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*Research Article*

# Antifungal Activity and Mechanism of Action of Different Parts of *Myrtus communis* Growing in Saudi Arabia against *Candida* Spp.

**Abdullah A. Alyousef**

*Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, P.O. Box 10219, Riyadh 11433, Saudi Arabia*

Correspondence should be addressed to Abdullah A. Alyousef; abalyousef@ksu.edu.sa

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Discovering new antifungal drugs from natural products is a key target for the treatment of infections, such as candidiasis and other *Candida*-related infections. As current therapeutic drugs for the treatment of infections, such as candidiasis and other *Candida*-related infections, have adverse effects on human health, discovering new antifungal drugs from natural products is urgently needed. The objective of this study was to evaluate the antifungal activity of the methanolic and sodium phosphate buffer extracts derived from various parts of *Myrtus communis*, a plant that is traditionally used in Saudi Arabia, against *Candida albicans* (ATCC 10213), *Candida glabrata* (ATCC 2001), *Candida kefyr* (ATCC 66028), *Candida parapsilosis* (ATCC 22019), and *Candida tropicalis* (ATCC 750). A well diffusion assay was performed to assess the antifungal activity through the measurement of the zone of inhibition. Of the extracts, those extracted with methanol from the roots and leaves displayed strong inhibitory activity against *Candida glabrata* ($23.5 \pm 0.12$ and $20.7 \pm 0.22$, respectively), at 50 mg/ml, with 5 mg/ml fluconazole administered as the standard control. The minimal inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values were 12.5 mg/ml and 25 mg/ml for the *M. communis* root extract and 25 mg/ml and 50 mg/ml for the *M. communis* leaf extract against *Candida glabrata*. The results were confirmed by scanning electron microscopy (SEM) imaging of the control and treated strains of *Candida glabrata*. Based on SEM, these extracts could alter the morphology and cause loss of cell integrity. The effect of *M. communis* root and leaf extracts on *Candida* cells was also determined by measuring the absorbance at 260 nm after treatment for 1 h at 37°C. Interestingly, the 260 nm absorbing material was higher in *Candida glabrata* than in the resistant strain, *Candida parapsilosis* (ATCC 22019). Based on our findings, the crude methanolic extract of *M. communis* roots and leaves exhibited good antifungal activity against the *Candida glabrata* strain. SEM results and estimation of the 260 nm absorbance material proved that the extract might act on the cell wall and cell membrane of *Candida* cells, further leading to cell death.

## 1. Introduction

Medicinal plants are employed as alternatives to Western medicine in developing countries to treat various health conditions. According to a survey by the World Health Organisation (WHO), 80% of the world's population uses natural remedies and traditional medicines [1, 2]. Plant extracts and their essential oils have shown exceptional biological activities against microorganisms. Globally, many countries, such as India, Jordan, and Mexico, have diverse flora and a rich tradition of using medicinal plants for antibacterial and antifungal applications [3, 4]. Screening of plant extracts against fungal strains has revealed that these plants contain active compounds with antimicrobial properties.

Invasive fungal infections are mainly caused by *Candida albicans* either locally or systemically [5]. These infections pose a serious threat to the public health sector economically and medically as they are associated with high

mortality rates and increased cost burden as well as hospital duration [6].

Some of the factors contributing to increased infections in immunocompromised patients include the use of intravenous catheters, invasive procedures, and total parenteral nutrition and the increasing use of broad-spectrum antibiotics, cytotoxic chemotherapies, and transplantation [7]. The virulence factors that lead to the pathogenicity of *Candida* species include their ability to evade host defences, adherence, promotion of hyphae, biofilm development (on host tissue and on medical devices), and the production of tissue-damaging hydrolytic enzymes, such as proteases, phospholipases, and haemolysin [8]. Abnormal hosts, such as immunocompromised persons or those with diabetes mellitus, are more prone to mucosal or systemic infections than healthy individuals [9].

Infections due to non-*albicans* species are rapidly increasing. In fact, *C. glabrata* is the second most common cause of candidiasis or vaginal candidiasis after *C. albicans* and *C. tropicalis*, which is the third most common species. In neonates, *C. parapsilosis* has become a dominant fungal pathogen in children and neonates in some centers [10].

Several antifungal agents have been discovered and are available for the treatment of invasive fungal infections, such as polyenes, pyrimidines, echinocandins, and triazoles. Fluconazole and voriconazole are the most commonly used antifungals. However, pathogenic microorganisms are constantly developing resistance to these agents [11, 12]. Antifungal drugs have undesirable side effects or are very toxic, induce drug-drug interactions, or lead to the development of resistance. Some drugs are also ineffective and have become less successful as therapeutic agents. Thus, searching for alternative antifungal drugs has been a major concern in recent years [13]. Natural products play an important role in drug development programs in the pharmaceutical industry [14]. As a result, several medicinal plants have been extensively studied in order to find safe, less toxic, and more effective drugs.

*Myrtus communis L.* (*M. communis*), also called myrtle (*Myrtaceae family*), is native to the Mediterranean basin and Arabian Peninsula. Since ancient times, different parts of myrtle, especially its leaves and fruit, have been used in food preparation and applied in traditional medicines as a general antiseptic, disinfectant, and therapy for many types of infectious diseases [15, 16]. Many previous studies on *Myrtus* demonstrated that different parts of this plant, such as leaves, branches, berries, and flowers, have different chemical compositions as well as pharmacological activities, such as antifungal, antibacterial, antiviral, anticancer, anti-inflammatory, analgesic, antioxidant, antidiabetic, and antimutagenic effects [17, 18]. Discovering more effective and less toxic novel antifungal agents is thus needed to overcome these disadvantages.

The objectives of this study were to determine the antifungal activity of different parts of the *M. communis* plant against known standard ATCC *Candida* strains using methanol and sodium phosphate buffer extraction methods and to investigate the mechanism of action of these extracts on the growth inhibition of different *Candida* species.

## 2. Materials and Methods

*2.1. Fungal Strains.* Five standard strains of *Candida* species were obtained from the Research Laboratory, Clinical Laboratory Sciences Department, College of Applied Medical Science, King Saud University: *Candida albicans* (ATCC 10213), *Candida glabrata* (ATCC 2001), *Candida kefyr* (ATCC 66028), *Candida parapsilosis* (ATCC 22019), and *Candida tropicalis* (ATCC 750). Each strain was subcultured on Sabouraud's dextrose agar (SDA) (Scharlau, Spain) medium and incubated at 37°C for 24 h to obtain inoculums for testing.

*2.2. Sample Preparation. M. communis* plants were procured from the Alfath nursery (Al-Qassim region, Saudi Arabia) in October 2019. Samples were transported to the Research Lab in a sterile bottle, washed with running tap water and later with distilled water, and air-dried under shade at room temperature. Subsequently, the samples were ground into a fine powder using an electric blender and stored in sterile 50 ml universal containers. Methanolic extraction was performed as described previously, with some modifications [15, 19, 20]. Briefly, a 25 g aliquot of each dried sample was extracted using 100 ml of methanol (95%) for 72 h at room temperature. Thereafter, the methanolic extracts were separated and filtered through Whatman filter paper No.1 and dried under pressure at 37°C with a rotator evaporator. The yield percentages were determined by dividing the weight of the extract by the weight of the sample multiplied by 100. The dried extracts were reconstituted in 1% dimethyl sulfoxide (DMSO; purity 99.7%) to prepare stock solutions, which were stored in a refrigerator at 4°C until the analysis.

Antimicrobial proteins and peptides were extracted as previously described [21, 22]. Briefly, a 25 g aliquot of each dried sample was soaked in sodium phosphate buffer (pH 6.5) at 30°C and left overnight. The next day, the extracts were filtered with Whatman filter paper No. 1 and subjected to 80% ammonium sulfate saturation. The collected saturated material was then separated by dialysis using a 3 kDa cut-off dialysis tubing (Sigma Aldrich, St. Louis, MO, USA), and the samples were subjected to spectrophotometric analysis at 280 nm to determine protein concentration.

*2.3. Preparation of the Inoculum.* Two to three colonies from 24 h old culture were used as the inoculum following suspension in 10 ml of 0.85% sodium chloride solution, which was autoclaved. The turbidity was adjusted to 0.5 McFarland standard units (i.e., $1.5 \times 10^8$ CFU/ml).

*2.4. Antifungal Susceptibility Test.* A well diffusion assay was used to determine the antifungal properties of medicinal plants, as described previously [20, 23, 24]. SDA was prepared according to the manufacturer's instructions and autoclaved. Medium (15-20 ml) was poured into sterile Petri dishes and allowed to solidify for 30 min. Thereafter, 0.2 ml of inoculum (fungal strain in saline) was spread on an agar plate, and the excess was removed via draining. The plates were then incubated at room temperature for 10 min. Using a sterile cork borer, 7 ditches of 4 mm were made in each plate. Methanolic plant extracts (50 mg/ml) were prepared

in dimethyl sulfoxide (DMSO). Each well was filled with either 50 $\mu$l of the methanolic extracts or 50 $\mu$l of crude protein; fluconazole (5 mg/ml) was used as a positive control while DMSO was used as a negative control. The plates were incubated at 37°C for 24 h. The zone of inhibition was measured in millimetres. The assay was repeated thrice for confirmation.

*2.5. Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC).* The minimal concentration of plant extracts that induced the inhibition of visible yeast growth or turbidity was referred to as the MIC. MIC was determined for the fungal strains that were sensitive to the extract in the well diffusion assay using the microdilution method and 2,3,5-triphenyltetrazohum chloride (TTC, tetrazolium red, purity min. 99%, SRL Pvt. Ltd. Mumbai, India) dye as a growth indicator, as described previously with some modifications [15, 25, 26].

Twofold serial dilutions of the extracts were prepared directly in a microtiter plate containing Sabouraud broth to obtain concentrations ranging from 100 to 1.56 mg/ml.

Thereafter, 100 $\mu$l of the strain inoculum, which was cultured overnight and adjusted to 0.5 McFarland units, was added to each well. The experiment was performed in triplicate. The culture plates were incubated overnight at 37°C for 24 h. The following controls, negative control (Sabouraud broth only) and positive controls (Sabouraud broth and microorganism), were tested to determine medium sterility and inoculum viability, respectively. The activity of fluconazole (5 mg/ml) was also compared with that of the extracts. To indicate fungal growth, 40 $\mu$l TTC (2 mg/ml) was added to each of the 96 wells in the plate. Thereafter, the plate was incubated for 30 min at 37°C. The lowest concentration at which there was no colour change was considered the MIC of the plant extract.

To determine the MFC, 20 $\mu$l of each of the wells with no turbidity or fungal growth was cultured on SDA. The plates were then incubated at 37°C for 24 h. The MFC was considered to be the lowest concentration cultivated in the plate that induced a fungicidal effect with no visible viable colonies on an agar plate.

*2.6. Scanning Electron Microscopy (SEM).* SEM was carried out to confirm the inhibitory effect of the plant extracts on sensitive strains using JSM-6380LA at an accelerating voltage of 10 kV. Two sets of samples were processed, control and treated. Overnight grown cultures of *Candida* spp. were inoculated in 10 ml Sabouraud dextrose broth (SDB) to derive the 0.5 McFarland standard. Suspensions of *Candida spp* were treated with 100 $\mu$l of the plant extracts at the MIC and incubated at 37°C for 24 h at 120 rpm; these samples were considered treated cultures. Suspensions of *Candida spp* that were not treated with the plant extracts were termed as the control cultures.

After incubation, the cultures were centrifuged at 1,500 $\times$ g for 5 min at room temperature to collect the pellet. Pellets were washed thrice with distilled water. A 200 $\mu$l volume of 2.5% glutaraldehyde was added to the Eppendorf tubes before sample preparation.

The sample pellets were fixed in buffer aldehyde (2.5% glutaraldehyde in phosphate buffer) solution for 3 h. After the removal of the glutaraldehyde solution, the samples were rinsed thrice in sodium cacodylate solution buffer for 5 min. Postfixation was performed using osmium tetroxide for 1 h, and the samples were rinsed in distilled water. Thereafter, the samples were dehydrated using a graded ethanol series of 25%, 50%, 75%, 90%, and twice with 100% ethanol for 10 min each. The sample was dried with a critical point dryer device, mounted on specimen stabs with gold coating, and viewed under an electron microscope.

*2.7. Measurement of the Release of 260 nm Absorbing Cellular Materials.* Spectrophotometer analysis of the 260 nm absorbing cellular materials was performed as described previously with some modifications [27, 28]. Briefly, an overnight grown culture of *Candida spp* was inoculated into fresh SDB to derive the 0.5 McFarland standard. Cells were harvested by centrifugation at 1,500 $\times$ g for 10 min at room temperature, and the pellet was resuspended in 10 ml phosphate buffer. Suspensions of *Candida spp* were treated with 100 $\mu$l of plant extracts at the MIC or the same volume of DMSO (control) and incubated at 37°C for 1 h. Samples were centrifuged at 12,000 $\times$ g for 1 min at 4°C, filtered through a 0.2 $\mu$m pore-size filter, and subjected to optical density measurement at 260 nm. Absorbance was estimated for the control and treated cells using a UV microplate reader (Bio-Tek), and the absorbance of DMSO control was subtracted.

*2.8. Statistical Analysis.* All studies were performed in triplicate. The data are presented as mean values. The difference between the control and treated samples was analysed using Student's *t*-test.

## 3. Results

*3.1. Extraction Yields of the Plant Extract.* The 25 g of dried plant material extracted with methanol (95%) yielded plant extract residues ranging from 0.7 to 2.4 g. The highest yield was obtained from *M. communis* leaves (2.4 g) followed by *M. communis* roots (1.53 g). The flowers had the lowest extract yield (Table 1).

As shown in Table 2, different concentrations of the protein extracts were successfully extracted from 25 g of dried plant materials. The flowers of *M. communis* had a low protein concentration of 50.1 $\mu$g/ml while the leaf extract had the highest protein concentration of 140.6 $\mu$g/ml when extracted with sodium phosphate buffer (pH 6.5).

*3.2. Antifungal Activity of the Plant Extracts.* An evaluation of the antifungal activity of different parts of the *M. communis* extracts against five standard *Candida* species was initially conducted using the well diffusion method. The *Candida* strains used in this study were *C. albicans* (ATCC 10213), *C. glabrata* (ATCC 2001), *C. keyfr* (ATCC 66028), *C. parapsilosis* (ATCC 22019), and *C. tropicalis* (ATCC 750). The effects of the extracts were compared with that of the standard antifungal agent, fluconazole (5 mg/ml), and the negative control, DMSO. The results for the

Table 1: Yield percentage of the methanolic extraction (%).

| Plant parts | Extract yield (%) |
|---|---|
| Stems | 4.2 |
| Flowers | 2.8 |
| Leaves | 9.6 |
| Roots | 6.12 |

Table 2: Protein concentration ($\mu$g/ml) in different parts of *Myrtus communis* extracted using sodium phosphate buffer.

| Plant parts | Protein concentration ($\mu$g/ml) |
|---|---|
| Stems | 76.2 |
| Flowers | 50.1 |
| Leaves | 140.6 |
| Roots | 109.8 |

antifungal activity of these extracts are presented in Table 3 and illustrated in Figure 1.

All *M. communis* extracts used in the study exhibited varying degrees of antifungal activity against the *Candida* strains. However, the methanolic extract of *M. communis roots* was the most effective among the extracts tested. In fact, this extract resulted in a zone of inhibition (ZOI) of 23.5 mm against *C. glabrata* compared with the standard antifungal, fluconazole (5 mg/ml). However, there was no significant activity against other *Candida* spp. The methanolic leaf extract resulted in a zone of inhibition of 20.7 mm against *Candida glabrata*; however, this extract did not exhibit any activity against other *Candida* spp.

*C. parapsilosis* and *C. keyfr* were found to be resistant to all methanolic extracts, whereas *C. albicans* showed very low sensitivity with zones of inhibition of 4 mm and 2 mm when treated with the methanolic extracts of roots and leaves, respectively. *C. tropicalis* showed very low sensitivity toward the methanolic root extract with a zone of inhibition of 5.2 mm.

Other methanolic extracts (stems and flowers) were not found to display antifungal activity against all tested strains.

The present study was conducted to investigate the efficacy of the crude extracts of antifungal proteins and peptides from different parts of *M. communis*. Extraction was carried out herein in sodium phosphate buffer at pH 6.5. The antifungal activities of these extracts were determined by the microbiological technique using the agar well diffusion assay.

The root extract displayed high activity against *C. glabrata* with a zone of inhibition of 14.5 mm and protein concentration of 109.8 $\mu$g/ml. The leaf extracts were found to be effective against *C. glabrata* and *C. albicans* with zones of inhibition of 13 mm and 6 mm, respectively.

*C. glabrata* showed very low sensitivity with a zone of inhibition of 4 mm when treated with the buffer extract of stems. Further, the extracts of stem and flowers had no activity against other *Candida* strains.

The antifungal activity of all the extracts suggested that all *Candida* strains were resistant to the methanolic and buffer extracts. The highest and most promising results against *C. glabrata* were obtained with the methanolic extract of *M. communis* roots and leaves. Hence, experiments were conducted to determine the MIC and MFC of this extract against *C. glabrata* (ATCC 2001), which were used for further characterization studies.

*3.3. Determination of MIC and MFC.* The effectiveness of the plant extracts in the standard *Candida* strains was confirmed by measuring the MIC and MFC. The MIC and MFC were only determined for organisms that had a zone of inhibition and were sensitive to the plant extracts in the previous antimicrobial assay carried out using the agar well diffusion method. In summary, the MIC and MFC values of the leaf extracts against *C. glabrata* (ATCC 2001) (MIC: 25 mg/ml, MFC: 50 mg/ml) were lower than those of the root extracts (MIC: 12.5, MFC: 25 mg/ml). *C. glabrata* (ATCC 2001) was found to be resistant to the other plant extracts.

*3.4. SEM Analysis.* SEM was performed to determine the morphology of the *C. glabrata* strain (ATCC 2001) after treatment with the *M. communis* roots and leaf extracts for 24 h. The cells that were not treated had normal *Candida* cell morphology, were oval-shaped, and had smooth outer surfaces. In contrast, the cells exposed to the MIC of the extracts showed considerable morphological alterations, including shrinkage, rough surface, and deformity, leading to the prominent loss of cell shape and integrity (Figure 2). This finding indicates that the *M. communis* root and leaf extracts might act on the cell wall and membrane of *C. glabrata*, which may be attributed to their high content of polyphenols and oxygenated monoterpenes [17], causing a loss in membrane integrity, leakage of cellular materials, and ultimately cell death.

*3.5. Measurement of the Release of 260 nm Absorbing Cellular Materials.* The effect of the methanolic extract of *M. communis* roots and leaves was also determined by confirming the leakage of 260 nm absorbing materials when *Candida* spp. were exposed to the MIC of the extracts (Figure 3). The OD of the sensitive strain, *C. glabrata* (ATCC 2001), significantly increased at 260 nm with the root extract and leaf extract relative to the control after 1 h, which may be attributed to leakage in the cytoplasmic membrane and release of cell materials, including nucleic acids, metabolites, and ions [29]. However, no changes in the optical density were observed for the resistance strain, *C. parapsilosis* (ATCC 22019), in this study.

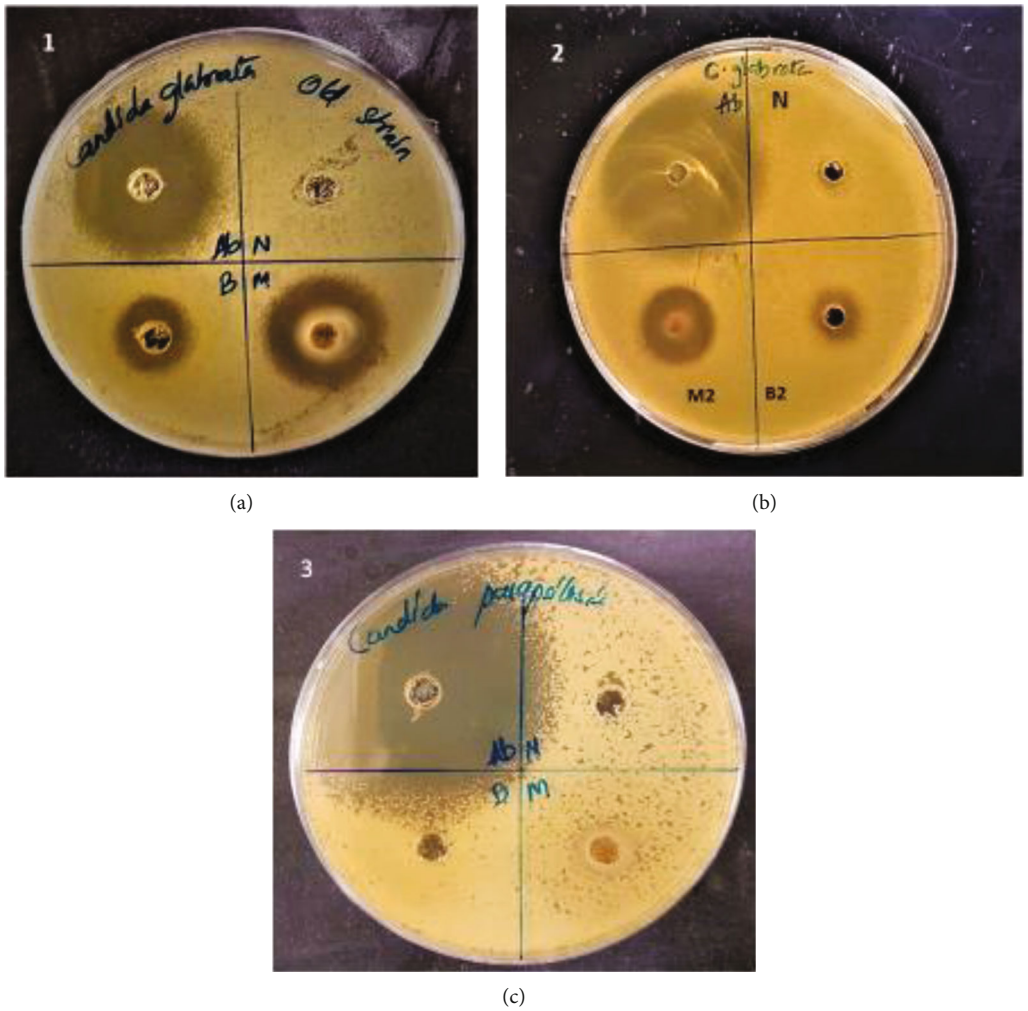
## 4. Discussion

The treatment of *Candida* infections is currently challenging owing to the limited number of available drugs, increased resistance to these drugs, high costs, and toxicity [30]. Consequently, novel alternative molecules that are more effective than conventional antifungal agents are urgently needed to prevent the emergence of fungal resistance. Only a few studies have assessed the antifungal activity of plants found in various regions of Saudi Arabia using ethanol, methanol,

Table 3: In vitro antifungal activity (mm zone of inhibition) of the methanolic (M) and sodium phosphate buffer (P) extracts derived from various parts of *M. communis* against standard *Candida* spp.

| Sample No. | Stems | | Flowers | | Leaves | | Roots | | Fluconazole (5 mg/ml) | DMSO |
|---|---|---|---|---|---|---|---|---|---|---|
| | P | M | P | M | P | M | P | M | | |
| *C. albicans* ATCC 10213 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 6.0 ± 0.12 | 2.0 ± 0.21 | 0.0 ± 0.0 | 4.0 ± 0.6 | 22.5 ± 0.22 | 0.0 ± 0.0 |
| *C. glabrata* ATCC 2001 | 4.0 ± 0.11 | 6.1 ± 0.5 | 0.0 ± 0.0 | 0.0 ± 0.0 | 13.1 ± 0.17 | 20.7 ± 0.22 | 14.5 ± 0.61 | 23.5 ± 0.12 | 29.5 ± 0.42 | 0.0 ± 0.0 |
| *C. keyfr* ATCC 66028 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 19.8 ± 0.37 | 0.0 ± 0.0 |
| *C. parapsilosis* ATCC 22019 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 30.8 ± 0.22 | 0.0 ± 0.0 |
| *C. tropicalis* ATCC 750. | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 5.2 ± 0.2 | 22.8 ± 0.31 | 0.0 ± 0.0 |

Data are means of three replicates ($n = 3$) ± standard error.




(a) (b)

(c)

Figure 1: Antifungal activity of the *Myrtus communis* extracts. The antifungal activity against the sensitive strain, *Candida glabrata* (ATCC 2001) (a, b), and the lack of antifungal activity of these extracts against *Candida parapsilosis* (ATCC22019) (c). M: methanolic root extract; B: buffer root extract; M2: methanolic leaf extract; B2: buffer leaf extract; Ab: fluconazole (5 mg/ml); N: DMSO (used as a negative control).

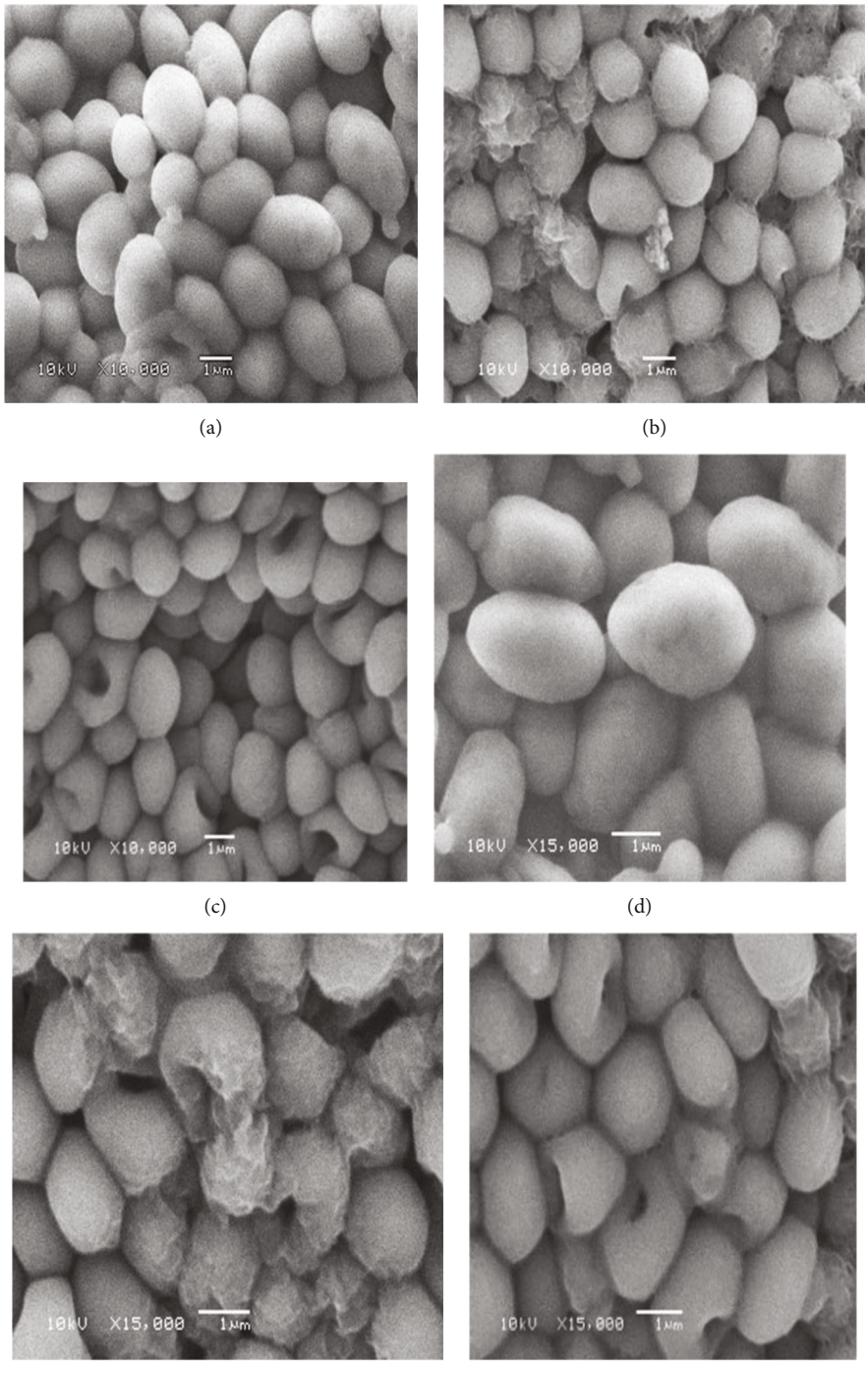



Figure 2: SEM analysis of the effects of plant extracts on the morphology of *Candida glabrata* (ATCC 2001). (a) Control cells (10,000x magnification); (b) morphological alterations in the cells, including shrinkage, rough surface, and deformity, when treated with the *Myrtus communis* root extracts (10,000x magnification); (c) morphological alterations in the cells, including shrinkage, rough surface, and deformity, when treated with the *Myrtus communis* leaf extracts (10,000x magnification); (d) control cells (15,000x magnification). Cell burst, rough surface, and deformity are indicated by arrows in cells treated with the *Myrtus communis* root (e) and leaf (f) extracts (15,000x magnification).

and other solvents to screen for alkaloids, terpenoids, and other antifungal agents.

Studies in the regions of Saudi Arabia, such as Abha, Dammam, Hail, Jeddah, and Najran, have revealed some significant results. A study carried out in Abha revealed that the antifungal activity of extracts from *Salvadora persica* and *Vigna fragrans* against *Aspergillus* sp. and *C. albicans* was higher than that of *Peganum harmala* and *Withania somnifera* extracts [31]. A study in Dhahran also revealed the antimicrobial activity of ethanol leaf extracts of *Catharanthus*

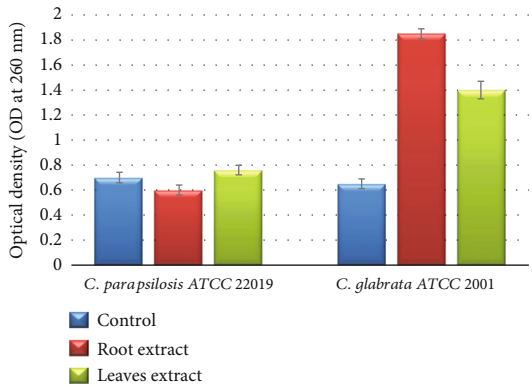



FIGURE 3: Absorbance of the cell material content of *C. glabrata* (ATCC 2001) and *C. parapsilosis* (ATCC 22019) at 260 nm following treatment with the MIC of the *M. communis* root and leaf extracts for 1 h. Values are expressed as the mean ± SD ($n = 3$).

*roseus* (Periwinkle) against *Staphylococcus aureus*, *Escherichia coli*, and *C. albicans* [32]. In another study, the antifungal activities of the aqueous and organic crude extracts of six medicinal plants collected from the markets of Jeddah, including *Azadirachta indica* (neem), *Zingiber officinale* (ginger), *Eucalyptus globulus*, *Lawsonia inermis*, *Lepidium sativum*, and *Rosmarinus officinalis*, were determined against different pathogenic fungi. The results revealed that some of the plant extracts had high antifungal activity against both *C. albicans* and *C. tropicalis* [33]. A study conducted by Bokhari revealed the remarkable antidermatophytic properties of the methanolic extracts of *Cymbopogon citratus* (lemon grass) and *Lantana camara* (lantana), which were collected from different localities of Jeddah city [34]. Further, a few studies have shown that different solvents (ethanol, ether, methanol, and chloroform) and aqueous extracts of *Commiphora myrrha* (myrrh), which is commonly used in Saudi Arabia, show broad-spectrum activity against pathogenic bacteria, moulds, and different *Candida* spp. [35, 36].

In a previous study, essential oils of *Ocimum basilicum* collected from the Jeddah region displayed antimicrobial activity against some bacteria and fungi [37]. Furthermore, the extract obtained from *Salvadora persica* (miswak) has been found to exhibit potent antifungal activity against all *Candida* strains. In this study, the plants were selected based on their traditional use among Middle Eastern communities for dental hygiene purposes and the prevention of tooth decay [38]. A review conducted in Najran, a city in Saudi Arabia, revealed the various therapeutic effects of *S. persica* on oral health, which can help elucidate the significance and importance of this indigenous oral hygiene tool [39].

In previous studies, the fruit (berry) extracts, leaf extracts, and essential oils of *M. communis* have been extensively analysed to determine their antimicrobial properties [17, 40]. However, only a few studies have reported the antifungal activities of these plant extracts. In a previous in-depth study that investigated the methanolic extracts of *M. communis* leaves, high antimicrobial activity was observed; the extraction procedures employed in this study included heat and a long incubation period of approximately 7 days [19, 41]. Previous studies have shown that methanolic extracts of the aerial parts of *M. communis* are highly active against three human pathogenic fungi, *C. albicans* (ATCC 10231), *C. tropicalis* (ATCC 13801), and *C. glabrata* (ATCC 28838) [42]. In another study, oil obtained from the leaves of myrtle exhibited strong antifungal activity against different *Candida* species [43]. Myrtle leaf oil has also shown significant antifungal activity against different strains of *C. albicans* when combined with the antifungal agent amphotericin B [44]. However, a study conducted by Mert et al. revealed that the n-hexane, ethanol, methanol, ethyl acetate, and aqueous extracts of the leaves of *M. communis* did not exhibit any antifungal activity against *C. albicans* (ATCC 10239) [45].

Many types of molecules with antimicrobial activity have been isolated from plants. Among these phytomolecules, proteins and peptides with antifungal activity have been recently reported [46]. The present study was conducted to investigate the efficacy of crude extracts of antifungal proteins and peptides from different parts of *M. communis*. The root extract displayed high antifungal activity only against *C. glabrata*, and the leaf extracts were found to be effective against *C. glabrata* and *C. albicans*. Furthermore, the stem and flower extracts displayed no activity against other *Candida* strains. However, our previous studies have revealed that the antibacterial activity of the crude protein extracted from leaves is highly active against methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii*, and *S. aureus*. In addition, this extract has been found to be more active than standard antibiotics [22].

The MIC and MFC of these extracts against sensitive *Candida* strains were determined to quantify their activity. Methanolic root extracts showed MIC and MFC values of 12.5 mg/ml and 25 mg/ml, respectively, against *C. glabrata* (ATCC 2001). Methanolic leaf extracts showed MIC and MFC values of 25 mg/ml and 50 mg/ml, respectively, against *C. glabrata* (ATCC 2001). Therefore, the results of the present study indicate some of the advantages of *M. communis* root and leaf extracts that could be applied for the treatment of microbial infections.

To examine the antifungal mode of action of the studied extracts, it is important to estimate changes in fungal cell morphology, surface structure, and cell membrane permeability and integrity [29]. SEM analysis was performed to understand the effects of the extracts on *C. glabrata*. SEM images showed that the cells exposed to the MIC of the extracts displayed numerous morphological alterations, which caused a loss in membrane integrity, leakage of cellular materials, and ultimately cell death. This finding indicates that *M. communis* root and leaf extracts might disrupt the cell wall and membrane of *C. glabrata*, which may be attributed to their high content of polyphenols and oxygenated monoterpenes [17]. SEM was employed in previous studies to demonstrate that plant extracts can cause morphological changes in the tested organisms. In a recent study, SEM analysis was used to determine the effect of 150 $\mu$l/ml of the ethanolic extract of *Ziziphus spina-christi* leaves and *Phoenix dactylifera* seeds on *C. albicans*, and the results showed reduced cellular activity and shrinkage of the cell wall in the fungus [47]. SEM analysis was also

performed on bacteria to understand the effects of plant extracts on their cell morphology. Changes in cell morphology have been reported in some bacteria, such as *S. aureus*, *E. coli*, and *Salmonella*, after treatment with various plant extracts [29, 48]. The findings of the present study are consistent with those of previous studies.

Leakage of cellular materials was analysed by detecting 260 nm absorbing materials. In the present study, the OD of the sensitive strain, *C. glabrata* (ATCC 2001), significantly increased at the wavelength of 260 nm following a 1 h treatment with the root and leaf extracts compared with the control, suggesting damage to the cell wall and cytoplasmic membrane of the yeast cells. Similar results have also been reported for the essential oil of *Aloysia triphylla* and ethanolic extract of *Salvia miltiorrhiza* when tested against *C. albicans*, showing increased absorbance at a wavelength of 260 nm [13, 28]. Therefore, the absorbance of the material and proteins at 260 nm wavelength can be used as an indicator of damage to the cell wall and membrane, which causes leakage of the cellular materials into the surroundings [49].

## 5. Conclusion

Based on the results of our study, it can be concluded that the methanolic extracts of the roots and leaves of *M. communis* could serve as potential sources of herbal drug preparations against *C. glabrata*. Further studies are necessary to determine the antifungal activity of different parts of the *M. communis* plant using different extraction methods, solvents, and conditions as well as identify the chemical identity and toxicity of the bioactive compounds in *M. communis* root extracts that are responsible for the significant antifungal activity observed in this study.

## Data Availability

Data used to support the findings of this study are included within the article.

## Conflicts of Interest

The author declares that there are no conflicts of interest.

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