

Anti-dermatophyte efficacy and environmental safety of some essential oils commercial and in vitro extracted pure and combined against four keratinophilic pathogenic fungi

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Anti-dermatophyte efficacy and environmental safety of some essential oils commercial and in vitro extracted pure and combined against four keratinophilic pathogenic fungi

Sahar Yassin Ibrahim^{1,2} · Magda Magdy Abd El-Salam^{2,3}

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Abstract

Aim Establish new biocontrol practices with low persistence in the environment against dermatophyte causing mycosis.

Methods Antimycotic activity of twenty-six plant-derived commercial essential oils (EOs) was evaluated against four dermatophyte keratinophilic fungi (*Microsporum canis*, *Epidermophyton floccosum*, *Trichophyton rubrum* and *Trichophyton mentagrophytes*). Commercial EOs which showed the strongest mycelial growth inhibitions were selected and re-extracted in vitro from fresh plant samples. Minimal inhibition concentration (MIC) and antifungal index (AI) of pure and combined extracted oils and were evaluated. All samples were collected and examined during the year of 2014.

Results The results revealed that commercial EOs of *Prunus armeniaca*, *Prunus dulcis* var. *amara*, *Olea europaea* and *Mentha piperita* were the most potent anti-dermatophyte. The mixture of the extracted four oils was the strongest fungicides followed by the alternative two-oil combined extractions then pure extracted oils. MIC was at

50, 25 and 12.5 µg/disc for pure oils, two-oil combinations and four-oil mixture, respectively. Achieved values of AI were found variable.

Conclusion Using of natural products like plant-derived EOs instead of chemotherapy on pathogens can be regarded as an environmental safety mode of diseases control.

Keywords Essential oils · Dermatophyte fungi · Antifungal efficacy · Antifungal index · Combined oils · *Prunus armeniaca* · *Prunus dulcis* var. *amara* · *Olea europaea* · *Mentha piperita*

Introduction

Millions of people throughout the world are affected by superficial fungal infections, which are the most common skin diseases. These infections, which occur in both healthy and immune-compromised persons, are caused mainly by dermatophytes. The dermatophytes, *Trichophyton* spp. and *Microsporum canis* are commonly involved in such infections [1, 2]. They cause common infections in humans which are difficult to control effectively, and the pharmaceutical arsenal currently available against them is rather limited. Hence, plant products that inhibit their growth without harming the host represent potential therapeutic agent [3]. Increasing social and health implications caused by dermatophytes means there is a constant striving to develop safe and new natural antifungal agents to cure human fungal disorders caused by dermatophytes [1].

Plant-derived essential oils (EOs) and extracts are considered as non-phytotoxic compounds and have antimicrobials and antidermatophytic properties [1, 4]. It is known that most of their properties are due to the EOs they

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contain as products of their secondary metabolism. Therefore, they can be used as a natural therapy to inhibit fungal pathogens causing superficial infections [1, 5]. However, there is only limited information in the literature on the antifungal activity of EOs toward human fungal pathogens.

The objective of this work was to evaluate the antifungal efficacy of 26 plant-derived EOs against four widely spread pathogenic keratinophilic fungal strains that cause superficial skin infections in humans trying to find more safely hygienic natural plant products.

Materials and methods

Study design

Twenty-six commercial EOs of different plant origins were screened for their antifungal efficacy against four filamentous fungal strains of hygienic significance. The most potent toxic commercial EOs were selected and re-extracted in vitro. Minimal inhibitory concentration (MIC) and antifungal index (AI) of plant extracted pure oils, their mixture and their interchangeably two-oil combinations were determined.

Essential oil and plant material

Fresh mint leaves, *Mentha piperita*, ripe olive drupes, *Olea europaea*, bitter almond seeds, *Prunus dulcis* var. *amara* and apricot seeds, *Prunus armeniaca*, were collected from local markets in Giza (Egypt) and Alkhatj (Saudi Arabia) cities. They were collected during April, May and June, 2014 and subjected to extraction process as soon as collected. At the same period time, twenty-six commercial plant-derived EOs mentioned in Table 1 were purchased from 2 brands “Al-Ahlam for Seeds Oil” (Production Jeddah, Saudi Arabia) and “Al Captain Company” (Cairo, Egypt). 4 months later, all EOs and extracted oils were then bio-assayed.

Oils extraction

The mint leaves, *M. piperita*, were air dried and then ground to semi-powdered state. The external hard covers of bitter almond, *P. dulcis* var. *amara*, and apricot seeds, *P. armeniaca*, were eliminated. The unshelled seeds were air dried for 2 weeks and then pulverized using laboratory miller into powder form. Fleshy parts of olive drupes, *O. europaea*, were separated, cut into small parts and minced into a paste. Mint semi-powder (100 g), bitter almond and apricot powders (500 g of each) and olive paste (200 g) were subjected to hydro-distillation for 2 h in case

of mint and 3 h for other samples using a Clevenger-type apparatus. Extracted oils were dried over anhydrous Na_2SO_4 , sterilized and preserved in a sealed vial at 4 °C prior to further studies [6].

Fungal strain references

Four pure cultures of local pathogenic dermatophyte isolates were kindly provided by Laboratory of Microbiology, King Khalid Hospital at Riyadh, Kingdom of Saudi Arabia; *Microsporum canis*, *Epidermophyton floccosum*, *Trichophyton rubrum* and *Trichophyton mentagrophytes*. These clinical keratinophilic references were isolated from patients diagnosed as having various infections. All strains were maintained on Czapek's dox agar (diffco) at 4 °C as stock cultures.

Antifungal toxicity screening assay

Antifungal toxicity screening of 26 EOs was assayed using method described by Adam et al. [7] with slight modification. Each of the 26 commercial EOs was diluted in 95 % ethanol. It was then mixed and homogenized with Czapek's dox agar growth medium with a pH of 5.5 to achieve final concentrations of 4, 2, 1, 0.5, 0.25 and 0.125 $\mu\text{l/ml}$ of medium. Twofold serial agar dilution method was applied according to Pandey et al. [4]. Triplicates of petri dishes of 9.0 cm diameter were then poured with aliquots of 25 ml sterilized media containing tested EO at a specific concentration. After solidification of agar, central well of 1.0 cm diameter was aseptically punctured in each Petri dish. Wells were then refilled by Czapek's dox agar discs of same diameter taken from a culture of 7-day-old reference fungal strains. Negative and positive controls were prepared to control the sensitivity of the tested fungus. In negative control, Czapek's dox agar was seeded with equivalent amounts of 95 % oil free ethanol. While, in positive control, tested oils was replaced with equivalent amounts of the standard antifungal, ketoconazole (Sigma). All plates were incubated at 28 °C for ≈ 7 –10 days. Antimycotic potential of EO was evaluated by visual monitoring of reference mycelial growth density on agar plate and scored as specified by Abd El Salam and Ibrahim [8]. At the end of incubation period, samples were compared with positive and negative controls. Data were collected and marked down.

Determination of MIC

MIC was surveyed by disc diffusion technique which based on agar diffusion method according to Bansod and Rai [9]. It was determined for extracted mint, olive, bitter almond

Table 1 Mycelial growth density of four dermatophyte references formed on applying 26 plant-derived commercial essential oils at different oil concentrations (µl/ml)

Essential oils	Microorganisms											
	Fungal growth density at different oil concentration (µl/ml)											
	<i>Microsporum canis</i>			<i>Epidermophyton floccosum</i>			<i>Trichophyton rubrum</i>			<i>Trichophyton mentagrophytes</i>		
	0.5	1.0	2.0	4.0	0.5	1.0	2.0	4.0	0.5	1.0	2.0	4.0
1 <i>Thyme vulgaris</i>	++++	++	++	+	++++	++	++	++	++++	++	++	+
2 <i>Nigella sativa</i>	++++	++++	++	++++	++++	++++	++++	++	++++	++++	++++	++
3 <i>Prunus armeniaca</i>	++	-	-	-	++	+	-	-	++	+	-	-
4 <i>Prunus dulcis var. amara</i>	++	-	-	-	++	+	-	-	++	+	-	-
5 <i>Curcume longa</i>	++++	++++	++	++	++++	++	++	+	++++	++	++	++
6 <i>Olea europaea</i>	++	+	-	-	++	+	-	-	++	+	-	-
7 <i>Allium sativum</i>	++++	++++	++	++	++++	++	++	++	++++	++	++	+
8 <i>Syzygium aromaticum</i>	++++	++++	++	++	++++	++	++	++	++++	++	++	++
9 <i>Zingiber officinale</i>	++++	++++	++	++	++++	++	++	++	++++	++	++	++
10 <i>Cinnamon vulgare</i>	++++	++++	++	++	++++	++	++	++	++++	++	++	++
11 <i>Aloe vera barbadensis</i>	++++	++++	++	++	++++	++	++	++	++++	++	++	++
12 <i>Mentha piperita</i>	+	-	-	-	+	-	-	-	+	+	-	-
13 <i>Ocimum basilicum</i>	++++	++++	++	++	++++	++	++	++	++++	++	++	+
14 <i>Sinapis alba</i>	++++	++++	++	++	++++	++	++	++	++++	++	++	++
15 <i>Amygdalus communis</i>	++++	++++	++	++	++++	++	++	++	++++	++	++	++
16 <i>Cuminum cyminum</i>	++++	++++	++	++	++++	++	++	++	++++	++	++	++
17 <i>Pimpinella anisum</i>	++++	++++	++++	++	++++	++	++	++	++++	++	++	++
18 <i>Elettaria cardamomum</i>	++++	++++	++	++	++++	++	++	++	++++	++	++	++
19 <i>Rosmarinus officinalis</i>	++++	++++	++	++	++++	++	++	++	++++	++	++	++
20 <i>Origanum vulgare</i>	++++	++++	++	++	++++	++	++	+	++++	++	++	++
21 <i>Petroselinum crispum</i>	++++	++++	++	++	++++	++	++	++	++++	++	++	++
22 <i>Crocus sativus</i>	++++	++++	++	+	++++	++	++	++	++++	++	++	+
23 <i>Citrus sinensis</i>	++++	++++	++	++	++++	++	++	++	++++	++	++	+
24 <i>Amoracia rusticana</i>	++++	++	++	+	++++	++	++	+	++++	++	+	+
25 <i>Coriandrum sativum</i>	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
26 <i>Trigonella foenum</i>	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++

++++, good growth; ++, moderate growth; +, weak growth; -, completely inhibited

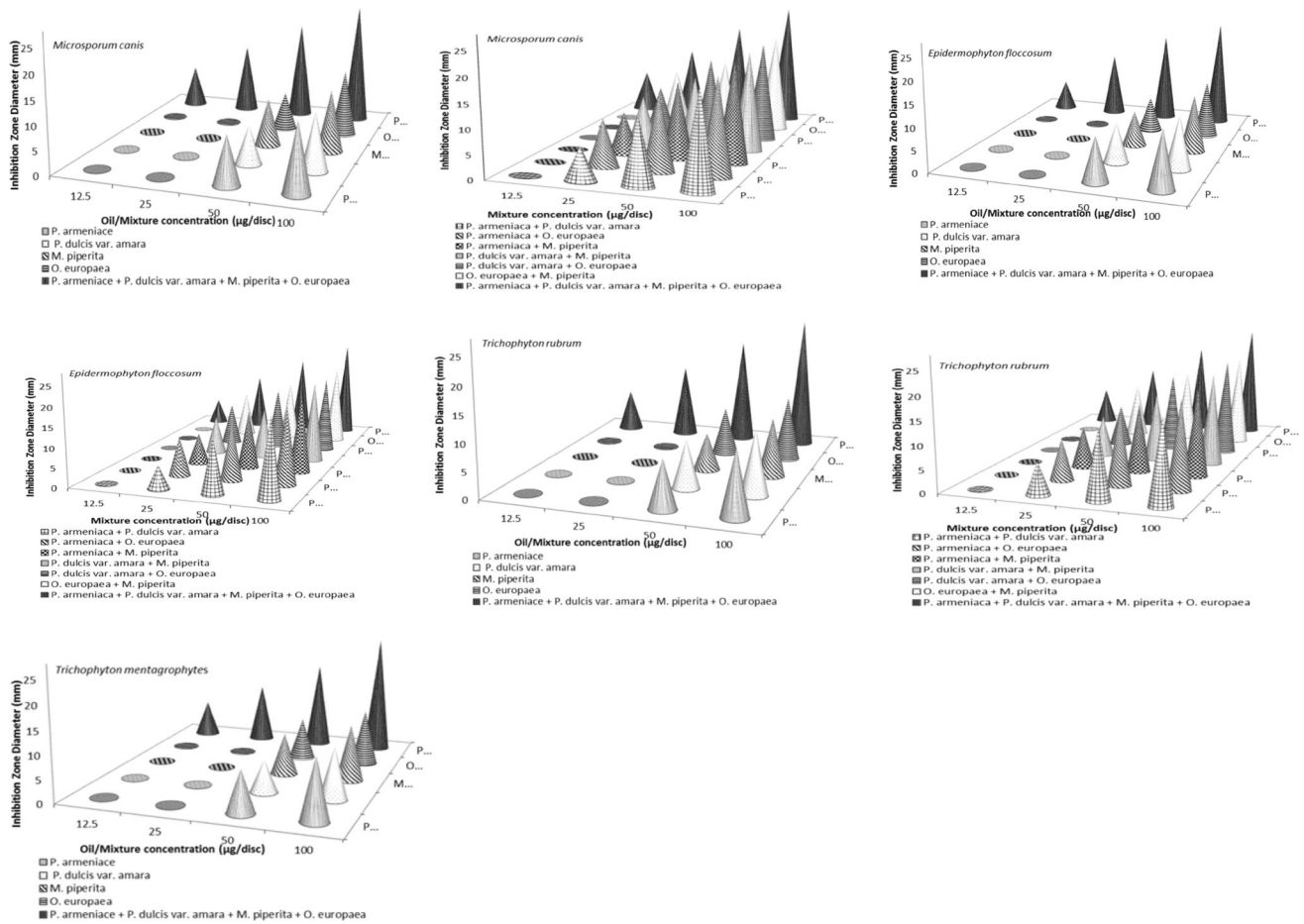


Fig. 1 Four dermatophyte growth inhibition zones (mm), including disc diameter, formed on applying extracted oils: pure, interchangeably two-oil mixtures and four-oil mixture at different concentrations ($\mu\text{g}/\text{disc}$)

and apricot pure oils, their interchangeably two-oil combinations (Fig. 1) and a mixture of the four oils. Extracted *M. piperita*, *P. dulcis* var. *amara*, *P. armeniaca* and *O. europaea* oil stock solutions were prepared as described by Abd El Salam and Ibrahim [8], 1.0 g extracted oil was dissolved in 10 ml 5 % dichloromethane and was then sterilized by filtration. For two-oil and four-oil mixtures, sterile filter paper discs (Whatman No.1 of 5 mm diameter) were loaded with equal ratios of each of oils in mixture to get final concentrations 100, 50, 25 and 12.5 μg . Also pure oils were loaded separately at the same concentrations. Soaked discs were then aseptically applied to the surface of Czapek's dox agar plate seeded with equal proportions of 7-day-old target dermatophyte inocula (adjusted at 10^4 CFU per ml). For each oil concentration, triplicates of petri dishes were prepared. Also, equivalent amounts of ketoconazole were applied in positive controls. All Petri dishes were incubated at 28 °C for 7 days.

Diameters of dermatophyte mycelial inhibition zones formed surrounding discs (included disc diameter) were measured in mm using a vernier caliper. Diameters mean values were calculated and figured. The MICs were

determined as the lowest concentration of oil showing a zone of inhibition. MIC was expressed in $\mu\text{g}/\text{disc}$.

Antifungal index evaluation

For this assay, the method followed by Cheng et al. [10] was employed. Briefly, different concentrations of extracted EOs and their mixtures, 400, 200, 100 and 50 $\mu\text{g}/\text{ml}$, were prepared in triplicates of 9 cm Petri dishes using sterile Czapek's dox agar. After transferring fungal mycelia, the testing dishes were incubated at 28 °C. When the mycelium of fungus reached the edges of negative control dish (without adding oil or mixed oils) for ≈ 10 –14 days, the antifungal indices were calculated.

$$\text{Antifungal index (\%)} = \left(1 - \frac{Da}{Db}\right) \times 100$$

where Da is the diameter of growth zone in the experimental dish (cm); Db, the diameter of growth zone in the control dish (cm). AI means and standard deviations were also calculated and tabulated.

Results

Antifungal toxicity screening assay

Variable antimycotic oil effectiveness was registered on screening antifungal toxicity of 26 commercial EOs against keratinophilic *M. canis*, *E. floccosum*, *T. rubrum* and *T. mentagrophytes* (Table 1). It was found that 24 oils were bioactive, while two oils were inactive.

All screened 26 commercial EOs marked down antifungal inactivity at oil concentrations 0.125 and 0.25 $\mu\text{l/ml}$ (not mentioned in the Table 1). This was detected by visual comparisons of treated reference strains growth; *M. canis*, *E. floccosum*, *T. rubrum* and *T. mentagrophytes* with their controls. Among the 22 bioactive EOs, four EOs registered the most fungicide toxicity. They caused a complete mycelial growth inhibition of the four references at concentrations of 2.0 and 4.0 $\mu\text{l/ml}$. These oils were *P. armeniace*, *P. dulcis var. amara*, *O. europaea* and *M. piperita* EOs (Table 1). On the other hand, all tested concentrations (4, 2, 1, 0.5, 0.25 and 0.125 $\mu\text{l/ml}$) of *Coriandrum sativum* and *Trigonella foenum* EOs allowed normal mycelial growth of four dermatophytes. This reflected the total inactivity of these two oils. Table 1 also demonstrated that 20 commercial EOs showed variable mycelial growth densities of *M. canis*, *E. floccosum*, *T. rubrum* and *T. mentagrophytes* at concentrations 4, 2, 1, 0.5, 0.25 and 0.125 $\mu\text{l/ml}$. Maximum antifungal toxicities of the 22 bioactive EOs were recorded at oil concentrations 2.0 $\mu\text{l/ml}$ and/or 4.0 $\mu\text{l/ml}$.

Both oil concentrations, 2.0 and 4.0 $\mu\text{l/ml}$, of *P. armeniace*, *P. dulcis var. amara*, *O. europaea* and *M. piperita* EOs showed the most antidermatophyte efficacy. The two concentrations recorded complete mycelial growth inhibition of *M. canis*, *E. floccosum*, *T. rubrum* and *T. mentagrophytes*.

Determination of MIC and antifungal index survey

Established MIC values of four-oil mixture, interchangeably two-oil combined mixtures and pure oils are demonstrated in Fig. 1. It was determined by the agar dilution method. Deduced MIC values of all pure extracted *M. piperita*, *P. dulcis var. amara*, *P. armeniaca* and *O. europaea* EOs were registered at 50 $\mu\text{g/disc}$. But in case of the two-oil alternative combinations, they were at 25 $\mu\text{g/disc}$. While, MIC of the four-oil mixture was at concentration of 12.5 $\mu\text{g/disc}$. Recorded diameters of dermatophyte growth inhibition zones indicated that the fungicidal efficacy of two-oil mixtures against *M. canis*, *E. floccosum*, *T. rubrum* and *T. mentagrophytes* was higher than that of pure oil extracts. Nevertheless, four-oil mixture registered the highest ever toxicity against four pathogenic references

as shown in Fig. 1. Widest fungal inhibition zone diameters (25–26 mm) expressing the most antidermatophyte efficacy were detected on applying four-oil mixture at 100 $\mu\text{g/disc}$ concentration. This was followed by two-oil alternatives inhibition zone diameters (18–21 mm) which were recorded at 100 $\mu\text{g/disc}$. Pure oils inhibition zones of (11–14 mm) were marked down at 100 $\mu\text{g/disc}$.

Table 2 demonstrated that among tested pure and mixed oils, four-oil mixture registered the highest, AI values at concentration 50 mg/ml (65.28–69.56 %), while it was the lowest in case of pure oils (26.96–33.46 %). AI of extracted *P. armeniaca* pure oil recorded the highest values at oil concentration 50 % (30.73–33.46 %) followed by *P. dulcis var. amara* (28.61–30.73 %), *M. piperita* (28.88–30.00 %) and then *O. europaea* (26.96–30.12 %) extracted oils. Antifungal indices of two-oil mixtures were 60.28 and 54.13 %; a percentage of 60.28 % for *P. armeniaca* and *P. dulcis var. amara* with *M. canis*, while it was 54.13 % for a mixture of *P. armeniaca* and *O. europaea* with *T. mentagrophytes*. Moreover, at concentration 100 mg/ml, four-oil mixture (*P. armeniaca*, *P. dulcis var. amara*, *P. armeniaca* and *M. piperita*) marked down 100 % AI with four studied dermatophytes. All pure extracted oils recorded high antifungal indices (98.00–82.99 %) against all the investigated fungal strains except *P. armeniaca* oil whose AI value was 100 % against *M. canis* only. Nevertheless, antifungal indices were equaled to 100 % at concentrations of 200 and 400 mg/ml of all pure oils and oil mixtures (not mentioned in Table 2) with all pathogenic references.

Four-oil mixture recorded the lowest MIC and highest AI values against all four dermatophyte references as illustrated in Fig. 1 and Table 2. This was followed by two-combined oil mixtures and then pure oils. The highest MIC and lowest AI values were obtained in case of pure oils.

Discussion

Antifungal toxicity screening assay

A great diversity of plant EOs documented their microbial effectiveness in many investigations. Some were carried out to discover plant products inhibiting pathogenic fungi without environmental health damage. Prakash et al. [11] recommended the use of *Ocimum gratissimum* EO in spices as a nontoxic antimicrobial and anti-aflatoxigenic agent.

Same tested 26 EOs, except *P. dulcis var. amara* as investigated by Abd El Salam and Ibrahim [8] whose results were consistent with ours. They screened antimicrobial properties of 39 diversified EOs against *Aspergillus niger*, *Aspergillus flavus*, *Fusarium lycopersici*, and

Table 2 Antifungal index (%) of pure and combined extracted oils at different concentrations ($\mu\text{g/ml}$)

	Oil extract and oil mixtures concentration ($\mu\text{g/ml}$)	Dermatophyte fungal strains			
		<i>Microsporum canis</i>	<i>Epidermophyton floccosum</i>	<i>Trichophyton rubrum</i>	<i>Trichophyton mentagrophytes</i>
		\bar{x} of antifungal index (%) \pm SD			
<i>P. armeniaca</i>	50	30.11 \pm 0.3	33.46 \pm 0.2	30.73 \pm 0.29	31.91 \pm 0.29
	100	100 \pm 0	98.00 \pm 0.29	97.00 \pm 0.5	96.01 \pm 0.25
<i>P. dulcis var. amara</i>	50	29.60 \pm 0.58	30.63 \pm 1	30.73 \pm 0.58	28.61 \pm 0.25
	100	89.34 \pm 0.02	88.56 \pm 0.05	90.49 \pm 0.29	88.00 \pm 0.76
<i>M. piperita</i>	50	30.00 \pm 1	28.88 \pm 1.2	29.11 \pm 1	29.35 \pm 0.9
	100	88.39 \pm 0.23	86.99 \pm 0.25	88.79 \pm 0.48	87.19 \pm 0.74
<i>O. europaea</i>	50	26.96 \pm 1	27.98 \pm 0.76	28.00 \pm 0.5	30.12 \pm 0.9
	100	82.99 \pm 0.35	85.00 \pm 0.4	84.89 \pm 0.45	83.45 \pm 0.26
<i>P. armeniaca</i> + <i>P. dulcis var. amara</i>	50	60.28 \pm 0.17	60.10 \pm 0.32	58.94 \pm 0.47	58.11 \pm 0.11
	100	100 \pm 0	100 \pm 0	100 \pm 0	100 \pm 0
<i>P. armeniaca</i> + <i>O. europaea</i>	50	55.09 \pm 0.15	56.66 \pm 0.26	55.95 \pm 0.58	54.13 \pm 0.36
	100	100 \pm 0	99.54 \pm 0.29	100 \pm 0	99.00 \pm 0.2
<i>P. armeniaca</i> + <i>M. piperita</i>	50	56.96 \pm 0.29	57.98 \pm 0.01	58.00 \pm 0.29	60.12 \pm 0.48
	100	100 \pm 0	100 \pm 0	100 \pm 0	100 \pm 0
<i>P. dulcis var. amara</i> + <i>M. piperita</i>	50	58.91 \pm 0.06	60.03 \pm 0.76	57.88 \pm 0.29	58.77 \pm 1.53
	100	100 \pm 0	100 \pm 0	99.54 \pm 0.33	98.98 \pm 0.5
<i>P. dulcis var. amara</i> + <i>O. europaea</i>	50	50.48 \pm 0.76	50.90 \pm 0.29	48.64 \pm 0.58	48.13 \pm 0.5
	100	100 \pm 0	100 \pm 0	99.22 \pm 0.11	98.99 \pm 0.005
<i>O. europaea</i> + <i>M. piperita</i>	50	40.28 \pm 0.58	40.10 \pm 1.29	38.94 \pm 0.82	38.11 \pm 0.39
	100	95.23 \pm 0.29	94.56 \pm 1.15	95.55 \pm 0.58	94.00 \pm 0.41
<i>P. armeniaca</i> + <i>P. dulcis var. amara</i> + <i>M. piperita</i> + <i>O. europaea</i>	50	68.56 \pm 0.06	67.34 \pm 0.06	69.56 \pm 0.06	65.28 \pm 0.06
	100	100 \pm 0	100 \pm 0	100 \pm 0	100 \pm 0
Z-test		11.868	12.051	15.808	16.142
p value		0.000 ^a	0.005*	0.007*	0.003*

* Significant results

Alternaria solani. On comparing data, relatively same results of antifungal efficacies were noticed; Bansod and Rai established different antimycotic activities on screening EOs extracted from fifteen variable medicinal plants against *A. fumigatus* and *A. niger*; Chuang et al. proved that *Moringa oleifera* Lam crude extracts and EO has antifungal effect on *T. rubrum*, *T. mentagrophytes*, *E. floccosum*, and *M. canis*; Shin and Lim detected a growth inhibition of six *Trichophyton spp* by some herbal EOs; Brunia et al. recorded antifungal activity of *Ocotea quixos*, Lauraceae EOs against dermatophyte *T. mentagrophytes*; Adam et al. found that EOs of *Origanum vulgare* subsp. *hirtum*, *Mentha spicata*, *Lavandula angustifolia*, and *Salvia fruticosa* exhibited antifungal activities against *Malassezia furfur*, *T. rubrum*, and *T. beigelii*; Kishore et al. screened fungitoxicity of sixteen EOs against *T. rubrum* and *M. gypseum* [1, 2, 7, 9, 12, 13]. They documented that *Artemisia nelagirica*, *Caesulia axillaris*, *Chenopodium ambrosioides*, *Cymbopogon citratus* and *Mentha arvensis* showed strong activity.

Screening results of 26 EOs in present study were largely supported according to many published findings. Prasad et al. [5] observed variable antidermatophytic activities by *Psoralea corylifolia* seeds extract against *T. rubrum*, *T. mentagrophytes*, *E. floccosum* and *M. gypseum*. Bansod and Rai [9] documented variable EOs antimycotic activity against *A. niger* and *A. fumigatus*. They found that EOs of *Cymbopogon martini*, *Eucalyptus globulus* and *Cinnamomum zylenticum* were of maximum activity followed by *Cymbopogon citratus*. While, oils of *Mentha spicata*, *Azadirachta indica*, *Eugenia caryophyllata*, *Withania somnifera* and *Zingiber officinale* exhibited moderate activity; and oils of *Cuminum cyminum*, *Allium sativum*, *Ocimum sanctum*, *Trachyspermum copticum*, *Foeniculum vulgare* and *Elettaria cardamomum* showed comparatively low activity. Our study results were also enhanced by Abd El Salam and Ibrahim [8] findings; they deduced that commercial *P. armeniaca* EO was the most antifungal effective oil among screened 39 EOs at 2000 $\mu\text{g/ml}$. Zohri et al. [14] studied the inhibitory effect

of onion oil against nine different species of dermatophytic fungi. They found that onion oil (200 ppm) completely inhibited the growth of *M. canis*, *M. gypseum* and *T. simii*. While, the growth of both *Chrysosporium queenslandicum* and *T. mentagrophytes* was completely inhibited by 500 ppm of onion oil, and the growth of other tested species of dermatophytic fungi was gradually reduced by increasing the concentrations of onion oil.

Determination of MIC and antifungal index survey

Many published studies investigated the effect of different concentrations, determined the MIC and screened AI values of various antimycotic active EOs. Cimanga et al. [15] studied the antifungal activity of EOs from 15 aromatic medicinal plant species. They indicated that all oils from fresh plant materials exhibited an antifungal activity at different levels against *Candida albicans*, *Candida tropicalis*, *A. niger*, *T. mentagrophytes* and *M. canis*. They recorded a high antifungal activity of inhibition zone diameter (15–22 mm) followed by (14–17 mm) then (11–17 mm) and (10–12 mm). This finding is consistent with the present study results. Abd El Salam and Ibrahim [8] found that MIC of *P. armeniaca* extracted oil against *F. lycopersici* and *A. solani* was obtained at 500 µg/ml, while MIC was documented with *A. niger* and *A. flavus* at 1000 µg/ml. Also, Cheng et al. [10] studied the antifungal activity and evaluated AI of eugenol and cinnamaldehyde against white-rot fungi, *Trametes versicolor*. At concentration 100 µg/ml, they deduced antifungal indices of 74.5 and 100 %, respectively. Both the materials totally inhibited the growth of *Laetiporus sulphureus* (brown-rot fungus) and *Lenzites betulina* at concentration 100 µg/ml. While at the level of 200 µg/ml, AI of both constituents was 100 % against all tested fungal strains.

Many EOs are only fungistatic and high concentrations are needed for fungicidal activity [16]. To enhance the efficacy of EOs, the combined use of different oils has been evaluated recently for potential synergistic effects. The combination of EOs with synthetic antifungals will probably result in a more effective therapy [17–20]. Shin and Lim [2] concluded that the antifungal effects of ketoconazole against *Trichophyton spp.* were enhanced significantly by administering it in combination with the EO fraction of *P. graveolens* or its main components. However; no published literatures studied the use of combined EOs with each other as antimicrobial agents. Thus, we decided to investigate this idea in our study by comparing between MIC and AI values of pure and combined extracted oils. We assayed these two parameters to be used as indicators for the fungicidal potential of tested oils. As we studied all achieved AI with MIC data, we deduced that four-oil mixture was the most potent effective fungicide

followed by two-combined oil mixtures and then pure oils. This proved the enhancing synergistic effect of the combined use of EOs.

When surveying data obtained in different studies, most publications provide explanations about the active antimicrobial compounds of EOs. They are generally terpenes, which are phenolic in nature and attack the pathogens through cell wall and cell membrane. Thus, active phenolic compounds might have several invasive targets which could lead to the inhibition of human infectious fungal pathogens [21]. The antifungal activity can be also attributed to the presence of some components such as carvacrol, α -terpinyl acetate, cymene, thymol, pinene, linalool which are already known to exhibit antimicrobial activity [6, 22–24].

In this study, medical safety point of applying extracted four EOs under investigation on human normal skin was not tested. At the same time, no publications were found examined or even discussed EOs hazards on public health. Accordingly, there was not any evidences showed that these EOs used in this study are not harmful for human normal skin. So, we recommend testing medical safety of these four EOs before applying them in curing of dermal diseases.

Conflict of interest No conflict of interest declared.

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