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Research article

Inhibitory effects of different fractions separated from standardized extract of *Myrtus communis L.* against nystatin-susceptible and nystatin-resistant *Candida albicans* isolated from HIV positive patients

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ABSTRACT

Background: and Purpose: Myrtle (*Myrtus communis L.*) is a medicinal herb that plays an essential role in treating fungal infections. The present study investigated the antifungal properties of different fractions of the *M. communis L.* leaf extract against *Candida albicans* (susceptible and resistant to nystatin).

Materials and methods: Total extract (TE) and petroleum ether (PE), chloroform (CH), ethyl acetate (EA), and methanol (ME) fractions were prepared using the sonication method. The study used the standard strain sample (ATCC 76645) and nystatin-resistant *C. albicans* from oral samples of HIV-infected individuals. The identification of resistant isolate was performed using phenotypic and molecular methods. Minimum inhibitory concentration (MIC) and Minimum fungicidal concentration (MFC) of the fractions along total extract were determined by microdilution method on nystatin-resistant and susceptible *Candida albicans*. The Folin-Ciocalteu method was used to determine the total phenolic content of the extract.

Results: The extraction yield (w/w) was 13.50 for TE, 2.10 for PE, 2.23 for Ch, 2.14 for EA, and 10.03 for ME fractions. Chloroform extract showed good anti-candida activity against nystatin susceptible and resistant *C. albicans* (62.5 μg/mL). Ethyl acetate fraction exhibited the greatest MIC against nystatin susceptible and resistant *C. albicans* (250 μg/mL). The MIC value of fluconazole was >64 μg/mL for both susceptible and -resistant strains. The amount of phenolic compounds of the total extract was reported to be equal to 5.4%, equivalent to gallic acid.

Conclusion: Results revealed that the PE and CH fractions showed greater antifungal effects than the total extract against both susceptible and resistant strains of *Candida albicans*. It can conclude that active antifungal compounds of the plant belong to a specific group of metabolites, which according to the type of solvent, probably have non-polar nature. Further separation is carrying out.

1. Introduction

Candidiasis is one of the most important infectious diseases in individuals infected with the human immunodeficiency virus (HIV) and is considered an independent predictor of immunodeficiency in patients with acquired immunodeficiency syndrome (AIDS). In addition, candidiasis accounts for about 25–50 percent of nosocomial infections in the intensive care unit and 8 to 15 percent of total nosocomial infections (the fourth leading cause of nosocomial infections) [1].

Candida albicans (C. albicans) is one of the most common opportunistic fungal infections. It has a worldwide distribution and is endogenous yeast of the gastrointestinal tract and mucosal surfaces of the body, but it can rarely be seen on human skin [2]. *C. albicans* and *Candida dubliniensis* are more commonly isolated from the mucosa of healthy individuals [2]. Candidiasis is considered an opportunistic infection that occurs following the host's initial physiologic and immunologic weakness; this does not contradict the possibility of pathogenicity candida and especially *C. albicans* [3].

The common antifungal drugs such as amphotericin B and nystatin, which are used to treat candida infections, have many side effects such as hepatotoxicity and nephrotoxicity [4]. The recent increased use of azoles such as itraconazole and fluconazole, has raised concerns over the potential for the emergence of resistance of *Candida* species (notably *C. albicans*, *C. glabrata*, *C. tropicalis* and *C. krusei*) to antifungals [5]. For

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these reasons, researchers focused their attention on the antimicrobial activity of natural compounds to find alternative treatments [4, 5]. In particular, scientists have seen herbs as one of the most valuable resources to produce medications.

M. communis L. is an aromatic evergreen perennial shrub in the Myrtaceae family that grows wildly in Iran, especially in Kerman province [6]. Apart from its traditional use, diverse biological and pharmacological activities such as antimicrobial, anti-oxidant, anti-leishmanial, anti-inflammatory, anti-diabetic, antimicrobial, and protective effects on pulmonary fibrosis have been reported for the plant [7, 8, 9]. Different components in the leaves have antimicrobial activity, scavenging free radicals and preventing the oxidation of unsaturated fatty acids. *M. communis L.* has traditionally been used as a disinfectant and hypoglycemic agent and is also useful in treating oral pest ulcers [10]. Although there are various reports on the anti-candida effect of *M. communis* extract, no study has been done on the effect of plant fractions. For isolating and achieving the active compounds of the plant, the first step is to fractionate the plant, which has been achieved in this study.

This study aimed to separate different fractions of the standardized extract of the *M. communis L.* leaves and compare their inhibitory effect against nystatin-susceptible and nystatin-resistant *C. albicans.*

2. Materials and Methods

This study was carried out based on the principles of the Declaration of Helsinki declared by the Ethics Committee of the Kerman University of Medical Sciences (IR.KMU. REC.1397.366).

2.1. Chemicals

Fluconazole was purchased from Merck (Darmstadt, Germany), nystatin was obtained from Sigma (Saint Louis, MO, USA), DMSO was bought from Merck (Darmstadt, Germany). Nystatin-susceptible *C. albicans* strain was obtained from Pasteur Institute. Methanol, ethyl acetate, petroleum ether, and chloroform were purchased from Merck (Darmstadt, Germany). Moreover, RPMI-1640 was purchased from Biosera (France).

2.2. Plant materials

The plant leaves were collected from Kerman province in June 2018 and authenticated in the Pharmacognosy Department, Faculty of Pharmacy. A voucher specimen was deposited in the Herbarium Center of Faculty (KF1356).

2.3. Extraction and fractionation

About 500 g of the plant leaves were milled and passed through a sieve (mesh 35) and divided into 2 parts of 250 g:

1 250 g of the plant was used for preparation of total extract (TE), so the plant was extracted with 80% methanol by sonication method (1000 rpm, 35 min and 35 °C). After filtering the extract with filter paper, the residue on the filter paper was extracted twice by the same method and this process was repeated for three times. The three obtained extracts were mixed thoroughly and concentrated in vacuum at 40–45 °C. The final residue on the filter paper was discarded.
2 The second 250 g of the plant was used for fractionation. To do this, the leaves of the plant were extracted with petroleum ether (PT) in the same way as described above (sonicate method and for three times). After collecting the extracts, the residue of the plant extract on the filter paper was pressed to completely remove the previous solvent, then extracted three times with chloroform (CH) and so on with ethyl acetate (EA), and methanol (ME). The total extract (TE) and all the fractions were stored at -20 °C until the antifungal experiments.

2.4. Total phenolic content of the plant

The Folin-Ciocalteu method was used to determine the amount of phenolic compounds. To do this, a mixture of 0.1 mL of a gallic acid stock (1000ppm), sodium carbonate (4 mL), Folin reagent (5 mL), and distilled water (3 mL) was incubated at room temperature for 40 min. The absorption spectrum of the solution was recorded at 200–800 nm, and the maximum wavelength of absorption was determined ($\lambda max = 765$ nm). The absorbance of at least five concentrations of gallic acid and two concentrations of total extract of myrtle was determined at λ max, as mentioned earlier. The total phenolic content of the plant was calculated using the calibration curve of gallic acid [11].

2.5. C. albicans strains

The standard strain of *C. albicans* (ATCC 76645) was purchased from Pasteur Institute. Nystatin-resistant *C. albicans* samples were collected from patients with HIV referred to Kerman Behavioral Diseases Center, southeast of Iran. Three oral samples were taken from these patients. Identifications of nystatin-resistant *C. albicans* isolates were performed using phenotypic and PCR-RFLP methods. Antifungal assay, the minimum inhibitory concentration (MIC), and the minimum fungicidal concentration (MFC) were determined using the M27-A3 broth microdilution method. Nystatin and fluconazole were used as positive controls.

2.6. Molecular identification of C. albicans isolates by PCR-RFLP

Genomic DNA was extracted by the Gene All extraction kit (Gene All, South Korea). The PCR assay was performed using primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC3′). The ITS1–ITS4 sequences were digested with restriction enzymes MSP1 and BLN1. The separation of the digested fragments was visualized on 2% agarose gel run in TBE buffer at 100 V for 45 min and stained with 0.5 μg mL -1 ethidium bromide.

2.7. Preparation of standard suspension Candida albicans

After sampling the oral cavity using the sterile swap and falcon tube by the specialist, specimens were isolated and transferred to the laboratory and cultivated on Sabouraud dextrose agar (SDA) at 30 °C for 24 h. Next, a suspension containing 1.5 × 104 cells/mL of *C. albicans* was prepared from the yeast colony [9].

2.8. Antifungals stock preparation

Nystatin and fluconazole were dissolved in dimethyl sulfoxide (DMSO, Merck, Germany) to produce a stock concentration of 3,200 μg/mL and 12,800 μg/mL, respectively. Stock solutions were kept at room temperature for complete dissolving and then stored at -70 °C until use.

2.9. Determination of minimal inhibitory concentration (MIC)

Antifungal susceptibility testing was done according to the Clinical and Laboratory Standards Institute (CLSI) M27-A3 protocol. A serial dilution of total extract and fractions (1.953–1000), nystatin (0.03–16 μg/mL) and fluconazole (0.06–64 μg/mL) in dimethylsulfoxide 2% (DMSO 2% in water) was prepared in working RPMI 1640 in microplate wells. The amount of 1500 yeast cells from nystatin-susceptible and nystatin-resistant *C. albicans* suspension was added to each and after 24 h of incubation at 37 °C. The MICs were measured as the lowest concentration of each agent that resulted in 50% inhibition of growth. A solution of DMSO 2% was used as a control [12]. According to CLSI guidelines, the resistance breakpoint for nystatin is 16 g/mL [13].

2.10. Determination of minimum fungicidal concentration (MFC)

The amount of 10 μl of each well was cultured in a plate of SDA and incubated at a 35 °C for 24 h. The MFC was the lowest concentration from which were observed ≤3 colonies on the agar plate [12].

2.11. Phytochemical study of petroleum ether (PT) and chloroform fractions (CH)

Given that the greatest anti-candida effect was related to PT and CH fractions, so the presence of phytochemicals including alkaloids, saponins, flavonoids, tannins and terpenoids was studied as explained previously [14]. The results indicated presence of terpenoids (+++) and small amount of alkaloids (+) in both fractions. Total terpenoids was performed as explained by Inmudati et al. An amount of 100 mg of the fractions was soaked in ethanol (9 ml) and after 24 h was filtered. The filtrate was extracted with PT (10 ml) using decanter funnel and was dried on water bath at 40 °C and was weighed after complete drying and expressed as % of terpenoid weight (g)/total fraction weight (g)×100.

2.12. Statistical analysis

Yield of extraction and specification of total extract and fractions separated from *M. communis L.* were mentioned based on percentage of extraction (W/W). MIC of total extract, different leaf fractions of *Myrtus communis L.* were used to represent the data based on (μg/mL) in *C albicans*. Standard curve of gallic acid was performed.

3. Results

3.1. PCR-RFLP

The results of PCR-RFLP by using MSP1 and BLN1 enzymes showed that, three clinical specimens, two strains were *C. albicans,* that one of them was resistant to nystatin. Also, one strain of *C. dubliniensis* was identified (Figure 1).

3.2. Extraction and fractionation of the plant

The results of extraction and fractionation of the *Myrtus communis L.* plant has shown in Table 1. As this table shows, the most significant percentage of fractionating was due to Methanol fraction.

Table 1. Yield of extraction and specification of total extract and fractions separated from *M. communis L.*

Fractions	Total extract	Petroleum ether fraction	Chloroform fraction	Ethyl acetate fraction	Methanol fraction
Variables					
Color	Dark green	Slime green	pistachio green	pistachio green	Slime green
Percentage of extraction (W/W)	13.50	2.10	2.23	2.14	10.03

3.3. Standard gallic acid curve

The calibration curve of gallic acid was plotted versus different concentrations (Figure 2). The total phenolic content of the plant was determined as 5.4% (w/w) equivalent to gallic acid.

3.4. MIC & MFC

Table 2 presents the MIC of the total myrtle extract, different separated fractions against *C. albicans* compared to nystatin and fluconazole. Among the different tested samples, the lowest MIC of *M. communis L.* against nystatin-susceptible *C. albicans* was due to CH fraction of the plant (MIC = 62.5 μg/mL) in comparison with nystatin and fluconazole (MIC = 2 μg/mL and >64 μg/mL respectively) (Figure 3). The MFC values were 32 μg/mL for nystatin and >64 μg/mL for fluconazole on

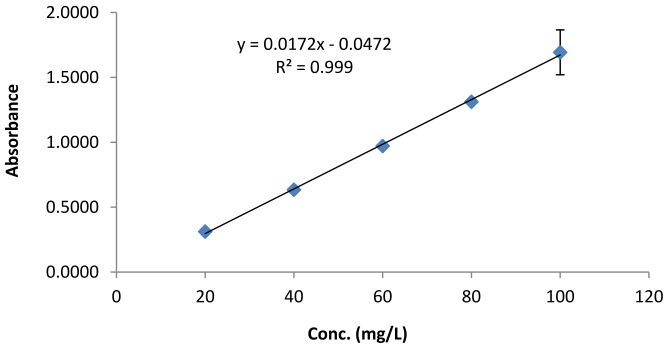

Figure 2. Standard curve of gallic acid at a wavelength of 765 nm.

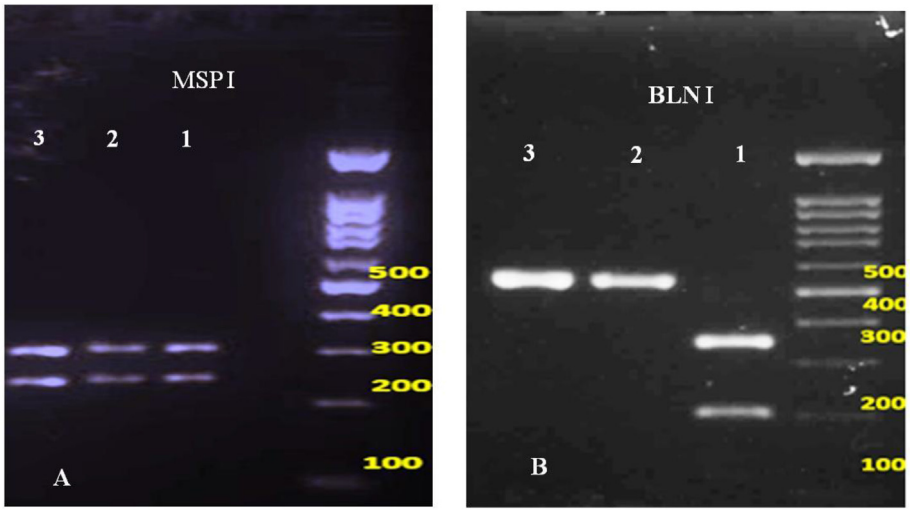

Figure 1. Patterns of ITS 1–ITS 4 sequences digested with restriction enzymes A: Using MspI, *C. albicans* and *C.dubliniensis* produce the same banding pattern (240 and 300 bp). B: Using BLNI, *C.dubliniensis* produce two fragments about 200 bp and 335 bp (sample 1) and there are no cutting sites for *C.albicans* (samples 2 and 3).

Table 2. MIC of total extract and separated fractions of *M. communis* against nystatin susceptible and resistant *C. albicans* in comparison to nystatin and fluconazole.

Antifungal compounds	*C. albicans*	MIC (μg/mL)	MFC (μg/mL)
Total extract	S	125	500
	R	125	>1000
Methanol fraction	S	125	1000
	R	62.5	>1000
Ethyl acetate fraction	S	250	>1000
	R	250	>1000
Chloroform fraction	S	62.5	1000
	R	62.5	1000
Petroleum ether fraction	S	125	250
	R	125	250
Nystatin	S	2	16
	R	>16	>16
Fluconazole	S	>64	>64
	R	>64	>64

nystatin-susceptible *C. albicans*. The PE fraction exhibited the lowest MFC (250 μg/mL) (Table 2). The fractions of ME and CH exhibited the least MIC on nystatin-resistant *C. albicans* (62.5 μg/mL) compared to MIC = 16 μg/mL and >64 μg/mL for nystatin and fluconazole, respectively (Figure 3). The least MFC value was due to PE fraction (250 μg/mL) against nystatin-resistant *C. albicans* in comparison to MFC >64 μg/mL and 256 μg/mL for nystatin and fluconazole, respectively (Table 2).

3.5. Phytochemical study and total terpenoid

Phytochemistry studies confirmed presence of terpenoids and a small amount of alkaloids in PT and CH fractions. Flavonoids and saponins were absent in these fractions. Quantitative study of PT and CH resulted into 67.5% and 58.3% of total terpenoids content respectively.

4. Discussion

More than 90% of patients with AIDS, experience oropharyngeal candidiasis at some point during their illness. In this condition, drug resistance to antifungals has been considered a global problem [15]. The use of alternative sources of drugs can lead to access the novel therapeutic agents with fewer side effects without the risk of drug resistance. *M. communis L.* is an easily accessible plant in Iran [16]. Traditionally leaf and fruit of myrtle have been used as a stomachic, hypoglycemic, anti-cough, and antimicrobial remedy [17, 18].

The results obtained from this study showed that *M. communis L.* fractions can inhibit *C. albicans* which is different from the total extract. In this work, CH fraction showed the least MIC (62.5 μg/mL) against nystatin-susceptible *C. albicans* (compared to 2 μg/mL nystatin and >64 μg/mL for fluconazole). In addition, the PE fraction exhibited the least MFC (250 μg/mL) on nystatin-susceptible *C. albicans*. The least MIC for nystatin-resistant *C. albicans* was due to the methanol and chloroform fractions. Petroleum ether fraction demonstrated the lowest MFC value (250 μg/mL) on nystatin-resistant *C. albicans*. Both PE and CH fractions exhibited more inhibitory effects than total extract. Up to our knowledge, this is the first study aimed to assess the antifungal activity of PE and CH fractions of *M. communis L.* on nystatin susceptible and resistant *C. albicans*.

Curini et al. showed the inhibitory effect of essential oils of *M. communis L.* against phytopathogenic fungi Rhizoctonia solani Kuhn, *Fusarium solani*, and *Colletotrichum lindemuthianum*. This essential oil demonstrated 60% inhibition of *R. solani* growth at 1600 ppm [19]. The methanolic extracts of *M. communis* leaves were reported to be weak [20]. Najib–Zadeh et al. reported antifungal effects of *M. communis L.* essential oils on oral candidiasis in immunosuppressed rats. However, the essential oil was not enough to remove oral candidiasis completely in immunosuppressed rats at concentrations two times that of MIC [21].

In agreement with our results, Cannas et al. studied the antimycotic activity of *M. communis L.* leaves against *Candida* spp. from clinical specimens. Good activity has been observed against *C. albicans* and *C. tropicalis* after 24–48 h [17]. Erdogan et al. demonstrated the intense anticandidal activity of methanol, acetone, ethanol, and ethyl acetate extracts of *M. Communis* leaves against *C. albicans* with MIC ranging from 0.187 to 1.5 mg/mL and MFC ranging from 0.375 to 3 mg/mL [22]. Sadeghi-Nejad et al. demonstrated that the most vigorous antifungal activity of *M. communis* against clinical isolates of *Candida* spp. and Aspergillus spp. were shown against *C. glabrata* with MIC values of 0.625–5.0 μg/mL [23]. In a study conducted by Zabka et al., antifungal effects of different phenols were investigated. This study showed that among the 21 phenolic compounds which were studied, thymol and carvacrol had the most substantial antifungal effects and the lowest MIC [24]. Although there are different reports for anti-candidiasis activity of myrtle extract, it is the first work that compared the activity of separated fractions of the plant. Medicinal plants contain a series of components and secondary metabolites responsible for the plant's medicinal effects.

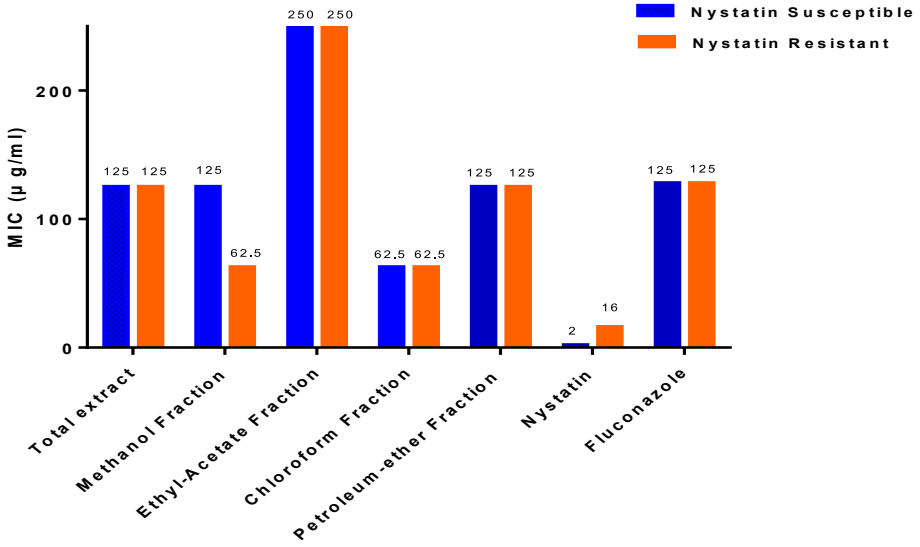

Figure 3. MIC of total extract, different leaf fractions of *Myrtus communis L.* and nystatin, fluconazole on susceptible and resistant *C. albicans*.

M. communis is composed of various phytochemicals such as alkaloids, flavonoids, tannins, terpenoids, which alone or in combination with each other cause different biological and pharmacological effects. Differences in the nature of these compounds are used as a guide for the bioassay-guided separation of biologically active fractions. In this research, plant fractionation has been done by increasing the solvent polarity; therefore, by comparing the anti-candida activity of different fractions and determining the active plant fraction, further research might explore the separation and determination and identification of the active compounds of the plant in the future.

5. Conclusion

In some cases, it has been reported that due to the synergistic effects of the phytoconstituents, the biological and pharmacological effect of the total extract is more prominent than each of the pure components. In contrast, our results indicated that the fractions of myrtle have more anticandidal activity than the total extract. So, we can conclude that the isolation could lead to active constituents with more inhibitory effects than the total plant extract. In this study, we researched for the first time *in vitro* anticandidal activity of PE and CH extracts of *Myrtus. communis.* In this regard, these fractions are a valuable candidate for further studies. Furthermore, due to the lack of effect of EA fraction, it can be concluded that the antifungal compounds of the plant are not likely flavonoids, and they are probably from the terpenoid category and non-polar.

Declarations

Author contribution statement

Iman Torabi and Seyed Amin Ayatollahi Mousavi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Fariba Sharififar and Alireza Izadi: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

[1] M. Pfaller, D. Diekema, Epidemiology of invasive candidiasis: a persistent public health problem, Clin. Microbiol. Rev. 20 (1) (2007) 133–163.

[2] M. Pfaller, D. Diekema, Rare and emerging opportunistic fungal pathogens: concern for resistance beyond *Candida albicans* and Aspergillus fumigatus, J. Clin. Microbiol. 42 (10) (2004) 4419–4431.

[3] R.A. Calderone, W.A. Fonzi, Virulence factors of *Candida albicans*, Trends Microbiol. 9 (7) (2001) 327–335.

[4] T. Kuriyama, D. Williams, J. Bagg, W. Coulter, D. Ready, M. Lewis, In vitro susceptibility of oral Candida to seven antifungal agents, Oral Microbiol. Immunol. 20 (6) (2005) 349–353.

[5] Exploring the relationship between drug side-effects and therapeutic indications, in: P. Zhang, F. Wang, J. Hu, R. Sorrentino (Eds.), AMIA Annual Symposium Proceedings, American Medical Informatics Association, 2013.

[6] Z. Mir Azadi, B. Pile Var, M.H. Meshaktosadat, R. Karamian, Site quality and Essential oil composition of M. communis L. (case study: cham moord site in Lorestan province), Agric. Biotechnol. J. 3 (2) (2012) 71–80.

[7] M. Samareh Fekri, A. Mandegary, F. Sharififar, H.R. Poursalehi, M.H. Nematollahi, A. Izadi, et al., Protective effect of standardized extract of *Myrtus communis L.*(myrtle) on experimentally bleomycin-induced pulmonary fibrosis: biochemical and histopathological study, Drug Chem. Toxicol. 41 (4) (2018) 408–414.

[8] W.A. Wannes, B. Mhamdi, J. Sriti, M.B. Jemia, O. Ouchikh, G. Hamdaoui, et al., Anti-oxidant activities of the essential oils and methanol extracts from myrtle (M. communis var. italica L.) leaf, stem and flower, Food Chem. Toxicol. 48 (5) (2010) 1362–1370.

[9] Z.F. Zahabi, F. Sharififar, P.G. Almani, S. Salari, Antifungal activity of different fractions of Salvia rhytidea Benth as a valuable medicinal plant against various species of Candida in Kerman Province, southeast Iran, Gene Rep. (2020 Feb 6) 100624.

[10] D. Yadegarinia, L. Gachkar, M.B. Rezaei, M. Taghizadeh, S.A. Astaneh, I. Rasooli, Biochemical activities of Iranian Mentha piperita L. and M. communis L. essential oils, Phytochemistry 67 (12) (2006) 1249–1255.

[11] K. Slinkard, V.L. Singleton, Total phenol analysis: automation and comparison with manual methods, Am. J. Enol. Vatic. 28 (1) (1977) 49–55.

[12] Clinical and Laboratory Standards Institute, Reference Method for Broth Diluition Antifungal Susceptibility Testing of Yeasts: Approved Standard, third ed., Clinical and Laboratory Standards Institute, Wayne, PA, 2008. M27-A3.

[13] National Committee for Clinical Laboratory Standards, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. Approved Standard. DocumentM27-A, second ed., NCCLS, Wayne, 2002.

[14] F. Sharififar, M. Mirtajadini, M.J. Azampour, E. Zamani, Essential oil and methanolic extract of Zataria multiflora Boiss with anticholinesterase effect, Pakistan J. Biol. Sci.: PJBS 15 (1) (2012) 49–53.

[15] R.S. Klein, C.A. Harris, C.B. Small, B. Moll, M. Lesser, G.H. Friedland, Oral candidiasis in high- risk patients as the initial manifestation of the acquired immunodeficiency syndrome, N. Engl. J. Med. 311 (6) (1984) 354–358.

[16] M. Mehrabani, A. Kazemi, S.A. Ayatollahi Mousavi, M. Rezaifar, H. Alikhah, A. Nosky, Evaluation of antifungal activities of *Myrtus communis L.* by bioautography method, Jundishapur J. Microbiol. 6 (8) (2013) 1–7.

[17] S. Cannas, P. Molicotti, M. Ruggeri, M. Cubeddu, M. Sanguinetti, B. Marongiu, S. Zanetti, Antimycotic activity of M. communis L. towards Candida spp. from clinical isolates, J. Infect. Dev. Ctries. 7 (3) (2013) 295–298.

[18] A. Deriu, G. Branca, P. Molicotti, G. Pintore, M. Chessa, B. Tirillini, B. Paglietti, A. Mura, L.A. Sechi, G. Fadda, S. Zanetti, In vitro activity of essential oil of *Myrtus communis L.* against Helicobacter pylori, Int. J. Antimicrob. Agents 30 (6) (2007) 562–563.

[19] M. Curini, A. Bianchi, F. Epifano, R. Bruni, L. Torta, A. Zambonelli, Composition and in vitro antifungal activity of essential oils of Erigeron canadensis and M. communis from France, Chem. Nat. Compd. 39 (2) (2003) 191–194.

[20] G. Sacchetti, M. Muzzoli, G.A. Statti, F. Conforti, A. Bianchi, C. Agrimonti, M. Ballero, F. Poli, Intra-specific biodiversity of Italian myrtle (M. communis) through chemical markers profile and biological activities of leaf methanolic extracts, Nat. Prod. Res. 21 (2) (2007) 167–179.

[21] T. Najib–Zadeh, M.H. Yadegari, H. Naghdi Badi, A. Salehnia, Antifungal efficacy of M. communis essential oils on oral candidiasis in immunosupressed rats, J.A.M.P. (J. Anim. Morphol. Physiol.) 2 (38) (2011) 102–116.

[22] E.A. Erdogan, G. Goksen, A. Everest, Anti-candidal activities of some M. communis L. extracts obtained using accelerated solvent extraction (ASE), J. Appl. Biol. 2 (5) (2014) 12–14.

[23] B. Sadeghi Nejad, M. Erfani Nejad, S. Yusef Naanaie, M. Zarrin, Antifungal efficacy of M. Communis linn, Jentashapir J. Health Res. 5 (4) (2014) 1–4.

[24] Zabka Martin, Pavela Roman, Antifungal efficacy of some natural phenolic compounds against significant pathogenic and toxinogenic filamentous fungi, Chemosphere 93 (2013) 1051–1056.