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Isolation of Candida albicans and Evaluation of Plant Extracts for Antifungal Activity.

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ABSTRACT

Candida albicans (C. albicans) is a common opportunistic fungal pathogen trustworthy for a variety of infections, especially in immunocompromised individuals. The increasing resistance to antifungal agents necessitates the search for alternative treatment options. In this study, we isolated C. albicans from clinical and environmental samples and evaluated several plant extracts, such as Allium sativm, salvia rosmarinus, Mentha, which we believed possessed antifungal activity. These plant extracts were tested for their ability to inhibit C. albicans growth using standard laboratory assays. Various plant extracts, including Allium sativm, salvia rosmarinus, Mentha yielded very similar results in terms of fungal inhibition, with the exception of the disc impregnated with peppermint extract, where no area of inhibition was observed. Several extracts exemplified promising inhibitory effects on fungal growth, suggesting that compounds derived from Allium sativm, salvia rosmarinus, Mentha could offer useful possibility or complement to current antifungal treatments. These findings paved the way for growth of plant based therapies to conflict drug reluctant fungal infections. The extracts exhibited great antifungal effects, notice on their potential as natural curative agents. These findings would assist further research into plant compounds as other possibility or complements to traditional antifungal drugs.

KEYWORDS: Candida albicans, Antifungal resistance, Allium sativm, salvia rosmarinus, Mentha, Natural antifungal agents, and Fungal pathogens.

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1.INTRODUCTION

Candida albicans(C. Albicans) is the most common causative agent of candidiasis, a fungal disease in humans. It is found as a commensal organism in the microbiome of mammals and can cause superficial infections in healthy individuals and severe disease in immunocompromised patients. C. albicans infections are associated with high morbidity and mortality, particularly among chronically ill and critically ill patients. The pathogen undergoes a morphological transformation from yeast to filamentous forms, leading to infection. The path to treatment remains long due to the increasing drug resistance of fungi, their small numbers, and their limited efficacy. Most antifungal treatments target azoles and ergosterol, a major amphiphilic lipid and a fundamental component of the fungal plasma membrane. However, resistance and tolerance to azole are becoming an increasing problem. In C. albicans, ergosterol-rich membrane domains are thought to interact with sphingolipids and membrane proteins, including multidrug transporters, further complicating treatment strategies.

The interaction between Candida albicans (C. albicans) and host cells is mediated by a range of virulence factors, including adhesions and invasins, secretion of hydrolytic enzymes, morphological transformation from yeast to filamentous fungal hyphae, and the formation of a robust biofilm. These properties collectively smooth efficient adhesion, invasion, and damage to host tissues [1]. Three bioactive compounds isolated from bogostemon axillaris have indicate potent inhibitory activity against fluconazole resistant C. albicans (MIC = 4 µg/ml). The main compound, pachisamin M, disrupted ergosterol synthesis and exhibited strong antifungal activity both in vitro and in vivo, suggesting that pachisamin M may be a promising compound for overcoming antifungal resistance, particularly in C. albicans [2]. The tannin rich N-butanol fraction extracted from plant extracts also exhibits antifungal and anti-biofilm activity, effectively and efficiently preventing C. albicans infections. [3] Hexane (Hex) and dichloromethane (DCM) extracts from the roots of tridax pentachaeta also exemplify strong photosynthetic activity against C. albicans. [4] Bacterial perfringolicin serves as a direct indicator for monitoring the cellular effects of fluconazole, revealing that highly polarized ergosterol distribution is not prerequisite for budding or filamentous growth in C. albicans. [5] Genetic or pharmacological inhibition of the tyrosine phosphorylation moderate dual kinase (DYRK) Yak1 productively prevents C. albicans spore formation and biofilm formation. [6] Calcium-activated calcineurin^{+}-calmodulin phosphatase plays a pivotal role as an inhibitor of key stress response pathways in C. albicans, reducing fungal persistence and its ability to cause disease within the host [7]. Heat shock protein 90 (Hsp90) is a highly conserved molecular cofactor that regulates the folding, stability, and function of a wide range of client proteins, many of which are substantial for signal transduction and stress responses in C. albicans. The Ras1-cAMP-PKA and MAPK chains, which control splicing and stress adaptation, are important for the fungus. Hsp90 contributes to resistance to antifungal agents, such as calcineurin, enhancing the host's tolerance to drug-induced stress. Hsp90 dysfunction affects morphogenesis, biofilm formation, and virulence in vivo, making it an attractive target for antifungal drug. Inhibiting fungal Hsp90, particularly in integration with existing antifungal agents, offers significant potential for numerous drug efficacy and overcoming fungal drug resistance mechanisms without affecting the host Hsp90, given the structural differences between fungal and human forms [8]. Baicalin (BE) is a small-molecule antifungal compound make distinctive by broad-spectrum activity, synergistic interaction with fluconazole, and low toxicity. Recent findings indicate that BE has antifungal activity against C. albicans by disrupting glycolysis. More specifically, BE targets and inhibits enolase 1 (Eno1), a key enzyme in glycolysis [9]. High-intensity violet light was applied to specific yeast species, including C. albicans. Cell suspensions were exposed to narrowband violet light with a wavelength of 405 nm, generated by an LED array. All fungi were

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effectively and efficiently inactivated using light with a wavelength of 405 nm without the need for external photosensitizers [10].

Exposure to tunicamycin induces a specific chromosomal aberration, a chromosomal abnormality resulting from endoplasmic reticulum (ER) tension, leading to trisomy 2 (Chr2x3) in C. albicans. This confers cross-tolerance to caspofungin, an antifungal agent from the echinocandin family [11]. Phosphatidylserine phosphatases are key enzymes in lipid metabolism, catalyzing the conversion of phosphatidic acid to diacylglycerol. In C. albicans, phosphatidylserine phosphatase Pah1 plays a crucial role in fungal physiology [12]. Two aminotransferases have been identified in C. albicans, Aro8p and Aro9p, each with distinct catalytic properties that play an important role in inhibiting fungal growth [13]. bioactive compounds as gold nanoparticles (Ca_AuNPs) used by C. albicans exhibit anti-azole activity, including against fluconazole, itraconazole, and voriconazole, making them effective against multidrug-resistant C. albicans strains [14]. The alginate CD aptamer complex nanosensor shows pledge in clinical applications, offering a sensitive and environmentally friendly alternative to conventional methods for diagnosing C. albicans [15]. Tested antifungal proteins (AFPs) have demonstrated significant antifungal activity against cutaneous C. albicans infections [16]. Results have shown that the crude methanolic extract of turmeric exhibits antifungal activity against C. albicans [17]. A series of novel 5-phenylthiophene derivatives have been designed and synthesized to address the high incidence of drug-sensitive and drug-resistant fungal infections [18]. Ethyl acetate exhibited fungicidal activity against C. albicans at a concentration of 1.0 mg/ml [19]. The incorporation of a photofluorescent protein with random optical oscillation (SOFI) effectively inhibited background fluorescence. Histone acetyltransferase is essential for achieving full virulence, and Erg11, a target of azole antifungals, has been identified in C. albicans [20]. Poly ([2 - (methacryloyloxy) ethyl) trimethylammonium chloride cross-linked) (PMETAC) and bacterial cellulose nanoparticles (BNCs) were used to determine their antifungal activity against C. albicans polymorphotypes [21]. 2,4-Dietyr-butylphenol, an antibiotic, has shown efficacy and safety against C. albicans and other pathogenic fungi [22]. Necessary oils have been tested against C. albicans [23]. Proteins of aspergillus fumigatus and aspergillus albicans, which are expressed or activated during environmental changes and compression conditions, have been used to detect potential virulence factors [24]. A microfluidic chip has been designed and fabricated for the fast and perfect detection of aspergillus albicans in clinical samples, and its potential has been utilized for early and accurate diagnosis [25]. Using molecularly printed polymers (MIPs) enhanced with nickel, iron oxide ($NiFe_2O_4$) nanoparticles, a novel electrochemical biosensor has been fabricated for the fast and accurate detection of aspergillus albicans [26]. A CRISPR-based platform targeting the internal interleaving gene 2 (ITS_2) of aspergillus albicans has been developed. This method combines Cas12a DNase cleavage activity with recombinant polymerase (RPA) amplification for fast and accurate detection [27]. Berberine inhibits the expression of azole impedance genes in fungi, reduces cell adhesion, and damage biofilm formation. Gene transcription analysis suggests that interference with the iron gain pathway is a key mechanism underlying berberine's inhibition of drug resistant fungal strains [28]. When PG, CA, and CAR compounds were individually applied to C. albicans biofilms for 5 minutes, their viability decreased by less than 0.50 at both 5 °C and 22 °C [29]. The yeast form of C. albicans utilizes glycosaminoglycans (GAGs), particularly heparan sulfate, as receptors for adhesion to corneal epithelial cells [30].

2.METHODOLOGY

2.1. Isolation of *C. albicans*

2.1.1.Sample Collection:

Clinical or environmental samples suspected to contain C. albicans species were collected using sterile cotton swabs.

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2.1.2.Culturing:

Samples were inoculated on sabouraud dextrose agar (SDA) plates and incubated at 30 – 37°C for 24 – 48 hours.

2.2. Materials used:

Plastic Petri dishes (9 and 6 cm in diameter), isolation needle, forceps, pipettes, assorted cups, cotton, cellophane paper, punch, filter paper, sterile swabs, a culture medium consisting of sabouraud dextrose agar, lactophenol blue cotton stain, distilled water, Parafilm, microscope slides and coverslips, and ethyl alcohol.

2.3. Equipment used:

Autoclave, balance, cooler, incubator, water bath, isolation chamber, microscope, Bunsen burner, and heat sterilizer.

2.4. Plant material:

The following plants were dried: garlic, rosemary, and wild mint (Allium sativm, salvia rosmarinus and Mentha). They were purchased from a local farm in Ujla, Libya, and identified by a farmer and vendor based on their color and aroma.

2.5. Preparation of plant extracts:

2.5.1. Aqueous extracts (cold):

To prepare the aqueous extract, Allium sativm, salvia rosmarinus and Mentha from garlic, rosemary, and mint plants were dried, then chopped into small pieces and ground in a ball mill to a powder. The powder was divided into several 10-gram portions, placed in a sterile screw-cap bottle, and 100 ml of sterile deionized distilled water was added. The extract was left to infuse for 48 h at 4 °C before the mixture was centrifuged at 2000 r.p.m for 10 min. The supernatant was passed through a 0.45 mm diameter membrane sieve. The extract was stored in sterile screw-cap bottles in the refrigerator until needed. Aqueous extracts were prepared using sterile distilled water for each plant.

2.6. Preparation of the inoculum for testing:

The inoculum was prepared by suspending a few fungal colonies in test tubes containing 9 ml of sterile normal saline. The turbidity of the fungal suspensions was visually matched to the equivalent turbidity of a $BaCl_2$ standard, which has a similar appearance to broth culture.

2.7. Antifungal activity testing using the disc diffusion method:

The modified agar diffusion method was used to determine antimicrobial activity. Sabouraud dextrose agar was inoculated with a microbial cell suspension (200 µL in 20 mL of medium) and poured into sterile Petri dishes. Sterile 6 mm diameter filter paper discs were impregnated with 20 µL of each extract, prepared using the same solvent used to dissolve the plant extracts. The aqueous extract was then sterilized by pasteurization and membrane filtration and placed on the surface of the inoculated agar. The plates were incubated at 30–37 °C for 4 –7 days. At the end of the incubation period, antimicrobial activity was assessed by measuring the inhibition zones [31].

2.8. Sample Collection , Fungi used, C. albicans, Fungi isolated from vagina

Samples collected for several persons from Awjila AL-qrawi Hospital and Red Crescent Clinic Jalo in June, May, and April 2025year.

This is done by taking a sample of vaginal secretions or a thin layer of the crust covering the dermatitis by Fungi C. albicans.

2.9. Work methods:

Culture media used for the growth and isolation of C. albicans fungus sabourud dextros agar of the medium consists of agar 65 g, the medium powder is weighed using a sensitive balance (the quantity is usually recorded on the package) then 1000 ml of distilled water is added to the medium. The contents are mixed well and then placed in a water bath for a period (35- 40 min.) until the contents are well dissolved and become transparent. The mixture is then distributed into small 250 ml beakers to facilitate the process of pouring into dishes. These beakers are sterilized in an autoclave for 15-20 min., after which they are ready to be poured into Petri dishes.

Isolation of C. albicans fungus from some patients visiting the " Isolation of a fungus from some patients at-

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tending Awjila AL-qrawi Hospital and Red Crescent Clinic Jalu ". Samples were taken from patients who complained of infections in the vaginal area in general at different intervals, by taking a swab from the infected area and culturing it on special nutritional media represented by the sabouraud dextros agar medium, which is a special medium for the growth of C. albicans fungi.

It was then placed in an incubator at a temperature of 30-37 °C. The plates were monitored periodically for 4 -7 days to observe the growing colonies. The plates were then incubated. After this, the plates were ready for microscopic examination.

A small percentage of the fungus growth is taken with an isolation of fungal growths from the nutrient media using a sterile isolation needle. The fungal smear is placed on a glass slide with a drop of phenolic blue cotton stain and covered with a glass slide cover for microscopic examination. The sample is examined under different power of the light microscope (X 10 ،X 40, and X 100) The obtained characteristics, both macroscopic and microscopic, were compared with the previously identified isolate and the results were recorded.

2.10. Preparation of tablets saturated with extract:

5 mm diameter discs were made from sterilized filter paper, these discs were dipped in plant extracts according to the species for 5 min. After removing them, the discs were placed in Petri dishes containing growths of the fungus under study, 5 discs were distributed in each dish, so that each disc was at a distance from the fungal growth. The dishes were left at 30-37 °C and monitored periodically for 4 - 7 days, and the results were recorded .[32]

2.11. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC):

Antifungal activity of Allium sativm, Salvia rosmarinus, and peppermint extracts on the growth and inhibition of C. albicans. For this experiment, we prepared 45 tablets of each Allium sativm, Salvia rosmarinus, and peppermint extract. Fifteen tablets were 100 % Allium sativm, 15 tablets were 100 % Salvia rosmarinus extract, and 15 tablets were 100 % peppermint extract. Five tablets were placed in each of three Petri dishes, each of which was left to contain a quantity of the extracts for 5 min. until saturated. Nine Petri dishes were prepared for testing, three for each extract. The dishes were inoculated with C. albicans at five locations on each dish. The discs were taken and distributed onto plates, each disc placed at a distance from the fungal isolate. The plates were incubated in an incubator at 30-37 °C. The plates were monitored after 4 -7 days in the incubator, and the results were recorded.

The MIC is defined as the lowest concentration of aqueous extracts capable of inhibiting fungal growth. Sabouraud dextrose broth was used to determine the MIC in the preparation of serial dilution test tubes. Serial dilutions of the two extract samples were performed in test tubes containing 2 ml of sabouraud dextrose broth medium to yield final concentrations of 2:2, 2:4, 4:6, and 6:8 mg/mL. 20 µl of the test organisms (108 CFU/ml) were distributed into the tubes. The negative control tube contained only 2 ml of the two extracts but did not contain any organisms. Positive control tubes contained only 2 ml of broth medium and each of the organisms, but neither extract was present. The tubes were incubated at 30 – 37 °C for 4 – 7 days. After incubation, the turbidity of each tube was visually checked. The clear test tube indicated the breakage point. From tubes showing no visible sign of growth / turbidity in the MIC determination, the test micro-organisms were inoculated onto sterile sabouraud dextrose agar plates using the stripe plate method. The plates were then incubated at 30 –37 °C for 4 – 7 days. The lowest concentration that did not show growth of the test organisms was considered to be [33].

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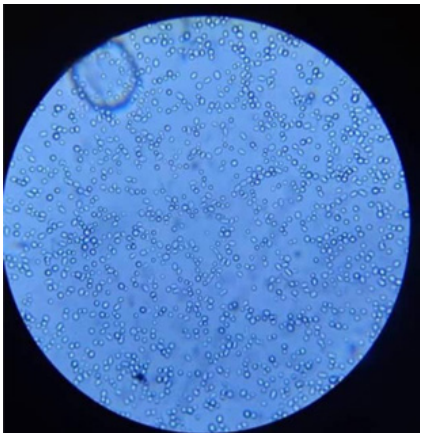

Figure. 1. Shows the C. albicans shape of the microscopic diagnosis.

Figure. 2. Shows the growth C. albicans on the media.

3.RESULTS AND DISCUSSION

Bonferroni test was used for statistical analysis to show if there any significant difference ($P \leq 0.05$).

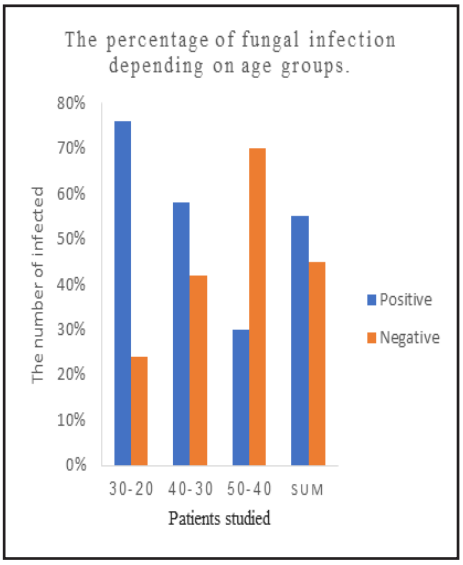

Figure.3. Shows the number of patients studied, the number of infected and healthy plates, and the percentage of fungal infection depending on age groups.

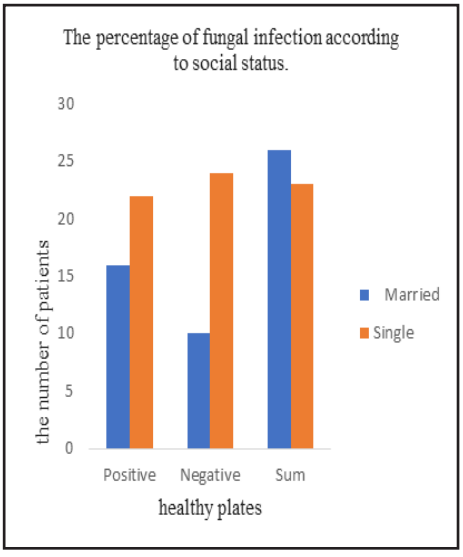

Figure.4. Shows the number of patients studied, the number of infected and healthy plates, and the percentage of fungal infection according to social status.

Table 1. Evaluations of the antifungal potential of the aquatic extract of plants Allium sativm, salvia rosmarinus and Mentha used.

Microorganism	**Concentration 100%**		
Fungi	Inhibition zone (mm)		
C. albicans	Allium sativum	Salvia rosmarinus	Mentha
Men inhibition zone(mm)	1.41	0.72	-

Table 2. Minimum inhibitory and bactericidal concentrations of aquatic extract of propolis against fungi .

Isolation	**fungal strain with inoculums density of 10^6CFU/m**	**Antimicrobial activity of Plant% (V/V)**			
		Allium sativm extract		**Salvia rosmarinus extract**	
		MIC	MBC	MIC	MBC
Vagiana	C. albicanas	2:4	2:2	2:2	-

After isolating the fungus from some patients attending Awjila Al-Qarawi Hospital and the Red Crescent Clinic in Jalu, the results are shown in Fig.3, for the period from April to July. Approximately 72 cases were studied, all of which underwent laboratory treatment, from the beginning of the sample culture on sabouraud dextrose medium until the end of the test. It was found that the number of positive results was highest among young people aged 20-30, while it decreased slightly among adults aged 30 - 40, and decreased by half between the ages of 40-50, as the table shows. This difference in infection rates among patients despite the fact that they all suffer from the same or nearly similar symptoms is explained by the fact that as people age, their cells become more resistant to the C. fungus, and the immune system strengthens and becomes more capable of fighting off infection, despite the fact that some cases are infected with bacteria whose symptoms are somewhat similar to those of C. fungi. Therefore, treatment is usually administered in some cases as a dual therapy consisting of doses of antibiotics that combat bacterial growth and those that combat fungal growth simultaneously, of the 72 samples, 42 % (31 samples) tested positive for Candida, while 57 % (41 samples) tested negative or tested for other microbial species. The results indicate that vulvovaginal candidiasis is widespread among women in the study area. This is consistent with a previous study conducted in North Africa (2022) [34], which showed an incidence rate ranging from 15-52 % depending on the region and diagnostic method. The results showed the highest infection rate (62.3 % of positive cases) in women aged 20-30 years. The study showed that women of this age group, which is considered reproductive age, are more susceptible to infection. This is consistent with a study conducted in 2022 [35], which recorded the highest infection rates among this group. This is attributed to hormonal changes, increased sexual activity, pregnancy, and the use of hormonal contraceptives. While the infection rate reached 29 % for the age group (30-40), the lowest infection rate was 9.7% for the age group above (40). This was evident in the decrease in infection with age due to the decline in estrogen levels after menopause. The study, in Fig.4, showes the distribution of infection by marital status, with 26 cases in the married group, of which 16 were positive (22.02 %), and 10 were negative. Forty-six samples were collected from the unmarried group, and the results showed that 22 samples were positive (30.5 %) out of 24 samples that were negative. The presence of infection in both groups is evidence of the presence of factors associated with infection, such as a humid environment, wearing tight clothing, vaginal microbial imbalance, the use of vaginal douching, as well as the excessive use of antibiotics, diabetes, and contraceptives [36].

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3.1. Evaluations of the antifungal potential of the Aquatic extract of plants Allium sativm, Salvia rosmarinus and Mentha used . Use of Salvia rosmarinus and mint(Mentha) extracts:

3.1.1.Effect of the aqueous extract on fungi isolated from the vagina:

Among the available methods for testing antifungal activity, the disk diffusion method (measurable inhibition zones) was used to determine the effectiveness of the aqueous extract against a single species of human-pathogenic fungi [37].

Table 1. Shows the results of tests of the antifungal activity of a 100 % v/v aqueous extract sample on fungi isolated from the vagina. The results showed that the fungal isolates obtained from the patients were C. albicans. This was confirmed by several methods, including the morphological description of the fungus or the general shape of the fungal colonies, which was confirmed by comparing the isolate we obtained with isolates identified in local research centers. We did not limit ourselves to morphology alone. All isolates were examined under a light microscope, and samples were sent to mycologists to confirm that they were the exact fungus. The isolates were also compared in terms of their morphology and microscopic appearance with previous studies on Candida fungi (C. fungi), and all data obtained were consistent with these studies. It became clear that the general "phenotypic" and microscopic appearance of the fungus was consistent with these studies. These are as follows: In terms of the fungus's physical appearance, it appears on nutrient media as white to pale yellow colonies with a waxy sheen. Colonies initially form on nutrient media in the form of circles that overlap with each other as the colony grows. Under the microscope, the fungus appears as oval cells, very similar in shape and reproduction to the yeast saccharomyces ceriveciae. Fungi are unicellular and reproduce by budding. They differ from yeast in that they are imperfect fungi, while yeast is a cyst-shaped fungus at 37 °C and a pseudofungi at 25-27 °C.

To determine the antifungal effect of Allium sativum, Salvia rosmarinus, and Mentha extracts on the growth and inhibition of C. fungi, it was found by monitoring dishes containing 15 tablets impregnated with plant extracts (Allium sativum, Salvia rosmarinus, and Mentha) five tablets per dish out of a total of three that during the first three days, no change in fungal growth was observed. However, after three days, slow fungal growth was observed toward the disc impregnated with either Mentha or Ocimum basilicum extract. The fungus continued to grow very slowly but did not directly reach the disc. The results showed significant inhibition of Allium sativum extract, with an average inhibition zone ranging from 1-2 mm, with an average inhibition zone of 1.44 mm in diameter. The results of Salvia rosmarinus extract showed a moderate inhibition zone, ranging from 0.6-1 mm, with an average inhibition zone of 9.89 mm in diameter. Mentha extract showed no inhibition zone at all. The reason for the low or absent inhibitory effectiveness of the studied plant extracts may be due to the presence of the plant extracts themselves in the fungal growth medium. These extracts may contain compounds that inhibit or inhibit the growth of some microorganisms, but some may lose their inhibitory capacity during extraction or storage. The method of preparing the plant extracts and their concentration are two important factors. This may be due to the nature of the extracts themselves. They may contain compounds that inhibit or inhibit the growth of some microorganisms, but some may lose their inhibitory capacity during extraction or storage [38].

3.2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for extracts aquatic were assessed on fungal strain for control and comparison purposes and was the result obtained showed in Table 2.

4.CONCLUSION

After isolating the fungus C. albicans from clinical samples in the oases area, three plant extracts were tested for their ability to inhibit the growth of C. albicans using

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standard laboratory tests. The plant extracts extracted from medicinal plants were Allium sativum, Salvia rosmarinus, and Mentha. Both Allium sativum, Salvia rosmarinus gave good and very similar results in terms of fungal inhibition, except for Mentha extract, which did not produce encouraging results in inhibiting C. albicans. This suggested that the compounds found in Allium sativum, Salvia rosmarinus may offer a valuable alternative or complement to antifungal treatments, especially for C. albicans. The method of preparation of the plant extracts, their concentration, storage method, and the duration of their extraction are important factors that affect the inhibition process.

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