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Drug discovery: a new bioactive compounds isolated from natural sources

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Abstract. *A rise in mortality due to fungal infections in immunocompromised population has been observed lately. Nowadays, due to increased fungal infections, the limitations encountered in their treatment like resistance, side-effects and high toxicity, the rising over prescription and overuse of conventional antifungals all stimulate a search for alternative natural drugs. Therefore we are in dire need of natural newer strategies that involve reliable agents for the treatment of fungal diseases such as essential oils (EOs) are known for their anti-microbial properties and are multi-component in nature. Soil samples (66 samples) were collected from different agricultural fields and animals habitat of Saharanpur (U.P.). Isolation of keratinophilic fungi was carried out by hair baiting technique. Extraction of Mentha piperita and Cinnamomum vernum EO was carried out by hydrodistillation method and chemical composition of both extracted EOs was determined by Gas Chromatography-Mass Spectrophotometry (GC-MS). Antimycotic studies of EO was done by standard disc diffusion method. In the present study, the antifungal potential of M. piperita and C. vernum EOs was evaluated against three human pathogenic fungi isolated from the soil of agricultural field and animals habitat of Saharanpur (U.P.) i.e. Trichophyton mentagrophytes, T. tonsurans and T. equinum. The chemical composition of M.piperita and C.vernum EOs were analysed by GC-MS. Menthol (53.28%) was the major compound of the M. piperita EO followed by menthyl acetate (15.1%) and menthofuran (11.18%). Major constituents of C.vernum EO were linalool (8%), (E)-cinnamaldehyde (7.2%), β-caryophyllene (7.4%), eucalyptol (6.4%), and eugenol (5.6%). EOs of M. piperita and C. vernum have been found to have a remarkable and excellent antifungal activity against these pathogenic fungi. Present findings conclude that natural products like plant-derived EOs instead of chemotherapy and emergence of resistance to antifungal drugs can be regarded as an environmental safety mode of diseases control against pathogens.*

Keywords: *essential oils, antimicrobial, drug, resistance, natural products*

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ФИЗИКО-ХИМИЧЕСКАЯ БИОЛОГИЯ

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Противогрибковый потенциал эфирных масел *Mentha piperita* и *Cinnamomum verum*

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Аннотация. *В последнее время наблюдается рост смертности от грибковых инфекций среди населения с ослабленным иммунитетом. Рост грибковых инфекций, ограничений, возникающих при их лечении, таких как резистентность, побочные эффекты и высокая токсичность, а также чрезмерное назначение и использование обычных противогрибковых препаратов стимулирует поиск альтернативных природных лекарств и новых стратегий. Эти стратегии предполагают использование таких надежных агентов для лечения грибковых заболеваний, как эфирные масла, известные своими антимикробными свойствами и являющиеся многокомпонентными по своей природе. В настоящем исследовании противогрибковый потенциал эфирных*

масел Mentha piperita и Cinnamomum vernum оценивался в отношении 3-х патогенных для человека грибов, выделенных из почвы сельскохозяйственных полей и среды обитания животных в г. Сахаранпуре (Индия): Trichophyton mentagrophytes, T. tonsurans и T. equinum. В общей сложности было взято 66 образцов почвы. Выделение кератинофильных грибов проводили методом с использованием волос в качестве «приманок». Экстракцию эфирных масел Mentha piperita и Cinnamomum vernum осуществляли методом гидродистилляции, а химический состав обоих экстрагированных эфирных масел определяли с помощью газовой ГХ-МС. Исследования противогрибковой активности эфирных масел проводили стандартным диско-диффузионным методом. Химический состав эфирных масел M. piperita и C. vernum анализировали методом ГХ-МС. Ментол (53,28%) был основным соединением эфирного масла M. piperita, за ним следовали ментилацетат (15,1%) и ментофуран (11,18%). Основными составляющими эфирного масла C. vernum были линалоол (8%), (E)-коричный альдегид (7,2%), β-кариофиллен (7,4%), эвкалиптол (6,4%) и эвгенол (5,6%). Было обнаружено, что эфирные масла M. piperita и C. vernum обладают высокой противогрибковой активностью против рассмотренных патогенных грибов. Результаты исследования показывают, что эфирные масла растительного происхождения можно рассматривать как безопасный для окружающей среды способ борьбы с патогенами и как альтернативу химиотерапии и противогрибковым препаратам, к которым возникла устойчивость.

Ключевые слова: *эфирные масла, противомикробные лекарственные препараты, резистентность, натуральные продукты*

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INTRODUCTION

Keratinophilic fungi are a group of microorganism that are able to decompose keratin remains in environment and pathogenic to humans and animals. The soil that is rich in keratin materials is most conducive for the growth and occurrence of keratinophilic fungi distribution is variable with the environment and depends on different environment factors such as humans and animals presence. Keratinophilic fungi include a variety of filamentous fungi mainly comprising hyphomycetes and several other taxonomic groups [1, 2]. Dermatophytes cause humans and animals mycoses and thus have drawn the attention of medical and veterinary epidemiologists. The majority of dermatophytes can live saprophytically and keratinophilic fungi can be considered as a potential pathogen [3]. They occur in many natural and man-made habitats. Keratinophilic fungi are unique in the sense that they require and utilize keratin for growth [4, 5]. Studies on keratinophilic fungi are now considerable significance for their important role on the breakdown of keratinous debris of animals and man in the nature and they have a worldwide distribution [6, 7]. Studies have been shown that soils are important sources of dermatophytes and keratinophilic fungi [8–10]. In the past two decades, the emergence of resistance to various antifungal drugs has accelerated dramatically. Azole-resistant *Candida* and *Aspergillus* species are the top pathogens responsible for nosocomial or food-borne infections [11, 12]. In addition, the formation of biofilms by *Candida* species have raised concerns due to their increased resistance to antifungal therapy and protects the microbial cells within biofilms from the host immune defenses [13, 14]. Moreover, Eos especially with known antibacterial effects have the potential to be used in food industry as preservatives and to increase the shelf life of products. Therefore, determining the antimicrobial properties of EOs might help to overcome microorganism resistance to antibiotics. The chemical composition of aromatic plants depends largely on the individual genetic variability and different plant parts. The presence and concentration of certain chemical constituents of EOs also fluctuate according to the season, climatic condition, and site of plant growth [15]. A review of literatures reveals that there are many EOs which acquire antifungal activity [16–19]. The development of antifungal resistance is complex and depends on multiple host and microbial factors [20]. According to Moreira et al, lipophilic compounds of the oils bond the phospholipid bilayer of the cell membrane increasing its permeability and spreading out the intracellular contents or damaging the enzymatic system of the cell [21, 22]. *Mentha piperita* oil is one of the most popular and widely used EOs, mostly because of its main components, Menthol, and menthone [23]. Previous studies have shown antiviral, antibacterial, antifungal, antibiofilm formation, radioprotective, antioedema, analgesic and antioxidant activities of the EO [24–26]. Cinnamon is a good source of the powerful antioxidant manganese. Therefore, much attention has been paid to the discovery and development of new antimicrobial agents that might act against these resistant microorganisms, and cinnamon could be an interesting candidate [27, 28]. With regards to volatile components, the chemical composition of cinnamon EOs depends on the part of the plant from which they are extracted [29–31]. The presence and concentration of certain chemical constituents of EOs also fluctuate according to the season, climatic condition, and site of plant growth [15,32].Theobjectiveofthisworkwastoevaluatetheantifungal efficacy of *Mentha piperita* and *Cinnamomum vernum* EOs against drug resistant three widely pathogenic fungal strains that cause superficial skin infections in humans and trying to find more safely hygienic natural plant products. The goal of this study was to investigate the chemical composition and *in vitro* antifungal activities of EOs of the leaves of *M. piperita* and *C. vernum.*

MATERIALS AND METHODS

Collection of Soil Samples. Sixty Six soil samples were collected from different agricultural fields and ani-

mals habitat of Saharanpur (U.P.) India. For this present study, different soil samples were collected in sterile polyethylene bags and brought to the laboratory for further microbiological analysis. Keratin substrates (Ks) were collected from different sources such as chicken feather, buffalo hairs, goat hairs, dog hairs, pig hairs, ship hairs, and horse hairs in clean sterilized plastic bags and transferred to the laboratory. Samples were transmitted to the laboratory as early as possible for further analysis like pH, temperature, humidity, salinity, total dissolved solids.

Collection of Plants & Extraction of Oil. Fresh leaves of *M. piperita* and *C. vernum* were purchased from Saharanpur, U.P. during summer season. Identification of both plant leaves was done from Herbarium office, NBRI, Lucknow. Extraction of oil from the fresh leaves of *M. piperita* and *C. vernum* were carried out by standard hydrodistillation method, Clevenger's apparatus and all operations were carried out at room temperature. The fresh leaves of *M. piperita* and *C. vernum* were washed to remove soil and cut into small pieces. Small pieces of leaves of *M. piperita* and *C. vernum* (250 gm) were placed in a separate flask together with distilled water (1L). After 5–6 h, oil was collected from the apparatus, anhydrous with sodium sulphate for removal of water traces, then this 100% pure essential oil were dispensed into dark bottles and stored at 4 °C until used. Essential oil was ready to use for disc diffusion test and determination of MIC. The essential oil thus obtained was subjected to antidermatophytic activity.

Isolation of keratinophilic fungi by Hair Bait Technique. Isolation of keratinophilic fungi was done using the hair bait technique (Vanbreuseghem R., 1952) [33]. Moist chamber were prepared using sterile soil samples. 2–3 cm short strands of sterilized defatted baits were spread over soil samples. 10–15 ml of sterile water was added to the soil to facilitate germination of fungal spores. Petri dishes were then incubated at room temperature at 28 °C for 15–20 days. Plates were examined periodically for the development of mycelium.

Identification of Keratinophilic fungi. Each single fungal colony was taken from the mixed culture. Pure culture was developed by repeated sub culturing which was transferred to agar slant and subcultured and then stored at 4 °C temperature. The fungal colonies were identified by microscopic and macroscopic charcteristics. The macroscopic examination of fungi was characterized by duration of growth, surface morphology and pigment production on the reverse and microscopically, the isolated fungi was stained with Lactophenol cotton blue staining and observe under the microscope at high power of objective lens.

Gas chromatography-Mass Spectrometry (GC-MS) analysis of M. piperita and C. vernum EOs. Quantitative and qualitative analysis of the EOs were performed using a GC-MS apparatus. The analysis was performed with an Rtx 5 MS column. For the GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Nitrogen gas was used as carrier gas with a flow rate of 1.21 ml/min. The column was raised from 50 to 320 °C at a rate of 3 °C min. The relative percentage of the oil constituents was expressed as percentage by peak areas and the identification of oil components was based on their retention time with available literature values [34].

Antimycotic Studies of EOs against Selected Fungi by Disc Diffusion. Oil was screened for their antifungal activity against *Trichophyton mentagrophytes, T. equinum* and *T. tonsurans* by disc diffusion method [35]. Standard size whatman no. 1 filter paper discs 6.0 mm in diameter, sterilized by dry heat at 140 °C in an oven for one hour were used to determine antifungal activity. SDA medium for disc diffusion test was prepared. After sterilization it was poured into sterilized petriplates and allowed to solidify. Then one day old fresh culture of isolated fungi were be used for inoculums preparation. A suspension was just tubid (~0.5 McFarland standard) by visual inspection was prepared by suspending fungus in 0.9% NaCl solution and the homogeneous suspension was used for inoculation. Using a sterile cotton swab, fungal cultures were swabbed on the surface of sterile Sabourauds Dextrose Agar plates. Sterilized filter paper discs were soaked in neat, undiluted (100, 75, 50, 25%) concentration of oils. Using an ethanol dipped and flamed forceps, oil saturated discs of 100, 75, 50, 25% concentration were aseptically placed over Sabourauds dextrose agar plates seeded with the respective test microorganism. The antibiotic discs of Clotrimazole and Ketoconazole (10 mcg per disc) were also aseptically placed over the seeded Sabouraud's dextrose agar plates as a standard drug for comparison of antifungal activity with EOs. The plates were incubated at 28 °C for 48–72 h. The diameter of the inhibition zones was measured in mm and the activity index was calculated on the basis of the size of the inhibition zone. Three replicates were kept in each case and average values were calculated.

RESULTS AND DISCUSSION

The present study reported that out of sixty six soil samples collected, three species of keratinophilic fungi were predominantly isolated from different animal's habitat and agricultural fields with different keratin substrates. These habitats contain lots of keratin debries. In the present work, *T. mentagrophytes, T. tonsurans* and *T. equinum* were isolated predominantly from the agricultural and animal habitat soils of Saharanpur (U.P.) and also found main etiological agent of zoonotic dermatophytosis in investigated area. Macroscopic and microscopic characteristics of isolated fungi are shown in Tab. 1. Various earlier workers were reported the diverse population of keratinophilic fungi and dermatophytes from India soil [36, 37]. The high prevalence of keratinophilic fungi from the soil of Saharanpur due to buffalo hairs, human hairs and dog hairs which come to the soil either as dead or dropped off serves as substrates and are subjected to microbial decomposition. The present study clearly indicates that the diverse existence of keratinophilic fungi in soil of Saharanpur and play major role in bioremediation in natural environment. The present study coincides with other workers who also reported diverse soil habitats have been screened from different countries Brazil, Kuwait, Iran and India indicating that these groups of fungi are distributed worldwide [38–40]. In urban areas, where there are high concentrations of people and animals, soil rich in organic matter may constitute a permanent or occasional reservoir for fungi and during this fungi can be a potential source of skin infection for animals and humans. These animals serve as reservoirs of

zoophilic dermatophytes and their zoonotic infections have considerable importance [41, 42]. Sandpits are thought to play a role in the epidemiology of human and animals mycoses and reported special care needs to be paid to identify potential pathogenic fungal species such as species from *Fusarium*, isolated from soil samples of recreational parks and in all elementary school [43]. Some workers reported that when infections due to other opportunistic fungal agents occur can lead to very unfavorable and even serious outcomes infection by fungi [44].

The leaves of *Mentha piperita* and *Cinnamomum vernum* were collected from Saharanpur (U.P.) and EO was extracted by Clevenger apparatus based on hydrodistillation method as shown in Fig 1, 2. Hence, antimycotic studies and chemical composition of es-

Table 1. Macroscopic and Microscopic Characteristics of Isolated fungi

Таблица 1. Макроскопические и микроскопические характеристики выделенных грибов

Isolated Fungi	Macroscopic Characteristics	Microscopic Characteristics
Trichophyton tonsurans	Colonies on Sabouraud's Dextrose Agar at 28 °C grow moderately and the color of the colony is initially white then creamy yellow rose at age with conidial production. Suede like with radial folds sometimes powdery or velvety. Reverse is pale lemon yellow to mahogany brown may dark diffusing in the agar.	Many pyriform microconidia on stalks ballon forms. Rare distorted macroconidia. Hyphae are relatively broad irregular, much branced with numerous septa. Microconidia are varying in size and shape from long clavate to broad pyriform is borne right.
Trichophyton mentagrophytes	Colonies on Sabouraud's Dextrose Agar at 28 °C grow moderately and velvety, flat, thin with powder and pale powder dry and white powder in color.	Round microconidia in grape-like clusters, Spiral hyphae and macro conidia narrowly attached to hyphae.
Trichophyton equinum	Growth rate moderately rapid texture suede like to downy flat. Thallus color cream to pale yellow deeps to pinkish or brown is in centre. Reverse deep yellow becoming red brown or dark pink may produce brown soluble pigment.	Pyriform microconidia lateral may be produced on stalks. Rare club shaped macroconidia smooth thin walled.

Fig. 1. *Mentha piperita* (Peppermint oil)

Рис. 1. *Mentha piperita* (масло мяты перечной)

Fig. 2. *Cinnamomum vernum* (Cinnamon oil)

Рис. 2. *Cinnamomum vernum* (масло корицы)

Table 2. Chemical components (%) of the EOs distilled from *Mentha piperita*

Таблица 2. Химический состав (%) эфирных масел, дистиллированных из *Mentha piperita*

Compounds	RI	% Oil
α-Pinene	930	0.32
Sabinene	970	0.26
β-pinene-1	978	0.58
β-pinene-2	1035	6.69
Cis-sabinene hydrate	1072	0.50
Menthone	1155	2.45
Menthofuran	1168	11.18
Neomenthal	1166	2.79
Menthol	1178	53.28
Neomenthy acetate	1274	0.65
Menthyl acetate	1298	15.10
Isomenthyl acetate	1308	0.61
β-Bourbonene	1389	0.37

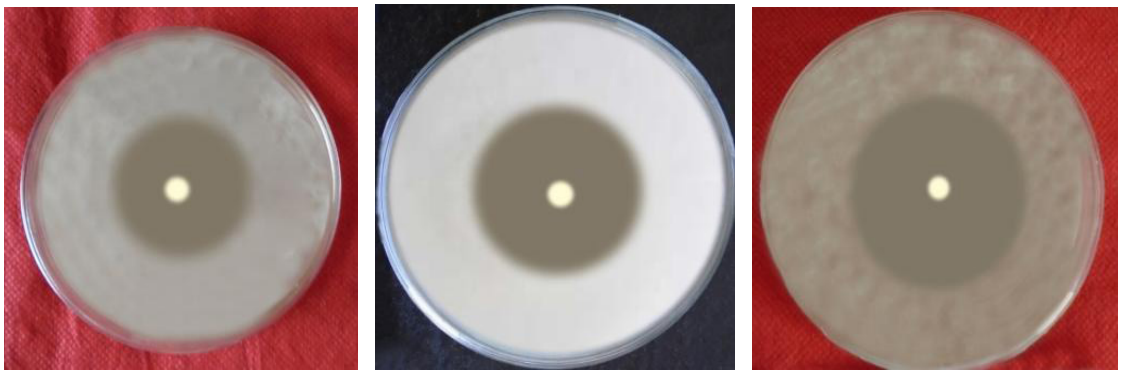
Table 3. Antifungal activity of *Mentha piperita* oil against selected isolated fungal strains

Таблица 3. Противогрибковая активность масла *Mentha piperita* в отношении отдельных изолированных штаммов грибов

Oil	Test strain	Concentration of oil, %	IZ* of sample, mm
Mentha piperita oil (Peppermint oil)	*T. mentagrophytes*	100	60
		75	50
		50	29
		25	12
	T. tonsurans	100	67
		75	40
		50	30
		25	15
	T. equinum	100	50
		75	39
		50	29
		25	15

Note. *– Inhibition zone (in mm) of standard antifungal drugs including the diameter of disc (6 mm).

sentinal oils of Cinnamon and Peppermint were carried out against selected fungi to eco-friendly control the fungal infections. In the present study, thirteen (13) compounds representing 99.37% area of the oil, were identified. The qualitative and quantitative compositions of the EO of *M. piperita* by GC-MS analysis and antifungal activity was carried out by disc diffusion method are presented in Tab. 2, 3 and Fig. 3. GC/MS analyses showed that the main constituent of the *M. piperita* EO was menthol (53.28%) followed by menthyl acetate (15.1%) and menthofuran (11.18%). The compositions of the EOs might be affected by the developmental stages of the plant. Some authors reported alpha terpinen as the dominant component of *M. piperita* EO (19.7%) while other previous studies identified menthol as one of the main constituents of the EOs [23, 45]. The higher concentration of menthol in this study as may reflect variations due to geographical location from which the plants were collected. In this study, the EOs *of M. piperita* exhibited strong antimycotic activities against *T. mentagrophytes* (60 mm), *T. equinum* (67 mm) and *T. tonsurans* (50 mm) at 100% concentration of pure EO than standard drugs. Similarly second oil i.e. *Cinnamomum vernum,* chemical constituents was identified by GC-MS analysis resulted in the identification of ten (10) chemical compounds for *C. vernum* EO, as indicated in Tab. 4 and Fig. 4. (*E*)-cinnamaldehyde (7.2%), linalool (8.00%), β-caryophyllene (7.40%), eucalyptol (6.40%), and eugenol (5.60%) were the main components of the *C. vernum* EO. The other important constituents were *p*-cymene (1.90%), α-humulene (1.70%), δ-cadinene (1.40%), α-pinene (1.30%), and limonene (1.20%). In accordance with our results, several studies have reported that cinnamaldehyde is the major chemical compound of *C. zeylanicum* bark EO [46, 47]. Antimycotic activity of *C. vernum* EO was also evaluat-



a b c

Fig. 3. Antidermatophytic activity of *Mentha piperita* EO at 100% concentration against selected fungal strains: a – *T. equinum*; b – *T. mentagrophytes*; c – *T. tonsurans*

Рис. 3. Противодерматофитная активность эфирного масла *Mentha piperita* в 100%-й концентрации в отношении отдельных штаммов грибов: a – *T. equinum*; b – *T. mentagrophytes*; c – *T. tonsurans*

ed by disc diffusion method and found excellent inhibition of zone at 100% concentration of pure EO against *T. tonsurans* (60 mm), *T. mentagrophytes* (59 mm) and *T. equinum* (58 mm) as compared to standard antifungal drugs i.e. Ketoconazole and Clotrimazole (Tab. 5).

Inhibition zone of Ketoconazole and Clotrimazole (10 mcg/disc) is 32 and 30 mm against *T. mentagrophytes*. Inhibition zone of Ketoconazole and Clotrimazole (10 mcg/disc) is 28 and 25 mm against *T. tonsurans*. Inhibition zone of Ketoconazole and Clotrimazole (10 mcg/disc) is 24 and 22 mm against *T. equinum*.

The traditional use of plants as medicines provide the basis for indicating which EOs may be useful for specific medical conditions. It is important to investigate scientifically those plants which have been used in traditional medicines as potential sources of novel antimicrobial compounds [48]. The present findings similar with Wuthi-Udomlert et al who reported the antifungal activity of turmeric oil against 29 clinical strains of dermatophytes and in screening of turmeric oil, diameter of inhibition zone was found to vary from 26.1 to 46 mm against 29 clinical strains of dermatophytes [49]. There are numerous scientific studies which proved the inhibitory effect of the EOs against different fungi [50]. It is important to analyze that the plants which have been used in the medicines as a potential source of normal antimicrobial compounds. The present work coincides with the work of Sharma et al who also reported the additive and inhibitory effect of *Curcuma longa* (Turmeric) and *Zingiber officinale* (Ginger) EOs against dermatophytes causing superficial skin infections [51]. The use of EOs in treatment and prevention from infection has been in demand in the field of research from the past. Natural products have served as a research resource for most drugs, providing a basis for chemical research and discovery of new drugs [52]. Several reasons have been offered to explain the success of natural products, among them is their great chemical diversity, the effects of evolutionary pressure in creating biologically active molecules, the structural similarity of protein targets in different species, among others [53]. The treatment of fungal disease is limited and part of the reason is due to the limited spectrum of the currently antifungal drugs and the expensive treatment particularly due to

Table 4. Chemical components (%) of the EOs distilled from *Cinnamomum verum*

Таблица 4. Химический состав (%) эфирных масел, дистиллированных из *Cinnamomum verum*

Compounds	RI	% Oil
***E*-cinnamaldehyde**	15.22	7.2
α-Pinene Linalool	5.66	1.3
Linalool	9.86	8.0
β-Caryophyllene	18.58	7.4
Eucalyptol	8.08	6.4
Eugenol	16.90	5.6
α-Humulene	19.47	1.7
δ-Cadinene	20.97	1.4
p-Cymene	7.82	1.9
Limonene	7.93	1.2

Table 5. Antifungal activity of *Cinnamomum verum* oil against selected fungi

Таблица 5. Противогрибковая активность масла *Cinnamomum verum* в отношении некоторых грибов

Oil	Test strain	Concentration of oil, %	IZ* of sample, mm
Cinnamomum verum oil	*T. mentagrophytes*	100	59
		75	50
		50	30
		25	20
	T. tonsurans	100	60
		75	46
		50	31
		25	19
	T. equinum	100	58
		75	45
		50	31
		25	19

Note. *– Inhibition zone (in mm) including the diameter of disc (6 mm).

a b c

Fig. 4. Antidermatophytic activity of *Cinnamomum verum* EO at 100% concentration against selected fungal strains: a – *T. equinum*; b – *T. mentagrophytes*; c – *T. tonsurans*

Рис. 4. Антидерматофитная активность эфирного масла *Cinnamomum verum* в 100%-й концентрации в отношении отдельных штаммов грибов: a – *T. equinum*; b – *T. mentagrophytes*; c – *T. tonsurans*

the need of prolonged therapy. Jayaprakasha et al also reported some alternative therapies including natural products are necessary to control the fungal infections [54]. Various workers have reported that due to high volatility and lipophilicity of the EOs they are readily attached to penetrate in to the cell membrane to exert their biological effect. Ali and Ali in Saudi Arabia investigate the antifungal activity of plants from the Jeddah region (*A. indica, Z. spina-christi*, and *O. europaea), P. dactylifera* seed, neem oil and other oils [55]. Another interesting investigation reports the antibacterial activity of cinnamon bark EO and its main constituents, *trans*-cinnamaldehyde and eugenol against *Cronobacter sakazakii* and *C. malonaticus*, which are opportunistic pathogens that cause infection in children and immunocompromised adults. Kizil et al reported the EOs exhibited fungistatic and fungicidal activities against both of the standard and clinical strains of *Candida* species at concentrations ranging from 0.5 μL/mL to 8 μL/Ml [56]. According to the study of Saharkhiz et al EOs killed the standard strain of *C. neoformance* is a well-known primarily opportunistic pathogen which produces chronic and life-threatening meningitis at concentration of 4 μL/mL [56]. The antifungal activities of the EOs of *Acantholippia seriphioides*, *Artemisia mendozana*, *Gymnophyton polycephalum*, *Satureja parvifolia*, *Tagetes mendocina*, and *Lippia integrifolia*, collected in the Central Andes area, Province of San Juan, Argentina, were investigated against *M. gypseum*, *T. mentagrophytes* and *T. rubrum* and were inhibited by the EOs of *G. polycephalum*, *L. integrifolia*, and *S. parvifolia*, with minimum inhibitory concentrations (MICs) between 31.2 and 1000 μg/ml. This study shows that these Cetral Andean area species might be used to treat superficial fungal infections [57]. In the study of Agarwal et al and Saharkhiz et al found that the formation of biofilm by *C. albicans* was inhibited completely at a concentration of up to 2 μL/mL in a dose-dependent manner [56, 58]. Shiming et al reported antifungal mechanism of EOs, inhibits the synthesis of ergosterol in fungal and cholesterol in mammalian cells [59]. The present results showed the excellent antidermatophytic efficacy of *M. piperita* and *C. vernum* against *T. mentagrophytes. T. tonsurans* and *T. equinum* as compared to standard antifungal drugs and can be used to control the superficial fungal infections of the skin as an eco-friendly agent and to combat the mechanism of antimicrobial drug resistance. The tested EO in the present study was rich in Menthol. It has been shown that this phenolic monoterpene has a hydroxyl group around the phenolic ring and exhibits its antimicrobial activity through the disruption of the cytoplasmic membrane and due to the main characteristics of EOs is their hydrophobicity, which enables their incorporation into the cell membrane [60, 61]. The great importance of natural products in developing new therapeutic tools is evident. In this aspect, medicinal plants and their derivatives are important for pharmacological research and drug development. Special attention has been directed to natural derivatives, based on the knowledge of antifungal compound production in nature.

CONCLUSION

The polluted area of agricultural fields of soil is a dynamic medium in which a large number of keratinophilic and non-keratinophilic fungi live in close association. Some keratinophilic fungi are pathogenic to man and animals. Dermatophytic infections are highly common and endemic disease in developing countries due to poverty, improper health facilities, illiteracy and other poorly developed diagnostic techniques. Also, some microorganisms which are resistant to newer or modern antifungal synthetic drugs and in some severely immunosuppressed patients. *M. piperita* and *C. vernum* EOs can be suggested as an alternative to synthetic antibiotics, especially for the treatment of antibiotic-resistant infections. These EOs can also be considered for developing products to controlling fungal infections. Thus, there is a need for an alternative therapy that is safe, cheaper, economical and easily available so wide range of EOs from plants are still unexplored for their antifungal activity. Hence, the present study conclude that to explore new antifungal agents alternative to synthetic drugs for the treatment of dermatophytic and other fungal diseases. This research study will be beneficial for the human health, human economy, environment safety and antimicrobial drug resistance for proper and timely treatment of fungal infections in immunocompromised patients.

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