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Original Article

Anti-fungal effects of Essential Oil and Nano-emulsion of some Medicinal Plants on the Human Pathogenic Fungus *Candida albicans*

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Abstract

Interest in essential oils is continuously increasing due to their biological activities in various fields, from pharmaceuticals to food and agriculture. Essential oil is unstable and hydrophobic under normal storage conditions. Thus, it can quickly lose its anti-fungal and antibacterial activity. The new method of nano-emulsion production has been proposed as an effective solution to increase their stability and activity. In this study, the nano-emulsions of *Myrthus communis*, *Rosmarinus officinalis*, and *Eryngium campestre* essential oils were formulated using different proportions and evaluated on *Candida albicans*. Nano-emulsions were prepared using an ultrasonic method, and the field emission scanning electron microscopy (FESEM) apparatus determined the size of the prepared nano-emulsion particles. The dilution method determined the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC). Also, the bioassay of plant compounds was investigated using the agar well diffusion method. Mean droplet size of *M. communis*, *R. officinalis*, and *E. campestre* nanoparticles were reported in 200-500, 100-200, and 200-300 nm, respectively. The lowest MIC value was obtained for the *M. communis* (12.5) and *R. officinalis* (12.5) nano-emulsion. The activity of the essential oil and nano-emulsion of the tested plants against *C. albicans* was confirmed with an inhibition zone diameter of 3.9-27.5 mm. Examining the time growth curve of *C. albicans* showed that nano-emulsions were significantly more effective on the fungal pathogen than essential oils. Results show that using nano-emulsions increases the antimicrobial properties of essential oils from medicinal plants.

Keywords: Anti-fungal; *Candida albicans*; Essential oil; Medicinal plants; Nano-emulsion; Pharmaceuticals.

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1. Introduction

Essential oils extracted from plants are a mixture of several efficient biosynthetic chemicals [1]. In recent years, attention to essential oil as an anti-fungal and insecticide has been shown to increase interestingly [2, 3]. Ecofriendly nature and environmentally safety,

are the most prominent and attractive properties of essential oils, so they generally recognized as safe status [4]. Because of the increase in drug resistance in microorganisms, one of the appropriate solutions is to replace essential oils with no side effects [5], and due to their unique compounds, they can resist a wide range of microbes, prokaryotes, and insects [6]. Numerous studies have been performed on the antimicrobial properties of these compounds and resulted in successful control of the pathogens [7-10].

Despite these advantages, several difficulties are associated with using essential oils due to their physical and chemical stability, thermal decomposition, and low solubility in water [11]. One of the proposed approaches to achieve purpose is using nano-emulsions, which are used to design and produce lipid transport systems [12]. Nano-emulsions are a system with thermodynamics and kinetic stability [13] and have special features such as easy preparation, simple composition, feasibility of production on an industrial scale, and low production cost [14, 15]. Due to the properties of nano-emulsion as tiny droplets, large areas, stable diffusion over time, high activity, and stability agents sediment, nano-emulsion of essential oils act as biologically safe, biodegradable, and environmentally compatible compounds [16, 17].

Candida species are the most prominent cause of fungal infections in humans, including mucosal, invasive, and fatal infections [18]. *C. albicans* is the most common cause of candidiasis infection among *Candida* species [19]. Due to the significant consequences of *Candida* spp. in human pathogenesis, principally in the appearance of anti-fungal resistance, the research conducted with different structures of essential oil is fascinating. It opens new directions for the design of new particles with more effective antimicrobial activity [20]. Recently, the effect of medical plants on the control of this pathogen has been investigated. Studies have shown that essential oils of lemongrass (*Cimbopogon citratus*) and kaffir lime (*Citrus hystrix*) have anti-fungal activity against *C. albicans*. The well-known compounds of limonene, α-terpineol, citronellyl acetate, terpineol, and β-pinene in the kaffir lime essential oil and citronella in lemongrass have been reported to inhibit the growth of *C. albicans* significantly. Therefore, combining these two essential oils has been recommended as an alternative trade for oral candidiasis [21]. These famous anti-fungal compounds could also restrict *C. albicans* biofilm development, such as those extracted from *Ocimum americanum* [22] and *Coriandrum sativum* [23], confirmed an unusual activity as biofilm inhibitors.

Also based on studies, the essential oils of *Thymus eriocalyx* and *T. hotschyanus* with the minimum inhibitory concentration of 4.2 and 3.3 µl/ml, respectively [24], and essential oil of Mint and Eucalyptus with the minimum fungicidal concentration of 0.15% and 0.12 µl/ml respectively [25], have an appropriate inhibitory effect on the growth of *C. albicans*. In addition, cinnamon essential oil can inhibit at least 50% of the mature biofilm protected by *C. albicans, C. parapsilosis,* and *C. glabrata* at a concentration of 500 µl/ml [26]. Nanoparticles of *Rosmarinus officinalis* essential oil could intensely inhibit the biofilm

development of *C. albicans*, and *C. tropicalis* [27]. Wu et al. (2016) also reported inhibiting biofilm development in *Penicillium digitatum* due to *Myrthus communis* active compounds [28].

However, the anti-fungal properties of nano-emulsion have been fewer studies in the control of *C. albicans*. According to the *in vitro* studies, nano-emulsion containing essential oil of clove [29] and green tea [30] showed good growth inhibition on this pathogenic fungus. Also, the effectiveness of clove essential oil nano-emulsion compared to its essential oil on *C. albicans* has been confirmed [31]. Due to the need to produce new anti-fungal agents that have the most remarkable effectiveness in inhibiting *C. albicans* and the most negligible cytotoxicity and side effects, the present study aimed to investigate the anti-fungal performance and inhibitory effect of nano-emulsions of some medicinal plants such as *Eryngium campestre*, *Myrtus communis*, and *Rosmarinus officinalis*.

2. Materials and Methods

2.1. Plants and fungal samples

In the flowering stage, the aerial parts of *E. campestre*, *M. communis*, and *R. officinalis* plants were collected from natural sites in Golestan province, northern Iran. They were identified and confirmed by the Biology Department of Gonbad Kavous University. One pathogenic isolate of *C. albicans* (A90029) was obtained from clinical specimens in the reference culture collection of the Dental Department of Hamadan University of Medical Sciences (UMSHA) and preserved on slant Sabouraud Dextrose Agar (SDA) at 4°C until further use. The fungal strain was identified by conventional and molecular methods by the UMSHA before.

2.2. Essential oils (Eo) preparation

The plant samples were washed with distilled water and air dried in a dark place at 25°C. Extraction of essential oil was performed using Clevenger for four hours. The essential oils were diluted at 1:1, sterilized through 0.22 µm microbiology filters, and stored in a sterile dark container at 4°C.

2.3. Analysis of the chemical composition of the essential oils

Chemical analysis was carried out by gas chromatography (GC) coupled with a mass spectrometer detector (GC-MS) (Agilent 5973, USA) with the capillary column of HP-5MS (column length: 30 meters, internal diameter: 0.25 mm and film thickness: 0.25 µm). This procedure set the split ratio to 1:50; mass spectra were recorded at 70 eV. Five µL of each essential oil was diluted with 500 µL of hexane, and 5 µL of diluted solution was injected. The peak area of spectrograms and their mass spectra were verified and compared to the Wiley mass spectral library information.

2.4. Nano-emulsion (NEo) preparation and determination of particle size

Homogenizer was used to generate nano-emulsions, using extracted essential oils (10% weight/volume) and Trriton-x-100 as a surfactant in distilled deionized water. The aqueous phase (distilled water) was acidified

with citric acid (3.0%) and stirred at 3000 rpm for 15 minutes (MEGA Kavosh 50000111, Iran). The essential oils and surfactant were mixed, and water was added to prepare a stubbly emulsion. The prepared emulsion was subjected to sonication using an ultrasonic sonicator (Elma, S30H, Germany) at 40 KHz and a power output of 100 W [32]. Sonicator reduces the droplet diameter of the emulsion by creating severe disrupting forces. The particle size of prepared nano-emulsions was determined by field emission scanning electron microscopy (FESEM) (Tescan, MiraIII-Republic Czech) apparatus.

2.5. Anti-fungal effects of Eo and NEo using disk diffusion method

To study the anti-fungal properties of prepared Eos and NEos, the agar disk diffusion method was used in different concentrations (1, 0.5, 0.25, and 0.125 v/v %) of *E. campestre*, *M. communis*, and *R. officinalis* essential oils and nano-emulsion essential oils. The fungal initial inoculum was prepared as described [33] using seven-day-old culture of the fungus in SDA and adjusted to 0.5 McFarland standard (equivalent to 10^6 cells/ml) at 530 nm using a spectrophotometer (Model 6300 Jenway UK). The fungal suspension was cultured in an SDA medium, and then sterile paper discs (Whatman No. 1) containing 20 µl of Eo or NEo of the test plants were added to the media surface. The plates were incubated for 24 hours at 37°C, and the diameter of the inhibition zone of *C. albicans* was measured compared to negative control (DMSO 10 µl) and positive control (Fluconazole 10% and Nystatin 5%). The test was performed in a complete randomized design with three replications and was analyzed using SAS 9.1 statistical software. For means comparison, Tukey ($p<0.01$) test was used.

2.6. Determination of MIC and MFC

The microdilution method in broth media assessed the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC). Tested Eo and NEo were diluted by Dimethyl sulfoxide (DMSO) in concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.125, and 1.562 (mg/L) and subsequently were inoculated with 20 µl of fungal suspension (10^6 cells/ml). The test tubes were incubated at 37° C for 24 hours. MIC was the lowest concentration of essential oil, which the fungus could not grow on and had no turbidity, and the minimum Eo concentration with no growth after plating on agar medium was mentioned as MFC [34]. Nystatin 5% was assessed as a positive control.

2.7. Inhibition kinetics of Eo and NEo

In order to assess the effect of tested Eos, NEos, and nystatin 5% on the growth curve of *C. albicans*, the fungal suspension was provided in the same way as the above anti-fungal tests and evaluated at sub-MIC concentration and incubated at 37° C for 24 hours. After that, the fungal population was measured at 2, 4, 8, 12, and 24 hours later as the number of cells per unit volume of sample (cell/ml) using a spectrophotometer and fungal growth curve was determined as affected by tested Eos and NEos.

3. Results and Discussion

3.1 Chemical composition of the essential oils

The chemical composition of the essential oil was investigated using GC-MS techniques (**Table 1**). Mass spectrum results revealed thirteen main compounds in the essential oils of *M. communis*, constituting 81.07% of essential oil. The most identified compounds of these essential oils were 1, 8-Cineol (23.56%), and alpha-pinene (17.70%). Based on the results, sixteen compounds were identified in *R. officinalis*, which constituted 80.95% of essential oil, and alpha-pinene (18.72%) had the maximum shared amount among these identified compounds in this essential oil. In *E. campestre*, eleven main compounds (97.66%) were identified, including alpha-pinene (16.32), Caryophyllene (16.65%), Caryophyllene (16.65), and 12-oxabicyclo [9.1.0] dodeca-3, 7-diene (14.21%).

3.2. Nanoparticle Dimensions

FESEM evaluated the average size of the NEo particles with 200000 magnifications (**Figure 1**). The NEo particle size was 100 to 500 nm, showing the accuracy of the nano-emulsion synthesis process. Scanning electron microscopy indicates that mean droplet sizes of NEo prepared from 10 (v/v %) of *M. communis* (**Figure 1-a**), *R. officinalis* (**Figure 1-b**), and *E. campestre* (**Figure 1-c**) essential oils were 200-500 nm, 100-200 nm, and 200-300 nm respectively.

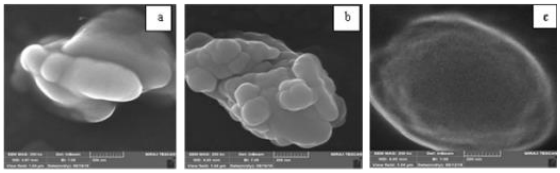

Figure 1. FE-SEM election microscopic synthesize nanoemulsions of a) *Myrtus communis*, b) *Rosmarinus officinalis*, c) *Eryngium campestre* using Field Emission Scanning Electron Microscopy method.

Table 1: Chemical composition (relative % of peak area) of essential oil of *Myrtus communis*, *Rosmarinus officinalis* and *Eryngium campestre* determined by GC-MS analysis.

Rosmarinus officinalis		***Myrtus communis***		***Eryngium campestre***	
Compound	%	Compound	%	Compound	%
α- Pinene	18.72	1,8 cineol	23.56	trans-Z-α-Bisabolene epoxide	0.3
linalool L	5.87	α- Terpinen	6.85	α-Pinene	16.32
trans-caryophyllene	8.23	Beta myrcene	0.72	o-Cymene	2.3
bicycle [3.1.1] hepta-3-en-2-one	7.40	Trans-Beta- Ocimen	0.4	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-o	11.16
pinocarvone	0.78	p- Cymene	14.05	Thymol	5.62
2-β-pinene	2.96	Linalyl acetate	8.89	carvacrol	9.64
trans-sabinene hydrate	0.54	3-Methyl	2.4	Isoaromadendrene epoxide	7.32
camphor	7.3	α-Pinene	6.71	Caryophyllene	16.65
5-isopropenly-2	0.81	Trans- geraniol	0.45	Caryophyllene oxide	10.23
β-myrcene	3.8	γ-Terpinene	0.64	Eucalyptol	3.91
borneol L	0.93	2-Hydroxycineol	0.23	2oxabicyclo[9.1.0]dodeca-3,7-diene	14.21
caryophyllene Oxid	5.62	Caryophyllene	0.87	--	--
bicycle [2.2.1] heptan-2-ol	3.68	Camphene	5.25	--	--
chrysathenone	6.2	--	--	--	--
1,6-octadien-3-ol	4.98	--	--	--	--
6,6-dimethyl-2-methylene bicycle	3.13	--	--	--	--

3.3. Anti-fungal Properties of Eo and NEo

The anti-fungal activities of Eo and NEo in different concentration (0.125-1 v/v %) against *C. albicans* was evaluated by measuring the inhibition zone diameter using the agar well diffusion method shown in **Table 2**. The Eo and NEo of the tested plants confirmed significant activity against *C. albicans* with inhibition zones ranging from 3.9–27.5mm. However, no inhibition zone can be measured in the negative control (DMSO). Positive control was evaluated using fluconazole and nystatin. Results revealed no significant difference ($p<0.01$) between these two anti-fungal drugs in controlling *C. albicans*, and both showed an inhibitory effect on tested microorganisms by inhibition zones ranging from 28.2–31 mm. Zones of inhibition with Eo in the maximum concentration (1 v/v%) varied from 18.4 to 24.2 mm, whereas zones of inhibition to NEo were slightly more (ranging from 18.9– 27.5 mm). Data indicated decreased susceptibility in the pathogen with intensifying Eo and NEo concentrations (**Table 2**). The most fungal inhibitory effect was for *M. communis*, with an inhibition zone of 27.5 mm.

The summarized results of MIC and MFC of Eo or NEo against the studied pathogen can be seen in **Table 3**. Results indicated that both Eo and NEo have anti-fungal effects against *C. albicans*. The MIC of the Eo or NEo ranged between 12.5 to 37.5 mg/L. The lowest MIC value was obtained for NEo of *M. communis* and *R. officinalis*.

Table 2: Means of the inhibition diameters (mm) of essential oil and nano-emulsion of *Rosmarinus officinalis*, *Myrtus communis and Eryngium campestre* against *Candida albicans* determined by agar diffusion compared to standard drugs.

Concentration (v/v)%	*Rosmarinus officinalis*		*Myrtus communis*		*Eryngium campestre*		FL 10%	NY 5%
	Eo*	NEo**	Eo	NEo	Eo	NEo		
1	20.9±0.1 a	23.3±0.1 a	24.2±0.1 a	27.7±0.1 a	18.4±0.2 a	18.9±0.2 a	28.2	31
0.5	11.9±0.2 b	13.5±0.2 b	14.5±0.3 b	16.0±0.3 b	10.9±0.2 b	11.5±0.2 b		
0.25	5.97±0.3 bc	6.5±0.3 c	8.1±0.3 bc	9.1±0.3 bc	5.1±0.2 bc	5.4±0.2 bc		
0.125	4.6±0.2 c	5.2±0.2 c	6.6±0.2 c	7.3±0.2 c	3.7±0.2 c	3.9±0.1 c		

*, **. Refer to essential oil and nano-emulsion of the essential oils respectively. FL: fluconazole, NY: nystatin. Data are the means of three replications± standard error .

Table 3: MIC and MFC (mg/L) of essential oils and nano-emulsion of *Eryngium campestre*, *Myrtus communis* and *Rosmarinus officinalis* against *Candida albicans*.

		MFC	MIC
Essential oil	*Eryngium campestre*	37.5 *	175
	Myrtus communis	28.12	137.5
	Rosmarinus officinalis	31.25	150
Nano-emulsion	*Eryngium campestre*	21.9	150
	Myrtus communis	12.5	100
	Rosmarinus officinalis	12.5	112.5
Control	Nystatin 5%	12.5	75

*data are the means of four replications.

3.4. Inhibition kinetics of the Eo and NEo

The present study measured the inhibition kinetics of *E. campestre*, *M. communis*, and *R. officinalis* against *C. albicans* in broth media for Eo or NEo (**Figures 2**). Results revealed that the viable count decreased by the first two hours of inoculation as affected by the studied Eo compared to the control. This trend continued slowly through the next 24 hours. NEo also had a reducing effect on the fungal growth curve. Nevertheless, NEo was more effective than Eo. At the end of the experiment, the time-dependent killing of *C. albicans* showed a decrease in fungal population by *M. communis* NEo followed by *R. officinalis* and *E. campestre* NEo, compared to the control. There was no measurable fungal inactivation in the untreated negative control (cells treated with the nonionic surfactant used to make the NEo). The initial population of fungal pathogens in the control and both treatment groups (Eo or NEo) was approximately 4.0 $\log_{10}$ cells/ml. In untreated control, the population of *C. albicans* reached about 10.5 $\log_{10}$ cells/ml during the 24 h incubation period. However, the presence of Eo in all three treatments reduced the fungal population to 5.1, 5.5, and 4.76 $\log_{10}$ cells/ml for *R. officinalis*, *E. campestre*, and *M. communis*, respectively, which was significantly different compared to the control.

Candida albicans is a common opportunistic fungal pathogen responsible for Candida infections, causing oral, vaginal, and systemic candidiasis. It may increase fungal infection due to acquired immunodeficiency syndrome (AIDS), cancer chemotherapy, and organ transplantation [34]. Treatment costs and the emergence of fungal resistance to common antibiotics and their side effects are significant limitations for chemical anti-fungal drugs. Essential oils, extracted from aromatic plants, are characterized by extensive activities, including antimicrobial properties, therefore, they are considered complementary and alternative medicine. Essential oils have unique antioxidant and antimicrobial properties [6].

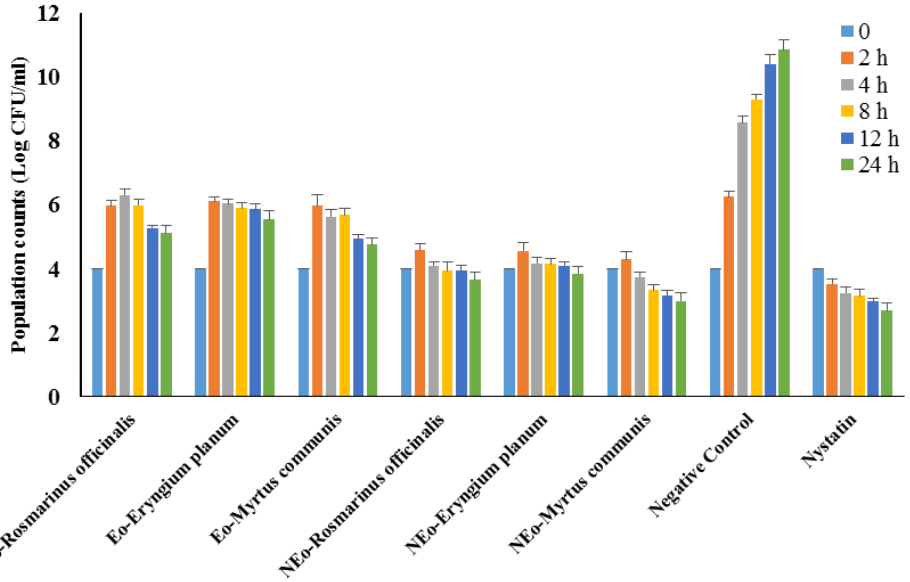

Figure 2. Survival curve of C. albicans in control and media containing sub-MIC concentration of essential oils for a variable period of time (0, 2, 4, 8, 12 and 24) at 37°C. Eo are essential oils and NEo are nanoemulsions.

On the other hand, the medicinal activities of different plant essential oils are closely related to their bioactive compounds and chemical substances. The results of the present study showed that terpenoid compounds like alpha-pinene are the most common bioactive compounds in all three studied essential oils. Based on almost all samples described in the literature, terpenoid compounded (1,8-cineol, α-pinene. myrtenyl acetate, limonene, linalool, α-terpinolene) is the most important compounds in the essential oil of *R. officinalis* leaves and *M. communis* [7, 36, 37]. Terpenes/ terpenoids are the main factor responsible for the anti-fungal properties and biofilm inhibition of essential oils due to their low molecular weight and highly lipophilic nature, which enable them to disrupt the cell membrane, inhibit the sporulation and germination of some fungi and cause cell death [38,39]. It has also been reported that the qualitative and quantitative differences in the chemical compositions of different essential oil might be varied depending on the geographic region, plant variety, plant age when the essential oil was produced, environmental and seasonal conditions during plant growing season, harvest timing, extraction methods, extraction organ, and finally genetic variability [40].

Plant essential oils have been perceived as a suitable antibacterial preservative. However, the low water solubility of essential oils is a technical restriction in drug production; thus, it requires new formulation approaches [41]. NEos are small droplet-sized emulsions with simple formulations and good functional properties which increase antimicrobial action [42]. Further, it has also been noted that a higher antimicrobial activity can be found in essential oils NEos [12, 43]. In the present study, NEo of *E. campestre*, *M. communis*, and *R. officinalis* were produced by sonication. Scanning electron microscopy of the NEos revealed that the mean droplet sizes of all NEo were 100-500 nm, not exceeding 500 nm. Nano-emulsions usually show droplet sizes smaller than 200nm, although there is no agreement on the size range of the NEo. Based on earlier studies, the droplet size of NEo prepared with surfactant can vary from 20 to 500 nm, a controversial issue in the classification of NEo [44-46]. Various studies showed that the size and stability of droplets depend on factors such as proportion approach, type and concentration of surfactant, and the type and amount of essential oil [9]. The surfactant concentration and the sonication period also affect the particle size of the nano-emulsion. A nano-emulsion system of caracole essential oil [47] and Arabic gum with a size range of 200nm [48] was also synthesized.

Results of the anti-fungal test showed that all Eos and NEos had an inhibitory effect on the tested pathogen. However, MIC and MFC values for NEo were lower than Eo's. This result was consistent with the research that demonstrated the four-fold antibacterial activity of rosemary NEo compared to Eo against *E. coli* and *S. typhi* [42]. Recent studies have shown that the nanometric size of the particles improved their bioavailability and the ability to penetrate materials [49]. In this study, the nonionic surfactant used to synthesize NEo had no anti-fungal activity on *C. albicans.* However, the MIC of 12.5 mg/L was reported in the nystatin anti-fungal agent as a positive control.

Interestingly NEo of *M. communis* anti-fungal activity was more like nystatin. MIC and MFC

assessment confirmed that NEo has fungicidal effects against *Candida* yeast cells. Recently, several reports have indicated that NEo is active against bacterial [50] and fungal [47] pathogens. Disruption of the cell wall formation and high levels of interaction with cell membranes were the main antimicrobial activity of NEo which enhanced the transport mechanisms through microbial cells [43]. Several investigations suggested the impact of the nanoparticle size and mixture of bioactive components on their anti-fungal activity [47, 51].

A study of the inhibition kinetics indicated that both NE and NEo could reduce pathogenic fungal growth. However, NEo was more effective than Eo. Numerous studies have shown that NEos had considerable fungicidal activity against *C. albicans*. For example, *Cleome viscosa* NEo could decrease foodborne *C. albicans* growth and significantly reduce biofilm production [47]. Consistently, Lemon kaffir NEo had remarkable growth inhibition effects on *C. albicans* [21]. *Origanum vulgare* NEo [41] and Pelargonium Eo [52] were also reported as effective fungicidal bio-agent against different pathogens and *C. albicans*. NEo of honey, curcumin, and piperine also showed desirable anti-fungal activity (more than 80%) against a wide range of *Candida* species [45]. In NEo treatments, the fungal population decreased sharply at all-time courses after the start of the experiment in comparison with the control. Although the more rapid inactivation effect was shown with nystatin, NEos were significantly more efficient on the fungal pathogen when compared to Eos. The significant anti-fungal activity of NEo might result from the synergism between Eo bioactive substances and nanodroplet structures [53, 54]. Numerous different studies have also shown that NEos are more effective than Eos. Recent studies demonstrated more antimicrobial activity of NEo of Sage oil [42], rosemary [35], and cinnamon [55] compared to Eo. The antimicrobial activity of NEo might be related to the hydrophilic and hydrophobic ability of surfactants and emulsifiers, along with the bioactive composition of Eo [49].

Eos, as well as the other phytochemicals, could reduce microbial growth and biofilm development through particular mechanisms. Essential oils disrupt the growth and development of fungal cells by damaging cell wall permeability [56] and also agglomerating reactive oxygen species (ROS) in the cell by altering the activity of fungal mitochondria, leading to fungal cell death [57]. In the case of *Candida*, the Eo monoterpenes may cause damage to the lipid bilayer of the cell membrane after passage through the cell wall. The usage of synthetic drugs together increases their activity and leads to cell damage by inhibiting biofilm development [25]. Recent studies also demonstrated that free Eo's antimicrobial activity was almost less than NEo [58, 16]. Generally, the NEo achieved by ultrasonification increases the essential oils' antifungal activity. In other words, the anti-fungal activity of essential oils' nano-emulsions increases transport mechanisms by damaging cell wall formation during growth [43]. Small droplet size and large surface area of NEos, improve the passage of the essential oil's active substances to the cell membrane's surface and its interaction with several molecular sites at the microorganism's cell membrane [59]. As mentioned in the results, the nano-emulsion form of essential oil showed a specific effect on *C. albicans*. However, more detailed studies are needed to detect the complicated mechanism.

4. Conclusion

This study compared the anti-fungal effect of *E. campestre*, *M. communis*, and *R. officinalis* Eo on *C. albicans* with NEo of these compounds. Based on the result, the Eo of Rosemary and Myrtus and their NEo had acceptable anti-fungal properties compared to fluconazole and nystatin (positive control). Rosemary and Myrtus are medicinal plants that have been found to have antimicrobial properties, and these natural compounds are used in the pharmaceutical and food industries. On the other hand, it is reasonably hard to prepare water-based solutions of plant essential oils, which is one of the limitations of using these compounds. Several methods, such as encapsulation and nanotechnology, have been designed to overcome this problem. In the present study, the anti-fungal investigations of Eo and the prepared NEo presented that all the studied Eo or NEo could inhibit *C. albicans*. At the same time, NEo exhibited more significant inhibitory effects. Therefore, it can be supposed that NEo of *M. communes* and *R. officinalis* essential oils are excellent options to inhibit the growth of candidate clinical isolates and can be used after clinical trials. The Food and Drugs Administration (FDA) generally accepts Eo as a safe product. Thus they are more widely recognized by consumers than synthetic agents. Recently, using NEo made from herbal essential oils as modern antimicrobial compounds has been considered. Manipulating materials at the nanoscale made it possible to conduct valuable interdisciplinary research.

Conflict of interest

The authors declare to have no conflict of interest.

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