The incorrect use of antibiotics in treatment leads to the emergence of highly resistant organisms, which has driven the shift towards traditional folk medicine by using medicinal plants due to their properties in directly affecting the growth and reproduction of these living organisms. The use of extracts from spices and medicinal plants has gained widespread acceptance because they are easy to use and highly effective. Spices possess beneficial biological functions, such as antimicrobial, antibacterial, antifungal, anthelmintic properties, and medicinal properties. **[33]**

The antifungal activity of *C. albicans* against extracts of medicinal plants (Ginger (*Z. officinale*), Garlic (*Allium sativum*), Myrrh (*Commiphora myrrha*) and propolis) was studied. The studies indicate garlic's ability to eliminate *C. albicans* and show a direct relationship between increased effect against the yeast with increased exposure, which is consistent with **Kumar *et al.*** (2012) **Agustantina and Soekartono** (2021) and show a significant difference in the inhibition rate (LSD: 0.332). **[34] [35]**

The presence of numerous active compounds in Garlic (*Allium sativum*) extract is attributed to its ability to inhibit the yeast. Previous studies have indicated that Garlic (*Allium sativum*) has a significant effect against pathogenic fungi, especially *C. albicans*, as it is a strong and natural antifungal agent. **[36]**

Garlic (*Allium sativum*) is one of the many natural compounds studied as an alternative treatment due to its antimicrobial properties, with allicin compound (the main thiosulfinate extracted from Garlic (*Allium sativum*)) identified as the main component associated with antimicrobial properties. Garlic (*Allium sativum*) arlic extract has shown the ability to significantly inhibit the metabolic activity of the biofilm of *C. albicans*. Garlic (*Allium sativum*) arlic extract can effectively reduce the metabolic activities of the yeast. It is believed that garlic's effect is significantly pronounced when it binds to the yeast's biofilms, enhancing the inhibition of hyphal formation. **[37]**

Allicin has been found to possess antifungal activity, and studies indicated that antifungal activity decreases when allicin is removed by certain solvents. Garlic (*Allium sativum*) contains other active compounds such as trisulfide dioxide, effective against various fungal and bacterial infections. **[38]**

Garlic (*Allium sativum*) possesses biologically active ingredients capable of inhibiting hyphal formation and altering the gene expression

levels of *C. albicans*. The compounds diallyl disulfide (DAS) and diallyl sulfide (DADS) found in Garlic (*Allium sativum*) can inhibit the secretion of protease, phospholipase, and morphogenesis in *C. albicans*. **[39]**

The current study yielded positive results for the effect of Ginger (*Z. officinale*) extract against *C. albicans*, and the results showed a significant difference in the inhibitory effect on yeast growth (LSD: 0.407).

Ginger (*Z. officinale*) is a medicinal plant considered an effective antifungal agent and is a member of the important Zingiberaceae family used in traditional folk medicine to treat various diseases, inflammations, and fungal activities. Ginger (*Z. officinale*) extract contains gingerol, which has the ability to inhibit the growth of many types of bacteria and fungi. **[40]**

The antimicrobial activity of Ginger (*Z. officinale*) is believed to be attributed to the presence of hydrophobic compounds, gingerol, zingerone, and others found in Ginger (*Z. officinale*), which affect fungal and bacterial growth by inhibiting DNA or RNA and protein synthesis. **[41]**

The antimicrobial activity of Ginger (*Z. officinale*) can be attributed to the presence of gingerol and shogaol (phenolic compounds), which are the active components. It is noted that the antimicrobial activity of Ginger (*Z. officinale*) depends on the chemical composition, extraction solvent, and extraction method. Studies indicate that the minimum inhibitory values of Ginger (*Z. officinale*) extract against *C. albicans* have the ability to inhibit the yeast's vital activities. The high inhibitory effect observed against *C. albicans* can be attributed to the presence of monoterpenes, which are said to possess a wide range of antifungal activity. **[42] [43]**

Studies have indicated that ginger extract had an inhibitory effect on *C. albicans*, and in agreement with other studies, it was noted that ginger's antifungal properties are stronger than some antibiotics such as fluconazole and nystatin. The use of medicinal plant extracts such as Ginger (*Z. officinale*) may represent a new development in treating fungi after they have developed resistance to antibiotics. **[44]**

The results of using Myrrh (*Commiphora myrrha*) extract against *C. albicans* showed a significant difference in the effect of inhibiting yeast growth. Myrrh (*Commiphora myrrha*) is a traditional medicine used in Arab regions and North Africa since ancient times to successfully treat various clinical symptoms. It is a secretion that comes from the stems of plants belonging to different species of the genus *Commiphora*, family Burseraceae. **[45]**

Many studies have indicated that the phytochemical analysis of Myrrh (*Commiphora myrrha*) has revealed its complex composition, which includes monoterpenoids, sesquiterpenoids, essential oils, diterpenoids, triterpenoids, and steroids. Diffusion tests have shown the inhibitory effects of Myrrh (*Commiphora myrrha*) against *C. albicans*. It is known that resins from Commiphora and sesquiterpenoids exhibit antimicrobial and antifungal properties. **[46]**

Monoterpenoids, sesquiterpenoids, essential oils, diterpenoids, triterpenoids, and steroids demonstrated potential antifungal activity and have the ability to inhibit various yeast forms, known for their capability to examine the cell wall. This also weakens cellular biological processes by interfering with the protein synthesis process in the cytoplasmic membrane, thus slowing down and halting the process. This also hinders the active transport of ions and salts across the membrane. **[47]**

Rivera-Yanez *et al.* (2021) indicated that propolis exhibited anti-*C. albicans* activity, and that propolis inhibits the growth and formation of biofilms in *C. albicans* isolates. This was confirmed by the ability of propolis to suppress the yeast-to-hypha transition. **Wolska** (2023) noted that propolis contains flavonoids and phenolic acids, and their esters are responsible for its antifungal properties, being effective against strains of fungi resistant to polyenes and azoles, particularly *C. albicans*. This was also pointed out by **Abdelati** *et al.* (2023), who stated that the antifungal activity of propolis can be attributed to its components, such as 3-acetylpinobanksine, pinobanksine-3-acetate, pinocembrin, p-coumaric acid, and caffeic

acid, which enhance its activity against *C. albicans*. **[48] [49] [50]**

Propolis extract inhibits the growth of *C. albicans* in a dose- and time-dependent manner, varying with the type, nature, and composition of the propolis. During the process of cell growth and division, *C. albicans* can reproduce by budding, resulting in the formation of a new cell from another cell, growing in a filamentous form. This morphological change plays a vital role in causing fungal infections. **[51]** Studies indicate that the biological activity of propolis interferes with germination processes and effectively prevents the formation of *C. albicans* hyphae depending on concentration and time. Phenolic compounds, including flavonoids, are considered one of the main bioactive components of propolis, and may be associated with the inhibition of germination and its antifungal activity, as it increases its antibacterial and antifungal activity with higher amounts of flavone and flavonol. **[52] [53] [54]**

Conclusions

In this study, which used C. albicans yeasts and cultured them in media enriched with Garlic (*Allium sativum*), Ginger (*Z. officinale*), Myrrh (*Commiphora myrrha*), and propolis, there was an inhibition of yeast growth. The concentrations used in this study are important because the higher the concentration, the greater the effect of the extract against the yeast, and the inhibition rate was better. Statistically, propolis recorded higher results than the other extracts, while Ginger (*Z. officinale*) recorded relatively higher inhibition rates than Garlic (*Allium sativum*). Based on this study, it is recommended to use propolis, Myrrh (*Commiphora myrrha*), Garlic (*Allium sativum*), and Ginger (*Z. officinale*) for therapeutic purposes and in pharmaceutical and food industries for preserving medicines, as they are low-cost materials available as natural substances. However, further pharmaceutical studies should be conducted to incorporate them into oral treatments and evaluate their cost-effectiveness.

References

1. Chen, Y., Gao, Y., Yuan, M., Zheng, Z., & Yin, J. (2023). Anti-Candida albicans effects and mechanisms of theasaponin E1 and assamsaponin A. *International Journal of Molecular Sciences*, *24*(11), 9350.
2. Araújo, D. E. (2021). *Application of Antisense Oligonucleotides to Prevent Candida Albicans Infections* (Doctoral dissertation, Universidade do Minho (Portugal)).
3. Won, E. J., Sung, H., & Kim, M. N. (2024). Changing Epidemiology of Clinical Isolates of Candida Species during the Coronavirus Disease 2019 Pandemic: Data Analysis from a Korean Tertiary Care Hospital for 6 Years (2017–2022). *Journal of Fungi*, *10*(3), 193.
4. Lohse, M. B., Gulati, M., Johnson, A. D., & Nobile, C. J. (2018). Development and regulation of single-and multi-species Candida albicans biofilms. *Nature Reviews Microbiology*, *16*(1), 19-31.
5. Macias-Paz, I. U., Pérez-Hernández, S., Tavera-Tapia, A., Luna-Arias, J. P., Guerra-Cárdenas, J. E., & Reyna-Beltrán, E. (2023). Candida albicans the main opportunistic pathogenic fungus in humans. *Revista Argentina de Microbiologia*, *55*(2), 189-198.
6. Alghamdi, A. M. (2022). Cholinergic modulation of Candida albicans virulence factors and the pathogenesis of candidiasis (Doctoral dissertation, University of Glasgow).
7. Arafa, S. H., Elbanna, K., Osman, G. E., & Abulreesh, H. H. (2023). Candida diagnostic techniques: a review. *Journal of Umm Al-Qura University for Applied Sciences*, *9*(3), 360-377.
8. Lee, Y., Puumala, E., Robbins, N., & Cowen, L. E. (2020). Antifungal drug resistance: molecular mechanisms in Candida albicans and beyond. *Chemical reviews*, *121*(6), 3390-3411.

9. Costa-de-Oliveira, S., & Rodrigues, A. G. Candida albicans antifungal resistance and tolerance in bloodstream infections: the triad yeast-host-antifungal. Microorganisms. 2020; 8 (2): 154.
10. Vaou, N., Stavropoulou, E., Voidarou, C., Tsigalou, C., & Bezirtzoglou, E. (2021). Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. *Microorganisms*, *9*(10), 2041.
11. Nwozo, O. S., Effiong, E. M., Aja, P. M., & Awuchi, C. G. (2023). Antioxidant, phytochemical, and therapeutic properties of medicinal plants: A review. *International Journal of Food Properties*, *26*(1), 359-388.
12. Chinedu, A. F. (2019). Comparative assessment of the antibacterial profile of Onion (Allium cepa) and Garlic (Allium sativum) against Pseudomonas aeruginosa, Staphylococcus aureus and Candida albicans. *International journal of Biotechnology and food science*, *7*(5), 65-71.
13. Agustantina, T. H., & Soekartono, R. H. (2021). Antifungal activity from garlic extract (Allium sativum) against Candida albicans growth. *Indonesian Journal of Dental Medicine*, *4*(2), 60-62.
14. Khan, A., Azam, M., Allemailem, K. S., Alrumaihi, F., Almatroudi, A., Alhumaydhi, F. A., ... & Khan, M. A. (2021). Coadministration of ginger extract and fluconazole shows a synergistic effect in the treatment of drug-resistant vulvovaginal candidiasis. *Infection and Drug Resistance*, 1585-1599.
15. Khalaf, A. A., al-Aedany, A. J. M., & Hussein, S. F. (2020). Activity Evalution of Ginger Alcoholic Extract against *Candida albicans*.
16. Alshibly, N. M., Mohamed, R. M., Al-Shaikh, T. M., & Soliman, A. M. (2022). Study of Commiphora myrrha (Nees) Engl. var. molmol Extract and Its Antibiogram Against Some Microbial Pathogens. *Egyptian Journal of Chemistry*, *65*(5), 13-22.
17. Alabdalall, A. H. (2023). Activity of oleo-gum resins of myrrha on the growth of Candidiasis. *bioRxiv*, 2023-12.
18. Zaher, Abdel & Shourbagy, Mohamed & Abdelaal, Mohamed & El-Badry, Anwer. (2024). Investigation of antifungal activity and histological effects of Myrrh on Candida corneal ulcers in vivo. 6. 7-14.
19. Abdulbary, M. (2018). Study of turmeric plant (Curcuma longa L.) rhizomes and myrrh (Commiphora myrrha L.) gums methanolic extracts effect on Candida albicans isolated from mouth.
20. Segueni, N., Boutaghane, N., Asma, S. T., Tas, N., Acaroz, U., Arslan-Acaroz, D., ... & Nieto, G. (2023). Review on Propolis Applications in Food Preservation and Active Packaging. *Plants*, *12*(8), 1654.
21. Kegode, T. M., Mokaya, H. O., Chogo, S. I., Noiset, P., Vereecken, N. J., Tamiru, A., ... & Kiatoko, N. (2023). Differences in the biochemical content and radical scavenging activity of propolis from different parts of a Meliponula ferruginea hive. *Royal Society open science*, *10*(12), 230241.
22. Berretta, A. A., Zamarrenho, L. G., Correa, J. A., De Lima, J. A., Borini, G. B., Ambrósio, S. R., ... & De Jong, D. (2023). Development and characterization of new green propolis extract formulations as promising candidates to substitute for green propolis hydroalcoholic extract. *Molecules*, *28*(8), 3510.
23. Deorukhkar, S. C., & Santosh Saini, S. S. (2014). Laboratory approach for diagnosis of candidiasis through ages.
24. Zafar, A., Jabeen, K., & Farooqi, J. (2017). Practical guide and atlas for the diagnosis of fungal infections.
25. Hospenthal, D. R., Beckius, M. L., Floyd, K. L., Horvath, L. L., & Murray, C. K. (2006). Presumptive identification of Candida species other than C. albicans, C. krusei, and C. tropicalis with the chromogenic medium CHROMagar Candida. *Annals of clinical microbiology and antimicrobials*, *5*, 1-5.

26. Yan, L. J., Thangthaeng, N., Sumien, N., & Forster, M. J. (2013). Serum dihydrolipoamide dehydrogenase is a labile enzyme. *Journal of biochemical and pharmacological research*, *1*(1), 30.
27. Yakasiri, H. P., & Siddabathuni, A. (2020). Utility of nonserum liquid media against conventional human serum in germ tube production test. *IP International Journal of Medical Microbiology and Tropical Diseases*, *6*(1), 54-57.
28. miruka, H. N., Eric, O. O., Musyoki, S., & Awuor, S. O. (2023). Incidence, antifungal resistance properties, and virulence traits of candida species isolated from HIV/AIDS Patients from the hospital system in Kenya. *Access Microbiology*, 000620-v1.
29. Bajac, J., Nikolovski, B., Kocić-Tanackov, S., Tomšik, A., Mandić, A., Gvozdanović-Varga, J., ... & Radojković, M. (2018). Extraction of different garlic varieties (Allium sativum L.)-Determination of organosulfur compounds and microbiological activity. In *International Congress Food Technology. Quality and Safety*.
30. Archin, T., Ownagh, A., Tehrani, A. A., & Keshipour, S. (2024). Application of Propolis Ethanol Extract and Propolis Nanoemulsion in Treatment of Cutaneous Infection in Rabbit. *Iranian Journal of Veterinary Surgery*, *19*(1), 8-18.
31. Kadry, A. A., El-Ganiny, A. M., & El-Baz, A. M. (2018). Relationship between Sap prevalence and biofilm formation among resistant clinical isolates of Candida albicans. *African health sciences*, *18*(4), 1166-1174.
32. Kakade, P., Mahadik, K., Balaji, K. N., Sanyal, K., & Nagaraja, V. (2019). Two negative regulators of biofilm development exhibit functional divergence in conferring virulence potential to Candida albicans. *FEMS yeast research*, *19*(2), foy078.
33. Oyinlola, K. A., Ogunleye, G. E., Balogun, A. I., & Joseph, O. (2024). Comparative study: Garlic, ginger and turmeric as natural antimicrobials and bioactives. *South African Journal of Science*, *120*(1-2), 1-7.
34. Kumar, A., Thakur, S., Thakur, V. C., Kumar, A., Patil, S., & Vohra, M. P. (2012). Antifungal activity of some natural essential oils against Candida species isolated from blood stream infection. *Journal of Krishna Institute of Medical Sciences University*, *1*(1), 61-66.
35. Agustantina, T. H., & Soekartono, R. H. (2021). Antifungal activity from garlic extract (Allium sativum) against Candida albicans growth. *Indonesian Journal of Dental Medicine*, *4*(2), 60-62.
36. Khounganian, R. M., Alwakeel, A., Albadah, A., Nakshabandi, A., Alharbi, S., & Almslam, A. S. (2023). The antifungal efficacy of pure garlic, onion, and lemon extracts against Candida albicans. *Cureus*, *15*(5).
37. Thomas, D. A., Lee, J. V. K., Choo, S., Rao, H., & Chong, P. P. (2022). Effects of fresh garlic extract on Candida albicans sessile cells, biofilms and biofilm associated genes, Flo8 and Ndt80. *Life Sci. Med. Biomed*, *6*(10.28916).
38. Sahidur, M. R., Islam, S., & Jahurul, M. H. A. (2023). Garlic (Allium sativum) as a natural antidote or a protective agent against diseases and toxicities: a critical review. *Food Chemistry Advances*, *3*, 100353.
39. Li, G., Ma, X., Deng, L., Zhao, X., Wei, Y., Gao, Z., ... & Sun, C. (2015). Fresh garlic extract enhances the antimicrobial activities of antibiotics on resistant strains in vitro. *Jundishapur journal of microbiology*, *8*(5).
40. Atai, Z., Atapour, M., & Mohseni, M. (2009). Inhibitory effect of ginger extract on Candida albicans.
41. Ozabor, Temilade & Olaitan, Janet & Oluwajide, Odunola & Akinde, Sunday. (2022). Antibacterial effect of Allium sativum (garlic) and Zingiber officinale (ginger) extracts against antibiotic resistant organisms isolated from chicken abattoir. 36. 5991-6000.
42. Hasan, H. A., Raauf, A. R., Razik, B. M. A., & Hassan, B. R. (2012). Chemical

composition and antimicrobial activity of the crude extracts isolated from Zingiber officinale by different solvents. *Pharmaceut Anal Acta*, *3*(9), 1-5.

43. Akullo, J. O., Kiage, B., Nakimbugwe, D., & Kinyuru, J. (2022). Effect of aqueous and organic solvent extraction on in-vitro antimicrobial activity of two varieties of fresh ginger (Zingiber officinale) and garlic (Allium sativum). *Heliyon*, *8*(9).
44. Aghazadeh, M., Bialvaei, A. Z., Aghazadeh, M., Kabiri, F., Saliani, N., Yousefi, M., ... & Kafil, H. S. (2016). Survey of the antibiofilm and antimicrobial effects of Zingiber officinale (in vitro study). *Jundishapur journal of microbiology*, *9*(2).
45. Al-Sabri, A. E., Moslem, M. A., Hadi, S., Yassin, M. A., & Ameen, F. (2014). Antifungal activity of Commiphora myrrha L. against some air fungi. *Journal of Pure and Applied Microbiology*, *8*(5), 3951-3955.
46. Alabdalall, A. H. (2024). Antifungal activity of Myrrh gum resin against pathogenic Candida spp. *Annals of Agricultural and Environmental Medicine*, *31*(3).
47. Al-Qaysia SA. Effect of Volatile Oil of *Myrtus communis* on growth and activities of some types of Pathogenic Bacteria and *Candida albicans*. J Baghdad Sci. 2008;5:8–13.
48. Rivera-Yanez, N., Rivera-Yanez, C. R., Pozo-Molina, G., Mendez-Catala, C. F., Reyes-Reali, J., Mendoza-Ramos, M. I., ... & Nieto-Yanez, O. (2021). Effects of propolis on infectious diseases of medical relevance. *Biology*, *10*(5), 428.
49. Wolska, K., & Antosik, K. (2023). The activity of propolis against pathogenic fungi isolated from human infections. *Brazilian Journal of Pharmaceutical Sciences*, *59*, e19978.
50. Abdelati, O., Anass, B. M., Zineb, M., & Lahoucine, H. (2023). Assessing the antimicrobial strength of Moroccan propolis from four regions on human infection-causing bacteria and yeast strains. *Journal of Central European Agriculture*, *24*(4), 942-952.
51. Zida, A., Bamba, S., Yacouba, A., Ouedraogo-Traore, R., & Guiguemdé, R. T. (2017). Anti-Candida albicans natural products, sources of new antifungal drugs: A review. *Journal de mycologie medicale*, *27*(1), 1-19.
52. Corrêa, J. L., Veiga, F. F., Jarros, I. C., Costa, M. I., Castilho, P. F., de Oliveira, K. M. P., ... & Negri, M. (2020). Propolis extract has bioactivity on the wall and cell membrane of Candida albicans. *Journal of ethnopharmacology*, *256*, 112791.
53. Gucwa, K., Kusznierewicz, B., Milewski, S., Van Dijck, P., & Szweda, P. (2018). Antifungal activity and synergism with azoles of polish propolis. *Pathogens*, *7*(2), 56.
54. Salas, M. P., Céliz, G., Geronazzo, H., Daz, M., & Resnik, S. L. (2011). Antifungal activity of natural and enzymatically-modified flavonoids isolated from citrus species. *Food Chemistry*, *124*(4), 1411-1415.