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# Antifungal Properties of *Zataria multiflora* on *Candida* species: A Systematic Review

**Ali Malekzadeh Shafaroudi, DDS[1], Nadia Elyassi Gorji, DDS[1], Pegah Nasiri, DDS[1], Javad Javidnia, PhD Candidate[2,3], and Mohammad Ebrahimi Saravi, DDS[4]**

## Abstract

**Background and purpose:** *Candida* infections have increased significantly in the antimicrobial resistance era, and synthetic antifungal drugs have limitations. The present work aimed to review the antifungal properties of *Zataria multiflora* (*Z. multiflora*) as an herbal remedy.

**Method:** PubMed, Scopus, ScienceDirect, Web of Science, SID, Civilica, and Magiran databases were searched for the antifungal activity on *in vitro*, *in vivo*, dental biofilm, and clinical studies of *Z. multiflora* on *Candida* species.

**Results:** Overall, 33 articles evaluated the effect of *Z. multiflora* on *Candida* species and classified them into four groups, as follows in vitro (23), dental biofilm (6), in vivo (2), and clinical studies (3). All studies considered *Z. multiflora* effective in reducing or even inhibiting the growth of *Candida* species. NoMFC significant differences were seen in the effect of *Z. multiflora* on susceptible *Candida* compared to the resistant groups of *Candida* in the studies. It was also influential in inhibiting *C. parapsilosis, C. glabrata, C. krusei, C. kefyer,* and *C. zeylanoides*.

**Conclusion:** Considering the side effects and resistance of current antifungal drugs as well as the benefits of using herbal medicines, such as lower cost, less likely to develop drug resistance, the absence of side effects, and toxicity compared with chemical ones**,** it is possible as a powerful alternative to replace or combine with the current antifungal for *Candida* infection therapy along with other therapies.

## Keywords

*Zataria multiflora*, Shirazi Thyme, *Candida*, antifungal properties, essential oil

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## Introduction

*Candida* consists of round, oval yeasts 3 to 30 micrometers in diameter and contains 150 different species. Of these, only a small number are isolated from the human body, which is colonized as an indigenous microbial flora on the mucosal surfaces of the human body.[1] *Candida* species are responsible for approximately 96% of opportunistic fungi; *Candida albicans* is the most common species that causes superficial, mucosal, and systemic infections in humans.[2] The prevalence of infections caused by *Candida* species has increased significantly in the last two decades due to the further spread of immunocompromised diseases, improper use of immunosuppressive drugs, endocrine disorders, malnutrition, and the widespread use of broad-spectrum antibiotics and medical devices, as well as aging.[3,4] Additionally, we are facing an emerging multidrug-resistant *C. auris*, which is causing outbreaks all over the globe.[5] Despite antifungal drugs therapy, morbidity and mortality rates of invasive candidiasis remain high.[6]

[1] Dentistry Student, Student Research Committee, Faculty of Dentistry, Mazandaran University of Medical sciences, Sari, Iran
[2] Student Research Committee Center, Mazandaran University of Medical Sciences, Sari, Iran
[3] Department of Medical Mycology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
[4] Assistant Professor, Department of Prosthodontics, Faculty of Dentistry, Mazandaran University of Medical Sciences, Sari, Iran

**Corresponding Author:**
Mohammad Ebrahimi Saravi, Assistant Professor, Department of Prosthodontics, Faculty of Dentistry, Mazandaran University of Medical Sciences, Sari, Iran.
Email: mohammadebrahimisaravi@gmail.com

There are currently three classes of antifungal drugs available to treat candidiasis: azoles, echinocandins, and polyene, which have limitations such as low susceptibility of some species or strains of *Candida*, resistant species, high cost, toxicity, drug interactions, or lack of oral drug formulation.[7,8] Treatment has become challenging with the emergence of Candida species resistant to common antifungal drugs.[9] Herbal derivatives have attracted much attention in the last decade since researchers believe herbal medicines have much fewer side effects and toxicity than chemical drugs and do not cause drug resistance.[10,11] Therefore, medicinal plants with antimicrobial properties help reduce synthetic substances, side effects, and toxic effects and are more economical.[12]

According to the literature, more than 258 plant species from 94 families have been studied for the survey anti-*Candida* Activity.[13,14] Shirazi Thyme belongs to the mint family (*Lamiaceae* or *Labiatae*) and is also known as *Zataria multiflora* (*Z. multiflora*). *Z. multiflora* grows wild in the central and southern regions of Iran, Afghanistan, and Pakistan.[15] The branches of this plant, collected for medicinal purposes in the early flowering period, contain essential oils, tannins, saponins, and plant disinfectants and are rich in peppermint tannins polymethoxy flavonoids, triterpenes, and polysaccharides. Two critical compounds in *Z. multiflora* essential oil are thymol and carvacrol, both of which are terpenoids. Most of this plant's antimicrobial properties have been attributed to these two substances.[16,17]

Given the above and the fact that so far, no secondary study has been conducted to put together the results of studies to evaluate the antifungal properties of the essential oils extracted from *Z. multiflora* on *Candida* species; In the present systematic study, we aim to classify the results of evidence-based methods of all published documents in a single systematic review.

## Methods

### Study Design

This Systematic review was performed according to the preferred reporting items for systematic reviews (PRISMA) guidelines.[18]

### Search Strategy

In this Systematic review, international electronic databases such as Scopus, PubMed, ScienceDirect, and Web of Science and national databases such as SID, Civilica, and Magiran were searched, restricted to articles in English and Persian, to find the relevant studies. Searches included articles published before 1 August 2021, using different keywords including "*Zataria multiflora*," "Thyme," "Shirazi thyme," "Avishan Shirazi," "Avishan-e-Shirazi," "*Candida*," and "candidiasis." The main MeSH terms were searched alone and combined with the other keywords. Two of the authors conducted the search independently. The third-party evaluated the searched items and hand-searched references of the papers to ensure that no study was missed.

### Inclusion and Exclusion Criteria

Inclusion criteria included studies that reported an association between *Z.multiflora* and *Candida* species, Articles in English and Persian were investigated.

The exclusion criteria included review studies, conference papers, book chapters, and articles and abstracts that did not contain the full text. In addition, articles that were impossible to quality evaluate were excluded from the study.

### Selection of Studies

After completing the search in the second stage, duplicate articles were removed using Endnote software version 20. Then the titles and abstracts of articles were reviewed, and irrelevant items were removed. Finally, the full text of the related articles was reviewed, and unrelated items were removed. Two researchers independently performed the selection of articles. Disagreements were also investigated and resolved by a third party.

### Data Extraction

The Excel software was used in order to extract the data based on the name of the first author, year of study, plant region, *Z. multiflora* chemical components, *Z. multiflora* formulation, Antifungal susceptibility testing method, *Candida* species, and Minimum inhibitory concentration (MIC) index ($MIC_{50}$, $MIC_{90}$), Minimum fungicidal concentration (MFC), from each of the initial studies.

The studies were reviewed based on the type of study and classified into four groups: *in vitro* assay, dental biofilm studies, *in vitro* studies, and clinical studies. All studies carried out to evaluate the antifungal susceptibility of *Z. multiflora* were included in the study.

## Results

### Study Characteristics and Search Results

In the initial systematic literature search in the databases, 2366 articles were found, and after eliminating the duplicates, the amount was reduced to 2248 articles. After reviewing the title and abstract, 2196 articles were excluded due to irrelevance. Finally, after reviewing the full text, 33 articles were selected as relevant articles. The process of article screening and selection is presented in Figure 1.

Finally, 33 articles were selected in this review and classified into four groups that performed antifungal activity of *Z. multiflora* on *Candida* species in different conditions, as follows:

1- *In vitro* studies (23 papers); 2- Dental biofilm studies (6 papers); 3- *In vivo* studies (2 papers); 4- Clinical studies (3 papers). One analysis has been classified in both *in vitro* studies and dental biofilm studies.[19]

A graphic summary of this study is shown in Figure 2.

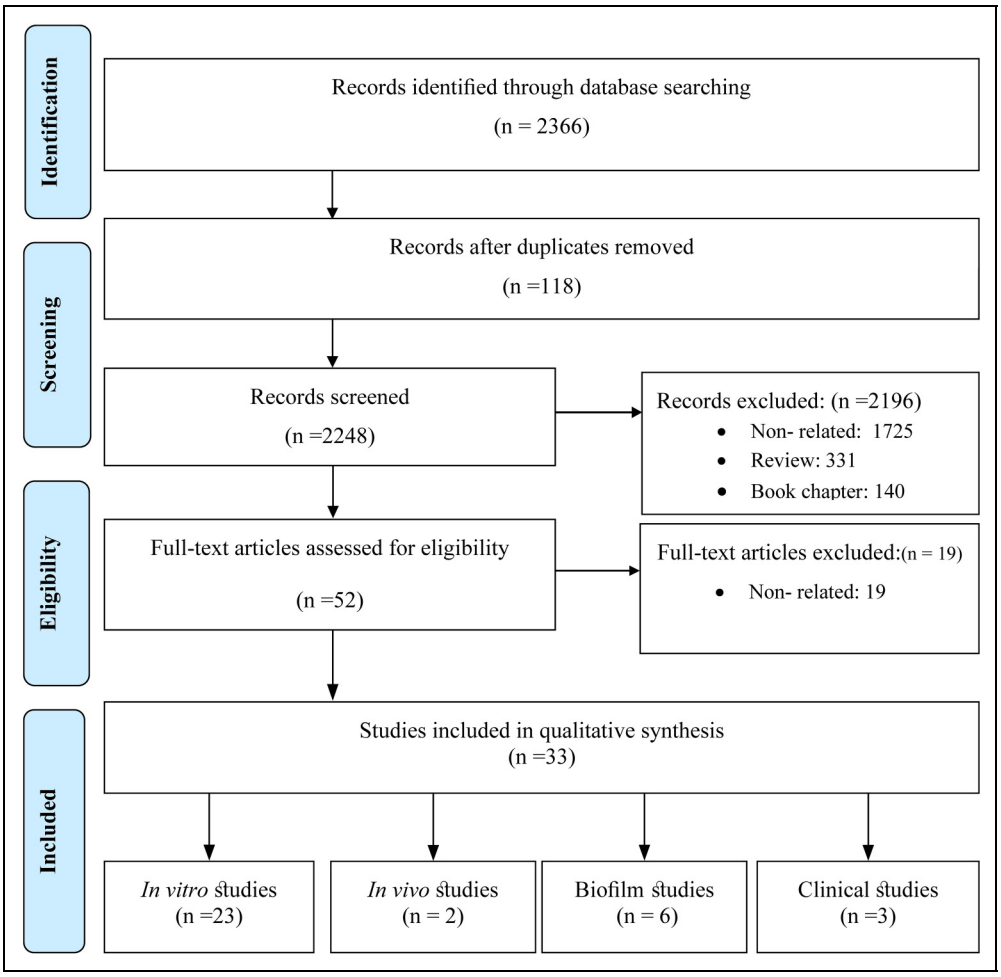


**Figure 1.** PRISMA flow diagram of the detailed process of selection of studies for inclusion in the systematic review.

## *Antifungal Activity of* Z. multiflora *on* Candida *species in* in Vitro *Conditions*

Among the 23 relevant articles[19–41] that surveyed the antifungal effects of Z. multiflora on Candida species, 23 articles reported that Z. multiflora has adverse effects on Candida species. This is demonstrated in Table 1. All studies have generally shown that *Z. multiflora* could reduce or even inhibit the growth of *Candida* species.

Resistant and susceptible species of *C. albicans* were used in two studies.[22,39] Consequently, these results indicated that no significant difference was seen in the effect of *Z. multiflora* on these two groups of *Candida*. For example, in the studies of Katiraiee et al,[22,39] which used two strains (susceptible and resistant) of *C. albicans*, no significant difference was observed in the MICs of these two strains to *Z. multiflora*.

The *Z. multiflora* extracts used in these studies have different formulations. These extracts include aqueous, ethanolic, methanolic, acetonic, ethyl-acetate, and hydroalcoholic extracts.

Among these, in three studies,[20,25,28] antifungal activities were not observed in *Z. multiflora* aqueous extract. However, in contrast to the three studies mentioned above, a study conducted by Rahimi et al[19] examined aqueous and ethanolic extracts and reported that they effectively inhibited the growth of *C. albicans*. In a study by Shokri et al,[29] *C. zeylanoides,* and a study by Esfandiary et al,[36] Clinical isolates of *C. parapsilosis, C. glabrata*, *C. krusei,* and *C. kefyr* species were examined. In both of these studies, *Z. multiflora* effectively inhibited the growth of these non-*albicans Candida* species.

In Moghim, Mahmoudabadi, Naini, Rahimi, and Rezaie Keikhaie studies,[19,20,25,33,40] the ethanolic extract of *Z. multiflora* and Mahmoudabadi, Arbabi- Kalati, Abedini, Nouri, and Rezaie Keikhaie studies,[20,30,37,40,42] the methanolic extract of *Z. multiflora* were examined. According to the MIC index, these extracts were effective in all cases, but the ethanolic extract was more effective than the methanolic extract. Also, the highest amount of MIC for the ethanolic extract was reported in Mahmoudabadi's study.[20]

Eleven studies used *Z. multiflora* essential oil to investigate its antifungal effects,[24,26,28,29,31,34–36,38,39,41] which all studies reported that *Z. multiflora* was effective in reducing the growth of *Candida* species.

In 9 studies, chemical compositions of *Z. multiflora* were analyzed using Gas Chromatography-Mass Spectrometry

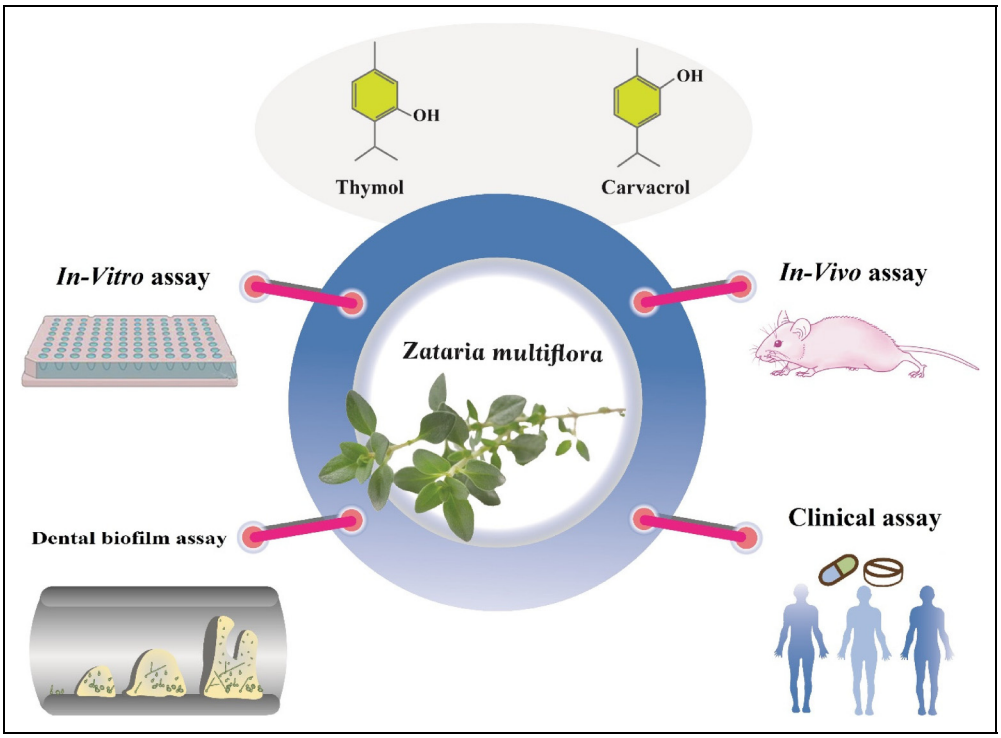


**Figure 2.** Graphic summary of the study on antifungal activity of *Z. multiflora* on *Candida* species.

(GC-MS), which among these, thymol, carvacrol, γ-Terpinene, and linalool had a more critical role in the antifungal properties of *Z. multiflora*.[24,26,28,31,34,35,38,39,41]

## *Antifungal activity of* Z. multiflora *on dental biofilm of* Candida *species derived from dental plaque and intraoral appliances*

6 studies[19,43–47] investigated the antifungal effects of *Z. multiflora* on dental biofilm containing *C. albicans* derived from the acrylic plates, root canals, and mobile orthodontic appliances, all of which were effective in reducing the growth of *C. albicans* species (Table 2). In a study by Jafari,[43] which used nystatin as a control group to compare its antifungal effects with *Z. multiflora*, the results showed that concentrations of 50 and 25 mg/mL of *Z. multiflora* essence effectively (removed 100%) removed *Candida* cells from acrylic plates similar to the control group. A study by Aghili et al,[44] used chlorhexidine mouthwash as a control group to evaluate the antimicrobial effects on the contaminated orthodontic elastomeric ligatures. The findings indicated that both *Z. multiflora* and chlorhexidine effectively eliminated *C. albicans* cells from the orthodontic elastomeric ligatures. Another study that aimed to compare the antifungal activity of *Z. multiflora* with sodium hypochlorite (NaOCl) as a root canal irrigant against *C. albicans* concluded that a 1 mg/ml concentration of *Z. multiflora* and NaOCl showed the highest antifungal efficacy in removing the biofilm of *C. albicans* from the surface of root canals.[45] Aghajani et al[46] compared the antifungal effect of *Z. multiflora* on the surface of acrylic resin dentures with the control groups (sodium hypochlorite, Deconex®) in two time periods (10 and 60 min). They concluded that the control groups disinfectant with higher potency in 10-min intervals compared to *Z. multiflora.* Still, after a 60-min period, *Z. multiflora* displayed similar effects as those of the chemical disinfectants.

Oshagh et al[47] compared the efficacy of 25 mg/ml *Z. multiflora* and 0.12% chlorhexidine in eliminating dental biofilm containing *Candida* from the acrylic baseplates of removable orthodontic appliances. They concluded that a 25 mg/ml concentration of *Z. multiflora* did not show desirable disinfectant properties compared to chlorhexidine. In another study that evaluated the effects of ethanolic and aqueous extracts of *Z. multiflora* on biofilm inhibitions of *C. albicans*, the results showed that both the two extracts significantly inhibit the fungal biofilm formation. Interestingly, the ethanolic extract has more ability (97%) to eliminate Candida biofilms compared to the aqueous extract (87%).[19]

## *Antifungal activity of* Z. multiflora *on* Candida *infection in* in vivo *studies*

Two studies examined the antifungal activity of *Z. multiflora* in animal models (Table 3). Fluconazole and itraconazole were used in the Khosravi[48] and Bayat[21] study as the control group, respectively. The results of both studies showed that *Z. multiflora* could be used as an alternative to antifungal drugs to inhibit the growth of *C. albicans* in mice models.

**Table 1.** Antifungal Activity of *Z. multiflora* on *Candida* species in *in Vitro* Conditions.

| First author. Refs | Plant region | Z. M chemical components (%) | Z.M Formulation | AFST method | Used *Candida* species (no.) | Disk diffusion result Z.M µl/Disk (mm) | Broth microdilution result Z.M MIC ± SD | MFC | $MIC_{50}$ | $MIC_{90}$ | Finding | Part of plant which were used |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Zarei Mahmoudabadi et al, 2007 [20] | Shiraz, Fars province | - | Aqueous | BD | Clinical *C. albicans* (7) | - | No activity | - | - | - | Methanolic extract showed more antifungal activities than ethanolic extract against 14 differnt *Candida* isolates, but Aqueous extract showed no activity against differnt *Candida* isolates. | - |
| | | | Ethanolic | BD | Clinical *C. albicans* (7) | - | 125.1 ± 10.8 mg/L | - | - | - | | |
| | | | Methanolic | BD | Clinical *C. albicans* (7) | - | 75.7 ± 9.5 mg/L | - | - | - | | |
| | | | Aqueous | BD | Clinical *C. tropicalis* (3) | - | No activity | - | - | - | | |
| | | | Ethanolic | BD | Clinical *C. tropicalis* (3) | - | 131.3 ± 5.6 mg/L | - | - | - | | |
| | | | Methanolic | BD | Clinical *C. tropicalis* (3) | - | 76.3 ± 11.9mg/L | - | - | - | | |
| | | | Aqueous | BD | Clinical *C. parapsilosis* (2) | - | No activity | - | - | - | | |
| | | | Ethanolic | BD | Clinical *C. parapsilosis* (2) | - | 125 ± 0.0 mg/L | - | - | - | | |
| | | | Methanolic | BD | Clinical *C. parapsilosis* (2) | - | 64.5 ± 1.5 mg/L | - | - | - | | |
| | | | Aqueous | BD | Clinical *C. glabrata* (2) | - | No activity | - | - | - | | |
| | | | Ethanolic | BD | Clinical *C. glabrata* (2) | - | 126.5 ±3.5 mg/L | - | - | - | | |
| | | | Methanolic | BD | Clinical *C. glabrata* (2) | - | 66.5 ± 3.5 mg/L | - | - | - | | |
| Bayat et al, 2008[21] | - | - | - | BD, DD | Clinical *C. albicans* (1) | 40 mm | 208/231707µg/ml | - | - | - | Z.M showed strongly antifungal activity against *C. albicans*. | - |
| Katiraee et al, 2008[22] | - | - | Essential oil | BD | Clinical Resistance *C. albicans* (16) | - | 0.187 ± 0.023 | - | - | - | Z.M esences has anti-*Candida* activity against azole resistance and azole susceptible *C. albicans* isolates, and they were similar. | - |
| | | | | | Clinical Susceptible *C. albicans* (14) | - | 0.174 ± 0.028 | - | - | - | | |
| Naeini et al, 2009 [23] | - | - | Essential oil | BD and DD | *C. albicans* ATCC 10231 (1) | 30 ml/ Disk (55 mm) | 150 µg/ml | 300 | - | - | The essential oil of Z.M exerted a strong activity in both BD and Disc diffusion tests. | Aerial parts and seeds |
| Saei-Dehkordi et al, 2010 [24] | Hajjiabad, Hormozgane, Iran | Thymol (47.46), p-Cymene (13.16), Carvacrol (9.64), Linalool (7.92), γ-Terpinene(2.72) | Essential oil | BD | *C. albicans* ATCC 10239 (1) | - | 0.5 µg/ml | - | - | - | Z.M from different geographical locations showed considerable antifungal activity against *C. albicans* and *C. tropicalis*. | Aerial parts |
| | | | | | *C. tropicalis* ATCC 13801 (1) | - | 0.125 µg/ml | - | - | - | | |
| | Farashband, Fars Province, Iran | Thymol (46.61), Carvacrol (17.26), p-Cymene (11.51), γ-Terpinene(4.01), Linalool (1.05) | Essential oil | BD | *C. albicans* ATCC 10239 (1) | - | 1 µg/ml | - | - | - | | |
| | | | | | *C. tropicalis* ATCC 13801 (1) | - | 0.5 µg/ml | - | - | - | | |
| | Yazd, Iran | Thymol (40.94), Carvacrol (22.39), p-Cymene (7.73), γ-Terpinene(5.43), Linalool (1.02) | Essential oil | BD | *C. albicans* ATCC 10239 (1) | - | 1 µg/ml | - | - | - | | |
| | | | | | *C.tropicalis* ATCC 13801 (1) | - | 0.5 µg/ml | - | - | - | | |
| | Najafabad, Isfahan Province, Iran | Thymol (64.87), γ-Terpinene(9.11), p-Cymene (5.63), Carvacrol (4.65), Linalool (0) | Essential oil | BD | *C. albicans* ATCC 10239 (1) | - | 0.25 µg/ml | - | - | - | | |
| | | | | | *C. tropicalis* ATCC 13801 (1) | - | 0.062 µg/ml | - | - | - | | |
| | Poldokhtar, Lorestan Province, Iran | Thymol (27.05), p-Cymene (9.49), Linalool (5.63), γ-Terpinene(3.96), Carvacrol (2.70) | Essential oil | BD | *C. albicans* ATCC 10239 (1) | - | 2 µg/ml | - | - | - | | |
| | | | | | *C. tropicalis* ATCC 13801 (1) | - | 0.5 µg/ml | - | - | - | | |
| Naeini et al, 2011[25] | - | - | Aqueous | DD | *C. albicans* ATCC10231 (1) | 0 | - | - | - | - | The essential oils of Z.M showed high strong activity against *C. albicans*., But aqueous essential oils of Z.M dosent showed any antifungal activity against *C. albicans*.. | Fresh aerial parts |
| | | | Ethanolic | DD | *C. albicans* ATCC10231 (1) | 30 | - | - | - | - | | |
| | | | Acetonic | DD | *C. albicans* ATCC10231 (1) | 35 | - | - | - | - | | |
| | | | Essence | DD | *C. albicans* ATCC10231 (1) | 55 | - | - | - | - | | |

*(continued)*

**Table 1.** (continued)

| First author. Refs | Plant region | Z. M chemical components (%) | Z.M Formulation | AFST method | Used *Candida* species (no.) | Disk diffusion result Z.M µl/Disk (mm) | Broth microdilution result Z.M MIC ± SD | MFC | $MIC_{50}$ | $MIC_{90}$ | Finding | Part of plant which were used |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Zomorodian et al, 2011[26] | Lamerd, Fars province, Iran | Thymol (38.88), Carvacrol (27.16), Limonene (15.76), p-Cymene (14.89) | Essential oil | BD | *C. albicans* ATCC 10261 (26) | - | - | 0.052 µL/mL | 0.012 µL/ml | 0.023 µL/mL | High concentration of carvacrol showed better antimicrobial activities. | Aerial parts |
| | | | | | *C. tropicalis* ATCC 750 (3) | - | - | 0.076 µL/mL | 0.023 | 0.047 µL/mL | | |
| | | | | | *C. dubliniensis* (4) | - | - | 0.035 µL/mL | 0.01 | 0.015 µL/mL | | |
| | | | | | *C. glabrata* ATCC 90030 (5) | - | - | 0.035 µL/mL | 0.0075 µL/mL | 0.0098 µL/mL | | |
| | | | | | *C. parapsilosis* ATCC 4344 (5) | - | - | 0.022 µL/Ml | 0.0098 µL/mL | 0.015 | | |
| | | | | | *C. krusei* ATCC 6258 (1) | - | - | 0.06 µL/mL | 0.015 µL/mL | 0.06 µL/mL | | |
| | Darab, Fars province, Iran | Carvacrol (82.7), Caryophyllene oxide (5.36), p-Cymene (4.7), γ-Terpinene(2.19), Thymol (0.1) | Essential oil | BD | *C. albicans* ATCC 10261 (26) | - | - | 0.118 µL/mL | 0.021 µL/mL | 0.049 µL/mL | | |
| | | | | | *C. tropicalis* ATCC 750 (3) | - | - | 0.153 µL/mL | 0.037 µL/mL | 0.075 µL/mL | | |
| | | | | | *C. dubliniensis* (4) | - | - | 0.06 µL/mL | 0.012 µL/mL | 0.03 µL/mL | | |
| | | | | | *C. glabrata* ATCC 90030 (5) | - | - | 0.12 µL/mL | 0.007 µL/mL | 0.015 µL/mL | | |
| | | | | | *C. parapsilosis* ATCC 4344 (5) | - | - | 0.08 µL/mL | 0.011 µL/mL | 0.017 µL/mL | | |
| | | | | | *C. krusei* ATCC 6258 (1) | - | - | 0.06 µL/mL | 0.03 µL/mL | 0.06 µL/mL | | |
| | Zarghan regions, Fars province, Iran | Linalool (87.35), alpha-Pinene (5.10), Thymol (0.0) | Essential oil | BD | *C. albicans* ATCC 10261 (26) | - | - | 0.765 µL/mL | 0.106 µL/mL | 0.29 µL/mL | | |
| | | | | | *C. tropicalis* ATCC 750 (3) | - | - | 1 µL/mL | 0.155 µL/mL | 0.391 µL/mL | | |
| | | | | | *C. dubliniensis* (4) | - | - | 0.84 µL/mL | 0.25 µL/mL | 0.2 µL/mL | | |
| | | | | | *C. glabrata* ATCC 90030 (5) | - | - | 0.287 µL/mL | 0.034 µL/mL | 0.068 µL/mL | | |
| | | | | | *C. parapsilosis* ATCC 4344 (5) | - | - | 0.57 µL/mL | 0.045 µL/mL | 0.091 µL/mL | | |
| | | | | | *C. krusei* ATCC 6258 (1) | - | - | 1 µL/mL | 0.12 µL/mL | 0.5 µL/mL | | |
| Arbabi klati et al, 2012[27] | - | - | Methanolic | DD | *C. albicans* Timm2640 (1) | 13 ± 0.82 | - | - | - | - | Z.M exhibited antifungal activity on *C. albincans.* | - |
| Mahammadi Purfard et al, 2012[28] | Arsenjan, Fars Province, Iran | Carvacrol (29.49), Thymol (25.70), p-Cymene (11.25), Linalool (9.36), γ-Terpinene(8.05) | Aqueous | BD and DD | *C. albicans* ATCC 10231 (1) | 24 µg/ml(0 mm), 50 µg/ml (0), 100 (0) | 0 | - | - | - | At concentration > 5 µg/ml, Z.M essential oil significantly reduced the growth of *C. albicans* by 100%. However, the aqueous extract did not show any activity against *C. albicans.* | Aerial parts |
| | | Carvacrol (29.49), Thymol (25.70), p-Cymene (11.25), Linalool (9.36), γ-Terpinene(8.05) | Essential oil | BD and DD | *C. albicans* ATCC 10231 (1) | 25 µg/ml(14.2 ± 0.84 mm), 50 µg/ml (23.8 ± 0.5), 100 (30 ± 1) | 2.8 ± 0.8 µg/ml | - | - | - | | |
| Shokri et al, 2014[29] | - | - | Essential oil | DD | *C. zeylanoides* (14) | 58.6 ± 2.6 mm | - | - | - | - | All *C. zeylanoides* were susceptible to Z.M essential oil. | - |
| Rahimi et al, 2014[19] | - | - | Aqueous | BD | *C. albicans* ATCC 10231 (1) | - | 1.5 µg/ml | - | - | - | The results showed that the aqueous and ethanolic extracts of Z.M were very strong and significant effect in preventing the growth of *C. albicans.* | - |
| | | | Ethanolic | BD | *C. albicans* ATCC 10231 (1) | - | 0.85 µg/ml | - | - | - | | |
| Abedini et al, 2014[30] | - | - | Methanolic | BD | *C. albicans* ATCC 10286 (1) | - | 312 µL/Ml | ≥1250 µL/mL | - | - | The best antifungal activities against *C. albicans* were obtained by Z.M (MIC < 0 3 µg/ml). | - |

*(continued)*

**Table 1.** (continued)

| First author. Refs | Plant region | Z. M chemical components (%) | Z.M Formulation | AFST method | Used *Candida* species (no.) | Disk diffusion result Z.M µl/Disk (mm) | Broth microdilution result Z.M MIC ± SD | MFC | $MIC_{50}$ | $MIC_{90}$ | Finding | Part of plant which were used |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gavanji et al, 2015[31] | - | Thymol (33.05), Carvacrol (25.88), p-Cymene (11.34), γ-Terpinene(4.73) | Essential oil | DD, BD | *C. albicans* ATCC 10231 (1) | 0.63/1.25/2.5 g/mL (0.0), 5 g/mL (0.17 ± 0.17), 10 g/mL(1.23 ± 0.20), 20 g/mL(1.23 ± 0.20),40 g/mL(1.23 ± 0.20),60 g/mL(1.23 ± 0.20),80 g/mL(1.23 ± 0.20), 100 g/mL(1.23 ± 0.20), 200 g/mL(1.23 ± 0.20), 300 g/mL(1.23 ± 0.20), 400 g/mL(1.23 ± 0.20), 500 g/mL(1.23 ± 0.20) | 34 µg/mL | 64 µg/mL- | - | - | Z.M essential oil had the best performance Z.M had the best MIC and MFC. | Fresh aerial parts |
| Yaghooti Khorasani et al, 2015[32] | - | - | Hydroalcoholic | DD | *C. albicans* ATCC 10231 (1) | 50 mg/l (34.31 ± 0.57), 100 mg/l (38.55 ± 0.72) | - | - | - | - | 100 mg/ml concentration of Z.M was most effective against *C. albicans*. | |
| Moghim et al, 2015[33] | - | - | Ethanolic | BD | *C. albicans* ATCC 10231 (1) | - | 0.13 µg/ml | 1.03 µg/ml, | 0.38 µg/ml, | 0.74 µg/ml | Z.M has the highest antifungal activity against *C. albicans*. | - |
| Zomorodian et al, 2015[34] | Darab, Fars, Iran | Thymol (37.88), Carvacrol (27.16) | Essential oil | BD | *C. albicans* ATCC 10261 (1) | - | 0.062 µg/ml | 0.125 | - | - | Z.M showed the highest antimicrobial activities against *Candida* species. | Aerial parts |
| | | | | | *C. dubliniensis* CBS 8501 (1) | - | 0.062 µg/ml | 0.125 | - | - | | |
| | | | | | *C. glabrata* ATCC 90030 (1) | - | 0.062 µg/ml | 0.125 | - | - | | |
| | | | | | *C. tropicalis* ATCC750 (1) | - | 0.25 µg/ml | 1 | - | - | | |
| | | | | | *C. krusei* ATCC 6258 (1) | - | 0.25 µg/ml | 0.5 | - | - | | |
| Kavoosi et al, 2015[35] | Fars province, Iran | Carvacrol (29.5), Thymol (25.7), p-Cymene (11.3), Linalool (9.4) | Essential oil | BD | *C. albicans* ATCC 10231 (1) | - | 2.8 ± 0.3 µg/ml | - | - | - | Carvacrolrich Z.M exhibited more antifungal activity than thymol-rich ZMO. | Aerial parts |
| | | Linalool (33), Carvacrol (26.7), Thymol (12.5), p-Cymene (4.5) | | BD | *C. albicans* ATCC 10231 (1) | - | 3.3 ± 0.4 µg/ml | - | - | - | | |
| | | Thymol (40.2), γ-Terpinene (38.7), p-Cymene (15.8), Carvacrol (0.73) | | BD | *C. albicans* ATCC 10231 (1) | - | 6.2 ± 0.6 µg/ml | - | - | - | | |
| | | Thymol (45.1), p-Cymene (20.2), γ-Terpinene (11), Carvacrol (3.7) | | BD | *C. albicans* ATCC 10231 (1) | - | 5.9 ± 0.7 µg/ml | - | - | - | | |
| | | Carvacrol (57.4), γ-Terpinene (22.6), α-Terpinene(4.5), Thymol (1) | | BD | *C. albicans* ATCC 10231 (1) | - | 2.8 ± 0.4 µg/ml | - | - | - | | |
| | | Carvacrol (52.3), γ-Terpinene (12.4), p-Cymene (4.3), Thymol (3.4) | | BD | *C. albicans* ATCC 10231 (1) | - | 2.5 ± 0.5 µg/ml | - | - | - | | |
| Esfandiary et al, 2015[36] | - | - | Essential oil | BD | Clinical *C. glabrata* (29) | - | 76747.73 µg/ml | - | 69750 µg/ml | 139500 µg/ml | Z.M has a favorable antifungal effect against non-*albicans Candida* | - |
| | | | | | Clinical *C. kefyer* (10) | - | 69750 µg/ml | - | 69750 µg/ml | 69750 µg/ml | | |

*(continued)*

**Table 1.** (continued)

| First author. Refs | Plant region | Z. M chemical components (%) | Z.M Formulation | AFST method | Used *Candida* species (no.) | Disk diffusion result Z.M µl/Disk (mm) | Broth microdilution result Z.M MIC ± SD | MFC | $MIC_{50}$ | $MIC_{90}$ | Finding | Part of plant which were used |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Clinical *C. krusei* (3) | - | 69750 µg/ml | - | 69750 µg/ml | 69750 µg/ml | species, despite having a | |
| | | | | | Clinical *C. parapsilosis* (2) | - | 69750 µg/ml | - | 69750 µg/ml | 69750 µg/ml | wide range of MICs (34875-139500 µg/ml). | |
| Nouri et al, 2016[37] | - | - | Methanolic | DD | *C. albicans* ATCC 10231 (1) | 2.5 µg/ml (13.25 ± 0.43 mm) | 13.25 ± 0.43 | - | - | - | The result showed extracts of Z.M have antifungal | - |
| | | | | | Clinical *C. albicans* (21) | 2.5 µg/ml (12.34 ± 0.46 mm) | 13.25 ± 0.43 | - | - | - | significant effects. | |
| Mahboubi et al, 2017[38] | Shahrood,Semnan Province, Iran | Carvacrol (34.30), Thymol (25.80), P-Cymene (5.67), γ-Terpinene (4.59), α-Pinene (3.13), Linalool (3.12) | Essential oil | BD and DD | *C. albicans* ATCC 710231 (1) | 0.75µL/mL (10.19 ± 1.30 mm), 1µL/mL (20.12 ± 1.28 mm) | 0.12 ± 0.18 µL/mL | 0.17 ± 0.60 µL/mL | - | - | Z.M showed antifungal activity against *C. albicans*. | Aerial parts |
| | Haji Abad, Fars Province, Iran | Thymol (41.16), Carvacrol methyl ether (28.32), Linalool (6.52), α-Terpineol (3.69), Carvacrol (1.50), | Essential oil | BD and DD | *C. albicans* ATCC 710231 (1) | 0.75µL/mL (9.28 ± 1.28 mm), 1µL/mL (21.64 ± 1.28 mm) | 0.12 ± 0.18 µL/mL | 0.17 ± 0.60µL/mL | - | - | | |
| | Jahrom, Fars Province, Iran | Thymol (29.40), Carvacrol (23.90), P-Cymene (12.10), γ-Terpinene (6.50), α-Terpinene (5.10), α-Pinene (4.36), Linalool (1.3) | Essential oil | BD and DD | *C. albicans* ATCC 710231 (1) | 0.75µL/mL (16.38 ± 1.30 mm), 1µL/mL (38.60 ± 1.28 mm) | 0.11 ± 0.18 µL/mL | 0.20 ± 0.60 µL/mL | - | - | | |
| Katiraee et al, 2017[39] | - | Thymol, Carvacrol, γ-Terpinene, p-Cymene, Linalool | Essential oil | BD | Clinical Susceptible *C. albicans* (20) | - | 0.156 µg/ml | - | 0.15 | 0.2 | Z.M essential oils showed high antifungal activity. | - |
| | | | | | Clinical Resistance *C. albicans* (20) | - | 0.161 µg/ml | - | 0.15 | 0.175 | Furthermore, there were no significant differences in the MICs of Z.M against the -azoles susceptible and -azoles resistant *C. albicans* isolates. | |
| Rezaie Keikhaie et al, 2018[40] | Zabol, Iran | - | Ethanolic | BD | Clinical *C. albicans* (12) | - | 50 mg/L | 100 mg/L | 50 mg/L | 200 mg/L | The results of this study showed that the extract | Leaves |
| | | | Methanolic | BD | Clinical *C. albicans* (12) | - | 100 mg/L | 200 mg/L | 100 mg/L | 400 mg/L | with different solvents inhibited the growth of | |
| | | | Chloroform | BD | Clinical *C. albicans* (12) | - | 50 mg/L | 100 mg/L | 50 mg/L | 100 mg/L | the fungus, however, different solvents in | |
| | | | Ethyl acetate | BD | Clinical *C. albicans* (12) | - | 25 mg/L | 50 mg/L | 25 mg/L | 50 mg/L | different concentrations inhibited fungal growth | |
| Niczad et al, 2019[41] | Estahban, Fars province, Iran | Thymol(45.85), γ-Terpinene(16.70), p-Cymene (9.49), Carvacrol(5.35) | Essential oil | DD and BD | *C. albicans* ATCC10231 (1) | 20 (80), 10(73), 5(64), 2.5(54), 1.25(42), 0.63(31), 0.32(22), 0.16(13) | 0.16 µl/ml | 0.16 µl/ml | - | - | The *Z. multiflora* essential oils obtained from different geographical location showed high | Fresh aerial parts |
| | Neyriz, Fars province, Iran | Thymol(54.35),p-Cymene (10.81), γ-Terpinene(8.33), Carvacrol(7.04) | Essential oil | DD and BD | *C. albicans* ATCC10231 (1) | 20 (80), 10(77), 5(69), 2.5(59), 1.25(42), 0.63(31), 0.32(20), 0.16(12) | 0.16 µl/ml | 0.16 µl/ml | - | - | antifungal activity against *C. albicans*. | |
| | Fasa, Fars province, Iran | Thymol(34.41), p-Cymene (17.11), γ-Terpinene(16.45), Carvacrol(6.06) | Essential oil | DD and BD | *C. albicans* ATCC10231 (1) | 20 (79), 10(69), 5(61), 2.5(51), 1.25(41), 0.63(29), 0.32(19), 0.16(9) | 0.16 µl/ml | 0.16 µl/ml | - | - | | |
| | Larestan, Fars province, Iran | Thymol(38.45), p-Cymene (19.85), Carvacrol(15.34), γ-Terpinene(7.34) | Essential oil | DD and BD | *C. albicans* ATCC10231 (1) | 20 (80), 10(71), 5(58), 2.5(47), 1.25(37), 0.63(24), 0.32(18), 0.16(7) | 0.16 µl/ml | 0.16 µl/ml | - | - | | |

.ZM; *Z. multiflora*, AFST; Antifungal susceptibility testing, BD; Broth microdilution, DD; Disk diffusion, MFC; Minimum Fungicidal Concentration, MIC; Minimum inhibitory concentration.

**Table 2.** Antifungal Activity of *Z. multiflora* on Biofilm of *Candida* species Derived from Dental Plaque and Intraoral Appliances.

| First author | Z. M Formulation | Application type (no.) | Fungal species (no.) | Control Agent | | *Zataria multiflora* | | Finding | Part of plant which were used |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Drug control | Removing Ability % | Concentration | Removing Ability % | | |
| Oshagh et al, 2014 [47] | Essential oil | Rremovable orthodontic appliance (20) | ND* | 0.12% Chlorhexidine | - | 25 mg/ml | - | Z.M** with the concentration and time used in this study cannot be a good alternative for chlorhexidine. | - |
| Rahimi et al, 2014 [19] | Aqueous | - | *C. albicans* ATCC 10231 (20) | - | - | 0.080, 0.10, 0.21, 0.42, 0.85, 1.50, 3.50, 6.25, 12.50, and 25 mg/ml | 87% | The ethanolic extract of Z.M was able to decrease 97% of growth, while the aqueous extracts was only 87%. | - |
| | Ethanolic | - | *C. albicans* ATCC 10231 (20) | - | - | 0.080, 0.10, 0.21, 0.42, 0.85, 1.50, 3.50, 6.25, 12.50, and 25 mg/ml | 97% | | |
| Aghili et al, 2015 [44] | 0.5 mg/ml Z.M mouthwashes | Resin acryl plates (Iranian ligature rings) (31) | *C. albicans* ATCC 10231 (1) | 0.2% Chlorhexidine mouthwash | 100 | 0.5 mg/ml | 100 | Z.M and chlorhexidine mouthwash were effective equally in completely eliminating the number of *C. albicans* cells from the orthodontic elastomeric ligatures. | - |
| | | Resin acryl plates (Ortho-technology ligature ring) (31) | *C. albicans* ATCC 10231 (1) | 0.2% Chlorhexidine mouthwash | 100 | 0.5 mg/ml | 100 | | |
| Jafari et al, 2015 [43] | Essence | Resin acryl plates (20) | *C. albicans* ATCC 10231 (1) | Nystatin | 100 | 50 mg/mL | 100 | Concentrations of 50 and 25 mg/ml of Z. M essence removed 100% of attached fungal cells similar to nystatin, while weaker Z.M removed 88%, 60.5% and 44.7% of attached fungal cells. | - |
| | | Resin acryl plates (20) | *C. albicans* ATCC 10231 (1) | Nystatin | 100 | 25 mg/mL | 100 | | |
| | | Resin acryl plates (20) | *C. albicans* ATCC 10231 (1) | Nystatin | 100 | 25 mg/mL | 90 | | |
| | | Resin acryl plates (20) | *C. albicans* ATCC 10231 (1) | Nystatin | 100 | 6.25 mg/mL | 60.5 | | |
| | | Resin acryl plates (20) | *C. albicans* ATCC 10231 (1) | Nystatin | 100 | 3.125 mg/mL | 44.7 | | |
| Sedigh-Shams et al, 2016 [45] | Ethanolic | Single-canal mandibular premolars (60) | Clinical *C. albicans* (1) | 5% NaOCl (3 mg/ml) | 100 | 0.5 mg/ml | 99.7 | Z.M has the potential to be used as a root canal irrigant. | - |
| | | | | 5% NaOCl (3 mg/ml) | 100 | 1 mg/ml | 100 | | |
| Aghajani et al, 2019 [46] | Essential oil | Resin acrylic dentures (32) | *C. albicans* (1) | Deconex, NaOCl | - | 0.1 | - | Chemical disinfectants with higher potency in 10-min intervals, compared to Z.M, But, after a 60 min, Z.M display similar effects as those of the chemical disinfectants. | - |
| | | | | Deconex, NaOCl | - | 0.01 | - | | |
| | | | | Deconex, NaOCl | - | 0.001 | - | | |
| | | | | Deconex, NaOCl | - | 0.001 | - | | |

*ND; Not Determine, ** Z.M; *Z. multiflora.*

**Table 3.** Effects of *Z. multiflora* on *Candida* Infection in *in Vivo* Studies.

| Study | Z.M Formulation | Type of Intervention | *Candida* species | BALB /C mice groups (no.) | | | Finding | Part of plant which were used |
|---|---|---|---|---|---|---|---|---|
| | | | | Total | Z.M* | Control | | |
| Khosravi et al, 2009[48] | Essential oil | Disseminated candidiasis (40) | *C. albicans* ATCC10261 | 40 | 32 | Itraconazole (8) | These data explain the increased rate of yeast clearance and reduced dissemination to the viscera of Z.M treated mice. | Air-dried aerial parts |
| Bayat et al, 2018[21] | Chloroform fraction of an ethanolic extract | Visceral candidiasis (42) | *C. albicans* ATCC10231 | 42 | 18 | Fluconazole (6) | The fraction of Z.M can be considered as a powerful alternative to *C. albicans* therapy along with other therapies. | Aerial parts |

* Z.M; *Z. multiflora*.

The results demonstrated that the fraction of *Z. multiflora* could be considered a powerful alternative to C. albicans therapy and other therapies.

### *Antifungal activity of* Z. multiflora *on* Candida *infections in clinical studies*

Three clinical studies were performed on the effects of *Z. multiflora* on *Candida* infections.[49–51] The result of these clinical studies is summarized in Table 4. Two studies with a total sample size of 80 concluded that 0.1% *Z. multiflora* vaginal cream was effective in treating vaginal candidiasis and significantly diminished the clinical signs and symptoms of vaginal candidiasis compared to the same control group (clotrimazole 1% cream).[50,51] Overall, Khosravi et al[50] and Fouladi et al[51] suggested that *Z. multiflora* cream can be considered a good antifungal agent in treating vaginal candidiasis.

In a study by Amanlou et al,[49] the effects of *Z. multiflora* were investigated to treat *Candida*-associated denture stomatitis. This study showed that *Z. multiflora* gel reduces the number of fungal colonies and mucosal erythema more effectively than the control group, a miconazole 2% gel.

## Discussion

The present systematic review evaluated the antifungal properties of various extracts and essential oils (EO) of Z. multiflora against different Candida species. It was shown that *Z. multiflora* inhibits the germination of *Candida* species, which leads to the deformation and destruction of these cells. Also, some studies reported that based on the MIC index, the antifungal properties of ethanolic extract were more significant than the methanolic extract, but due to the presence of various compounds in *Z. multiflora*, variant harvest locations, and preparation methods, this result cannot be generally considered. In some studies, resistant and susceptible species of *Candida* were used to examine the antifungal properties of *Z. multiflora*, but no significant changes were seen in the effect of *Z. multiflora* when it was used against either resistant or susceptible species of *Candida*. Generally, *Z. multiflora* has been an effective alternative in eliminating *Candida* rather than chemical antifungal drugs. This finding suggests that the antifungal properties of *Z. multiflora* essential oils are independent of changes associated with resistance to azole drugs.[52] In general, in order to eliminate *Candida* colonies, *Z. multiflora* is a more suitable alternative than chemical drugs.[43]

However, Sharifzadeh et al reported that FLU-susceptible species were more susceptible to essential oils.[53] Essential oil is a volatile, natural aromatic oil obtained from various parts of the plant. Mainly, the biological activity of EOs, such as antibacterial, antiviral, anti-inflammatory, antifungal, antimutagenic, anti-carcinogenic, antioxidant, and other activities, has been attributed to its compounds.[54] The main issue associated with using EOs is their instability to light, air, moisture, and volatility, which can easily lead to evaporation and reduce their efficiency.[55] Phenolic compounds such as carvacrol, thymol, and eugenol are the main components of *Z. multiflora* essential oil, which are generally stored in young leaves during plant growth.[56] Studies have shown that thymol, carvacrol, and γ-Terpinene are the main components for inhibiting *Candida* species. Differences in EO compositions may be due to differences in concentrations and factors such as the plant species, solvents, raw materials used to prepare the EO (dried / fresh *Z. multiflora*), the soil in which the plant grows, the time of harvesting and extraction techniques, which all can affect the antifungal power of the EO.[57] It should also be noted that higher altitudes of areas where the plant is collected reduce carvacrol and increases the amount of thymol.[58] Therefore, the difference in these two compounds also

**Table 4.** Effects of *Z. multiflora* on *Candida* Infections in Clinical Studies.

| Study | Type of *Candida* disease | Patients' groups (no.) | | | Outcome | Part of plant which were used |
|---|---|---|---|---|---|---|
| | | Total | Control | Z. M * | | |
| Amanlou et al, 2006[49] | *Candida*-associated denture stomatitis | *Candida*-associated denture stomatitis (24) | Miconazole 2% Gel (12) | 0.1% gel (12) | Z. M gel reduced the surface erythema of the palate more efficiently than miconazole gel but did not reduce the colony count of the denture surface as efficiently as miconazole. | - |
| Khosravi et al, 2008 [50] | Vaginal candidiasis | Acute vaginal candidiasis (86) | 1% Clotrimazole cream (31) | 0.1% vaginal cream (30) | The rates of improvement in the Z.M and clotrimazole groups were found to be about 90.0% and 74.8%, respectively. | - |
| Fouladi et al, 2009[51] | Vaginal candidiasis | Vaginal *Candida*cies (73) | 1% Clotrimazole cream (38) | 0.1% vaginal cream (35) | Z.M cream 1% (Cure: 54.3%), Clotrimazole vaginal cream (Cure: 47.4%) | - |

* Z.M; Z. *multiflora*.

depends on ecological and geographical factors.[59] This can be considered an explanation for the difference in the number of compounds in the EO of *Z. multiflora* in different studies. Therefore, in a study by Niczad et al,[41] despite the different compositions of *Z. multiflora* harvested from different Fars province cities, no difference was seen in the amount of their antifungal effects. Kavoosi's study showed that the higher amounts of thymol result in a more significant antifungal effect and a higher MIC.[35] In a study by Zomorodian et al, *Z. multiflora* species gathered from Darab, Fars province, had higher carvacrol levels and had more significant antifungal effects based on MIC than species collected from Lamerd and Zarghan.[26]

In Saei-Dehkordi 's study,[24] *Z. multiflora* gathered from Najafabad, Isfahan had the highest amount of thymol compared to *Z. multiflora* species in the present study. Still, its antifungal effects on *C. albicans* based on MIC index were less than other types of *Z. multiflora* in this study. Also, *Z. multiflora* species gathered from Poldokhtar Laarestan had the lowest amounts of thymol and carvacrol compared to other areas but had the most antifungal effects based on the MIC index.

Suppose a lower concentration of Z. multiflora essential oil can exert its antifungal properties. In that case, it may be due to the resistance of microorganisms or differences in the effective composition of the Eos.[31] Monoterpenes, such as thymol, found in *Z. multiflora* EO, are derived from isoprene hydrocarbure (2-methyl-1,3-butadiene) by binding two or more isoprene molecules. Studies have shown that thymol can affect the structure and electrostatic surface of cell membranes and lead to asymmetric membrane tension.[60] The ability to disrupt the adhesion of *C. albicans* has also been attributed to terpenes. They also inhibit the cellular respiration of *Candida*, which has destructive effects on the mitochondria.[61] According to Zia et al's study, if the concentration of *C. albicans* suspension decreases, the number of colonies growing in the culture medium will also decrease by reducing the concentration of *Z. multiflora* extract in the culture medium, *C. albicans* colonies will increase.[62]

Scanning and transmission electron microscopy have explained the mechanism of action of *Z. multiflora* compounds as follows: at a concentration of 50 ppm of *Z. multiflora* essential oil, the integrity and uniformity of the fungal cell wall is destroyed in some areas. In some areas, the cell membrane separates from the cell wall and invaginates into the cytoplasm.[56,63]

Loss of density and vacuolization also occur at this concentration. At a concentration of 100 ppm, a greater degree of destruction occurs along with a detachment of cell membranes from the cell wall, which have collapsed and undergone herniation at various intervals, leading to mycelia deformation. At 150 ppm, however, the damage is caused by depletion of the cytoplasmic content, which causes the mycelia to appear electron-lucent. Detachment and fragmentation of the plasma membrane and the formation of lysosomes (small vesicles attached to the membrane) could also be observed beneath the cell wall, and membrane fragments spread throughout the cytoplasm. Also, swelling and deformation of mycelia are other changes observed in this concentration. Also, at a concentration of 200 ppm, the germination of *Candida* is effectively inhibited, and no mycelia are formed at this concentration.[63]

Some *Candida* cells have a standard shape at this concentration, but others show changes in the range from vacuolization to cell membrane detachment and deformation; therefore, by increasing EO concentration up to 400 ppm, further degradation, including the destruction of the plasma membrane and the cell wall, are evident. Hyphae are entirely free to enter the cytoplasm.

Usually, mycelia have a homogeneous and dense cytoplasm, and their intracellular septa are completely intact. Also, hyphae enclosed by a cell wall have a fully integrated structure, and the plasma membrane is wholly attached to the cell wall and is smooth and Non-wrinkled.[64,65] On the other hand, the starting point for neutralizing the invasive state of *C. albicans* is the reduction of adhesion and its dimorphic transition to a filamentous state, both of which are significant factors in the pathogenesis of candidiasis.[61]

Although the antifungal mechanism of thymol or carvacrol is still unclear, their antifungal activity is likely due to the lipophilicity of thymol and eugenol, which can co-occur with carvacrol in the fatty acid chain of cell membranes and impair cell membrane fluidity and permeability.[66] This process, especially in *C. albicans*, affects the regulation and function of essential enzymes that bind to membranes and catalyze the synthesis of cell wall polysaccharide compounds (such as beta-glucans, chitin, and mannan) and eventually disrupts cell growth and envelope morphogenesis.[67] According to Braga et al, During envelope morphogenesis, thymol alone exerts more destructive effects than eugenol, which may be related to the degree of infiltration of monoterpenes into the fungal cell wall and cell membrane structure.[67] Thymol and eugenol have different molecular properties; These differences include molecular volume, surface area, polarity, hydrophilic/lipophilic balance, percentage of hydrophilic surface area, hydrogen bond capacity, and hydrogen bond acceptor capacity. Therefore, the difference between these two compounds is different in terms of strength and their mechanism of action, which can explain the synergistic effect of thymol and eugenol in combination with each other. Disorder in envelope morphogenesis is also functional, leading to dysfunction in important pathogenic factors such as adhesion.[67]

Other mechanisms theoretically cause damage to the cell wall and membrane along with rupture due to morphological deformation, the spread of this rupture, collapse, and damage to fungal filaments[68]; Therefore, *Z. multiflora* essential oil leads to irreversible destruction of the cell wall, cell membrane and cell organs of the fungal microorganism, which affects its growth and morphology[69] and the resulting essential oil can suppress fungal colony size and sporulation.[63]

The evidence obtained from this systematic review can guide researchers in discovering novel antifungal agents from *Z. multiflora*.

## Conclusion

Considering the side effects and resistance of current antifungal drugs as well as the benefits of using herbal medicines, such as lower cost, less likely to develop drug resistance, the absence of side effects, and toxicity compared with chemical ones**,** it is possible as a powerful alternative to replace or combine with the current antifungal for *Candida* infection therapy along with other therapies. Therefore, the data obtained in this systematic review suggests *Z. multiflora* is a suitable anti-*Candida* drug and disinfectant for controlling *Candida* infections.

## Limitations and Suggestions

This systematic review highlighted that the reviewed studies lack consensus and standardization of MIC, $MIC_{50}$, and $MIC_{90}$ values for defining antifungal susceptibility testing and biofilm assay for *Candida* species. Since different extracts or essential oils were evaluated in the studies extracted from different regions, the number of differences in chemical compounds in the extracted plant extracts or essential oil was affected by this topic. Thus, it is impossible to report a single concentration or MIC to be more effective. Furthermore, each researcher determines his/her own measurement scale for what could be considered as a significant inhibition zone or MIC and what could not.

Moreover, the difference in *Z. multiflora* concentrations, plant region, formulation, incubation time, incubation temperatures, and duration of treatment are not equivalent in these studies, which will significantly affect the results and outcomes of studies and make it hard to compare.

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### Authors' contributions

AMS., N.E.G. and J.J. collected the data, M.E.S. and J.J. performed the statistical analyses, interpreted data, and drafted and revised the manuscript for important intellectual content.
AMS., P.N. and N.E.G. reviewed the analyses and the final version of the manuscript. M.E.S., J.J. and AMS. interpreted data, revised the manuscript for important intellectual content, and approved the final version.
All authors have read and approved the manuscript.

### Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Consent to participate and publish

Not applicable.

### Declaration of Conflicting Interests

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### ORCID iDs

Pegah Nasiri https://orcid.org/0000-0002-0968-2584
Javad Javidnia https://orcid.org/0000-0001-9336-2518

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