Journal de Mycologie Médicale (2012) 22, 308—315

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ORIGINAL ARTICLE / ARTICLE ORIGINAL

Chemical composition and antifungal activity of *Matricaria recutita* flower essential oil against medically important dermatophytes and soil-borne pathogens

Composition chimique et activité antifongique de l'huile essentielle de fleurs de Matricaria recutita *contre des dermatophytes médicalement importants et pathogènes du sol*

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Received 13 July 2012; received in revised form 5 September 2012; accepted 12 September 2012
Available online 23 October 2012

KEYWORDS
Matricaria recutita;
Hypericum sp.;
Foeniculum vulgare;
Essential oil;
Antifungal activity;
Dermatophytes;
Aspergillus;
Trichoderma;
Fusarium

Summary
Objective. — Fungal infections are potential public health threats all over the world. In the present study, effect of *Matricaria recutita* flower essential oil (EO) was evaluated against medically important dermatophytes and opportunistic saprophytes using microbioassay technique.
Materials and methods. — Flower essential oil (EO) of *M. recutita* prepared by hydrodistillation was analyzed by gas chromatography/mass spectrometry (GC/MS). The effect of plant EO on the growth of pathogenic dermatophytes and opportunistic saprophytes was assessed using microbioassay technique. In the bioassay, fungi were cultured in 6-well flat-bottom microplates in presence of various concentrations of plant EO (2.5—1000 μg/mL) for 4—10 days at 28 °C.
Results. — A total of 14 compounds were identified in the plant oil by GC/MS accounting for 97.5% of the oil composition. The main compound identified was chamazulene (61.3%) followed

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1156-5233/$ — see front matter
http://dx.doi.org/10.1016/j.mycmed.2012.09.003

by isopropyl hexadecanoate (12.7%), *trans-trans*-farnesol (6.9%) and E-β-farnesol (5.2%). Growth inhibition for the dermatophytes exposed to serial two-fold concentrations of plant EO (2.5 to 80 μg/mL) was reported in the range of 3.24 to 68.15% for *Microsporum gypseum*, 24.48 to 100% for *M. canis*, 11.40 to 96.65% for *Trichophyton mentagrophytes*, 27.79 to 100% for *T. rubrum* and 45.73 to 100% for *T. tonsurans*. *M. recutita* EO inhibited the growth of opportunistic saprophytes by 3.98 to 64.29% for *Aspergillus flavus*, 6.38 to 93.62% for *A. fumigatus*, 3.52 to 89.45% for *A. niger*, 6.38 to 77.66% for *Trichoderma harzianum* and 17.41 to 89.41% for *Fusarium oxysporum* in serial two-fold concentrations of 15.62 to 1000 μg/mL.
Conclusion. — Results of the present study indicate that *M. recutita* could be considered as a potential candidate for designing effective antifungal formulations suitable for treatment of dermatophytosis and other fungal infections.

MOTS CLÉS
Matricaria recutita ;
Hypericum sp. ;
Foeniculum vulgare ;
Huile essentielle ;
Activité antifongique ;
Dermatophytes ;
Aspergillus ;
Trichoderma ;
Fusarium

Résumé
Objectif. — Les infections fongiques sont des menaces potentielles pour la santé publique partout dans le monde. Dans la présente étude, l'effet de l'huile essentielle de fleurs de *Matricaria recutita* (EO) a été évalué contre des dermatophytes médicalement importants et saprophytes opportunistes en utilisant la technique microbioassay.
Matériel et méthodes. — L'huile essentielle de fleurs (EO) de *M. recutita* préparée par hydrodistillation a été analysée par chromatographie en phase gazeuse/spectrométrie de masse (GC/MS). L'effet de l'EO sur la croissance des dermatophytes pathogènes et saprophytes opportunistes a été évalué en utilisant la technique microbioassay. Dans l'essai biologique, les champignons ont été cultivés dans des microplaques à six puits à fond plat en présence de diverses concentrations d'EO (2,5 à 1000 μg/mL) pendant quatre à dix jours à 28 °C.
Résultats. — Un total de 14 composés a été identifié par GC/MS représentant 97,5 % de la composition de l'huile. Le principal composé identifié était le chamazulène (61,3 %) suivi par l'hexadécanoate isopropylique (12,7 %), le *trans-trans*-farnesol (6,9 %) et l'E-β-farnesol (5,2 %). L'inhibition de la croissance pour les dermatophytes exposés à une double série de concentrations d'EO (2,5 à 80 μg/mL) se situe dans la gamme de 3,24 à 68,15 % pour *Microsporum gypseum*, de 24,48 à 100 % pour *M. canis*, de 11,40 à 96,65 % pour *Trichophyton mentagrophytes*, de 27,79 à 100 % pour *T. rubrum* et de 45,73 à 100 % pour *T. tonsurans*. L'EO de *M. recutita* a inhibé la croissance de saprophytes opportunistes de 3,98 à 64,29 % pour *Aspergillus flavus*, de 6,38 à 93,62 % pour *A. fumigatus*, de 3,52 à 89,45 % pour *A. niger*, de 6,38 à 77,66 % pour *Trichoderma harzianum* et de 17,41 à 89,41 % pour *Fusarium oxysporum* en série double des concentrations de 15,62 à 1000 μg/mL.
Conclusion. — Les résultats de la présente étude actuelle indiquent que *M. recutita* pourrait être considéré comme un candidat potentiel pour la conception de formulations efficaces antifongiques appropriées pour le traitement dermatophytose et d'autres infections fongiques.

Introduction

Fungi are ubiquitous in the environment, and infection due to fungal pathogens has become more frequent [16,45,46]. Fungal diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide [10]. Dermatophytoses, although very fastidious and difficult to manage, are less serious problems with respect to life-threatening invasive fungal infections [40]. A steady rise in the incidence of superficial and invasive fungal infections in past decades has been closely associated with particular infectious diseases such as AIDS, intensive chemotherapy and solid organ transplantation [9,12]. Recipients of organ transplants, leukemic patients and specific conditions such as prolonged neutropenia, corticosteroid therapy, diabetes and age are considered as the most important risk factors facilitate the onset of fungal infections [9,12,15,25,39]. Despite the improvement of diagnostic procedures, particularly non-cultural methods, the early diagnosis of invasive fungal infections remains difficult. The complexity and high cost of therapy and most of all the high case fatality rate of systemic fungal infections are reasons for the ongoing prophylactic approaches [9,25]. Non-absorbable polyenes for superficial mycoses and amphotericin B, echinocandins and newly developed azoles for invasive fungal infections have been successfully used in prevention and treatment programs; however the value of such antifungal prophylaxis in high-risk patients remains to be further studied.

In recent years, researchers have focused on finding novel antimicrobials from natural sources including higher plants, microorganisms, insects, nematodes and vertebrates. Plants are rich sources of beneficial secondary metabolites. Their essential oils (EOs) and extracts have a wide array of biological activities, especially antimicrobial effects on different groups of pathogenic organisms [1,2,42,44]. Plants with antimicrobial activity are also known to be numerous; yet prior to a decade ago, minimal research had been conducted in the area of antifungal medicinal plants [20,27,28,46]. Despite emphasis being put in research of synthetic drugs,

a certain interest in medicinal plants has been reborn, in part due to the fact that a lot of synthetic drugs are potentially toxic and are not free of side effects on the host [17,24]. This has urged microbiologists all over the world for formulation of new antimicrobial agents and evaluation of the efficacy of natural plant products as a substitute for chemical antimicrobial agents [18,32].

Matricaria recutita L. (syn: *M. chamomilla*; *M. suaveolens*; Hungarian chamomile; German chamomile; *Chamomilla chamomilla*; *Chamomilla recutita*) resides in the Asteraceae (Compositae) family and is one of the most widely used medicinal plants in the world [41]. *M. recutita* is being cultivated commercially as a medicinal herb with several applications in traditional medicine in different parts of Iran especially in Isfahan and Kerman provinces. The plant is an annual herb with erect branching and finely divided leaves growing between 50—90 cm tall. The flowers are daisy-like, with hollow conical yellowish centre surrounded by silver-white to cream colored florets. *M. recutita* is a safe plant used in different commercially available forms such as tea, infusion, liquid and capsules in human nutrition. It has a stable natural monocyclic sesquiterpene alcohol named α-bisabolol as the main component (high molar mass of 222.4 g/mol and a high boiling point of 153 °C at 12 mm Hg), so the plant essential oil has a long shelf life of 6 to 24 months. There are only a few reports of allergenic responses such as skin reactions and dermatitis after topical use of *M. recutita* as well contra-indications for its use in persons with known sensitivity to other members of the Asteraceae/Compositae family (e.g. ragweed, echinacea, feverfew, milk thistle) [26,34,38]. Despite that, the plant has been listed as generally regarded as safe (GRAS) by the FDA because it has no proven potentially toxic compound and therefore, no acute toxicity for human and animals [6,30,44]. A diverse range of pharmacological actions have been recognized for the plant including antimicrobial, anti-inflammatory, antioxidant, antispasmodic, antiviral, craminative, sedative and antiseptic properties [19]. Potentially active chemical constituents of *M. recutita* including terpenoids, flavonoids, coumarins, and spiroethers which are believed to be responsible in part for such a wide range of biological activities [30].

To our knowledge, little has been documented about the antifungal activity of *M. recutita* essential oil. In this paper, we evaluated the inhibitory effects of *M. recutita* essential oil on the growth of some pathogenic fungi from the genera *Aspergillus*, *Fusarium*, *Trichoderma* and the dermatophytes.

Materials and methods

Fungal strains and culture conditions

All fungal strains used in this study including *A. flavus* PFCC50041, *A. fumigatus* PFCC50091, *A. niger* PFCC50101, *F. oxysporum* PFCC12-86, *T. harzianum* PFCC11-89, *M. canis* PFCC 50691, *M. gypseum* PFCC50701, *T. rubrum* PFCC51431, *T. tonsurans* PFCC88-1352 and *T. mentagrophytes* PFCC50541, were provided from Pathogenic Fungi Culture Collection of Pasteur Institute of Iran (http://en.pasteur.ac.ir/pages.aspx?id=586). Fungal strains were cultured on Potato Dextrose Agar (PDA; E. Merck, Darmshdt, Germany) slants for 7—14 days at 28 °C. Spore suspensions were prepared by gently scraping the culture surfaces using a sterile glass rode after adding adequate amounts of 0.1% aqueous solution of Tween 80. Potato Dextrose Broth (PDB; Scharlau Chemie S.A., Barcelona, Spain) was the medium used for submerged cultures of fungi in presence of *M. recutita* oil.

Plant materials and preparation of essential oils and extracts

As indicated in Table 1, a total of 10 plant species belonging to three different families was included in the study. Besides *M. recutita* as the only bioactive plant with considerable antifungal activity in our bioassay (prepared from Esfahan province, central of Iran), eight *Hypericum* species and *Foeniculum vulgare* were tested. The plants were collected during April—July 2010. Voucher specimens were deposited in the herbarium of the Research Institute of Forest and Rangelands, Tehran-Iran. Plant materials (leaves, flowers, roots, stem) were steam distilled for 90 min in a fully glass apparatus. EOs were prepared by hydrodistillation of sterilized plant parts using a Clevenger-type apparatus during a 4-h time period [6]. The extraction was carried out for 120 min in 500 mL water. The EO yield for *M. recutita* and *F. vulgare* was around 1.0% of total weights, and were kept at 4 °C until use.

Table 1 General features and preliminary data of antifungal potential of *M. recutita*, *F. vulgare* and *Hypericum* species. *Caractéristiques générales et données préliminaires du potentiel antifongique de* M. recutita, F. vulgare *et de diverses espèces d'*Hypericum.

Family	Plant species	Part used	Month of harvest	Antifungal activity
Asteraceae	*Matricaria recutita* L.	Flowers	May	+
Hypericaceae	*Hypericum perforatum* L.	Leaves	June	—
	Hypericum hirtellum	Leaves/Flowers	June	—
	Hypericum helianthemoides	Leaves	June	—
	Hypericum triquetrifolium	Leaves/Stem	July	—
	Hypericum scabrum L.	Leaves/Flowers	July	—
	Hypericum dogonbadanicum	Leaves/Flowers	May	—
	Hypericum lysimachioides	Leaves	June	—
	Hypericum hyssopifolium	Leaves	July	—
Apiaceae	*Foeniculum vulgare*	Roots	June	—

To prepare extracts, leaves and flowers of *Hypericum* species were air-dried in room temperature and then powdered using a homogenizer. Amounts of 10 g of each powdered plant material were extracted separately with 100 mL methanol (MeOH) and *n*-hexane in Erlenmeyer flasks for 24 h. The extracts were filtered through Whatman No. 1 filter papers and evaporated near to dryness by a rotary evaporator. Extracts were kept at 4 °C until use.

GC/MS analysis of *M. recutita* (flowers) and *F. vulgare* (roots) essential oils

GC/MS analyses were performed using a Varian 3400 GC/MS apparatus coupled to a Saturn II ion trap detector and Quantitation was performed using Euro Chrom 2000 software from KNAUER by the area normalization method neglecting response factors. GC analysis was carried out using a DB-5 fused silica capillary column (60 m × 0.25 mm× film thickness 0.25 μm; J & W Scientific Inc., Rancho Cordova, CA, USA). The operating conditions were as follow: injector and detector temperature, 250 °C and 265 °C, respectively; Helium as carrier gas. Oven temperature program was 40—250 °C at the rate of 4 °C/min. Mass spectrometry conditions were an ionization potential of 70 eV and electron multiplier energy equal to 2000 V. The identities of the components of the oil were established from their GC retention indices, relative to C7-C25 *n*-alkanes, by comparison of their MS spectra with those reported in the literature [11] and by computer matching with the Wiely 5 mass spectra library, whenever possible, co-injection with a standard available in the laboratories.

Antifungal assay

Fungal strains were cultured on PDB in 6-well flat-bottom microplates (Greiner bio-one, well diameter 36.0 mm) in the presence of plants EOs and methanolic extracts using a microbioassay technique [37]. Culture medium (5 mL/well) was added to the microplates, which were inoculated with fungal spore suspension (5×10^6 spores/well) prepared in an aqueous solution of 0.1% Tween 80. Serial two-fold dilutions of the EOs and/or extracts (from 15.62 to 1000 μg/mL for opportunistic saprophytes and from 2.5 to 80 μg/mL for pathogenic dermatophytes) prepared in methanol (final concentration of 1.0%) were added separately to the test wells. The control wells for each fungus were treated in the same manner except that they did not contain plant EOs and extracts. Methanol at a concentration of 1.0% was added to separate wells as solvent control. Triplicate microplates were incubated for 4 days for opportunistic saprophytes and 10 days for dermatophytes at 28 °C under static conditions in two separate experiments.

As a control, antifungal susceptibility of the dermatophytes and opportunistic saprophytes to ketoconazole was determined by an agar-based disk diffusion method according to Nweze et al. [31]. Briefly, the conidial suspension (1×10^6 conidia/mL) of 7 day-old cultures of strains on PDA was streaked by a swab on the surface of Muller-Hinton agar plates. Commercial disks of ketoconazole (10 μg/disk, MAST Diagnostics, Merseyside, UK) were placed on the inoculated plates. The plates were inversely incubated at 30 °C for 4—7 days to allow for fungal growth. Inhibition zone diameters (IZDs) of two separate experiments in triplicate were measured and reported as in millimeters.

Determination of fungal growth

In microbioassay, the total contents of each well including culture medium and fungal biomass were filtered through a thin layer of cheese cloth and then thoroughly washed with distilled water. A known weight of fungal biomass was placed in a stainless steel container and allowed to dry at 80 °C to constant weight. The growth inhibitory effect expressed as percent inhibition of growth by the following formula:

Inhibition of growth (%) = Dc − Ds/Dc × 100; Where Dc was the dry weight of fungal biomass in control sample, Ds is the dry weight of fungal biomass in treated sample.

Statistical analysis

Data of fungal growth was subjected to analysis of variance (one-way Anova) in Tukey range using a SPSS Version 13.0 Program for Windows (http://www.spss.com/). Differences with $P < 0.05$ were considered significant.

Results

Plant characteristics

General features of plants used in the present study are summarized in Table 1. A total of 10 medicinal plants belonging to three different families in relation to their antifungal activities on the growth of pathogenic dermatophytes as well as some opportunistic saprophytes were evaluated using microbioassay technique.

Essential oil analysis

The main constituents of Eos of *M. recutita* (flowers) and *F. vulgare* (roots) identified by GC/MS are summarized in Table 2 according to their retention indices (RI) and percentage composition. Fourteen compounds were found in *M. resutita* EO of which chamazulene (61.3%) was the principal component followed by Isopropyl hexadecanoate (12.7%), *Trans-trans*-farnesol (6.9%), E-β-farnesol (5.2%), Z, E-farnesol (4.8%), α-bisabolol (2.0%) and α-bisabolol oxide A (1.7%). Other compounds comprised 2.9% of total oil. Eleven compounds were identified in *F. vulgare* roots EO of which dillapiol was the principal component comprised 87.5% of the oil. Other compounds constituted about 10% of the total oil.

Inhibitory effects on the growth of dermatophytes

Based on the obtained results, various concentrations of *M. recutita* essential oil inhibited the growth of all dermatophytes dose-dependently. As indicated in Table 3, inhibition of the growth of dermatophytes exposed to serial two-fold concentrations of *M. recutita* oil (2.5 to 80 μg/mL) was in the range of 3.2 to 100% including 3.2 to 68.1% for

Table 2 Chemical composition of *M. recutita* and *F. vulgare* essential oils.
Composition chimique des huiles essentielles de M. recutita *et de* F. vulgare.

Number	*Matricaria recutita* L. (Flowers)			*Foeniculum vulgare* L. (Roots)		
	Compound	RI	%	Compound	RI	%
1	α-phellanderene	985	0.3	α-pinene	925	0.8
2	(E)-β-ocimene	1053	0.3	Myrcene	980	0.5
3	γ-terpinene	1072	0.7	1,8-cineole	1024	1.3
4	P-cymenene	1095	0.2	γ-trpinene	1043	1.7
5	α-terpinene	1289	0.3	Fenchone	1066	3.6
6	Isomenthyl acetate	1310	0.7	Estragole	1181	0.5
7	E-β-farnesol	1459	5.2	*Cis*-carveole	1219	0.4
8	Spathulenol	1561	0.4	*Trans*-anethole	1265	0.5
9	α-bisabolol oxide A	1647	1.7	Piperitenone oxide	1348	0.5
10	Z, E-farnesol	1706	4.8	Elemicin	1531	1.1
11	α-bisabolol	1719	2.0	Dillapiol	1619	87.5
12	Chamazulene	1738	61.3	—	—	—
13	*Trans-trans*-farnesol	1829	6.9	—	—	—
14	Isopropyl hexadecanoate	1968	12.7	—	—	—
	Total		97.5	Total		98.4

M. gypseum, 24.5 to 100% for *M. canis*, 11.4 to 96.6% for *T. mentagrophytes*, 27.8 to 100% for *T. rubrum* and 45.7 to 100% for *T. tonsurans*. These inhibitions were significant comparing the control groups for all the dermatophytes in all concentrations except 2.5 and 5 μg/mL concentrations for *M. gypseum* (Anova, $P < 0.05$). EC_{50} values of plant oil were calculated as 3.0, 10.6, 13.6, 44.7, and 61.0 μg/mL for *T. tonsurans*, *M. canis*, *T. rubrum*, *T. mentagrophytes* and *M. gypseum*, respectively (Table 3).

As shown in Table 3, ketoconazole as a control synthetic antifungal drug inhibited the growth of all tested dermatophytes by different extents. Inhibition zone diameter for the

Table 3 Antifungal activity of *M. recutita* flower essential oil (2.5 to 80 μg/mL) against major pathogenic dermatophytes.
Activité antifongique de l'huile essentielle de fleurs de M. recutita *(2,5 à 80* μg/mL*) contre des dermatophytes pathogènes majeurs.*

EO concentration (μg/mL)	Fungal growth (total dry weight; mg)									
	M. canis		*M. gypseum*		*T. tonsurans*		*T. mentagrophytes*		*T. rubrum*	
	Mean ± SD	Inhibition (%)	Mean ± SD	Inhibition (%)	Mean ± SD	Inhibition (%)	Mean ± SD	Inhibition (%)	Mean ± SD	Inhibition (%)
0.0	38.0 ± 2.8	0.00	74.0 ± 0.1	0.0	25.7 ± 4.0	0.0	60.5 ± 0.9	0.0	30.4 ± 2.6	0.0
2.5	29.4 ± 0.5*	24.5	71.60 ± 2.0	3.2	13.9 ± 2.9*	45.7	53.6 ± 1.5*	11.4	21.9 ± 3.9*	27.8
5	22.9 ± 3.8*	41.3	65.5 ± 3.1	11.4	7.2 ± 0.7*	72.1	49.5 ± 0.5*	18.2	18.6 ± 2.4*	38.8
10	20.2 ± 1.2*	48.2	59.5 ± 3.0*	19.6	4.8 ± 0.4*	81.3	46.2 ± 2.1*	23.7	16.2 ± 0.2*	46.6
20	5.3 ± 1.1*	86.3	56.3 ± 1.3*	23.9	1.0 ± 0.5*	96.1	44.2 ± 3.2*	27.0	13.0 ± 1.0*	57.2
40	0.0*	100	51.9 ± 5.5*	29.8	0.0*	100	31.7 ± 3.1*	47.7	0.8 ± 0.1*	97.3
80	0.0*	100	23.5 ± 10.0*	68.1	0.0*	100	2.0 ± 0.6*	96.6	0.0*	100
EC_{50} (μg/mL)	10.6		61.0		3.0		44.7		13.6	
EC_{90} (μg/mL)	27.1		> 80		16.5		72.0		36.8	
Ketoconazole (10 μg/disk)**	16.2 ± 2.9		35.3 ± 8.3		26.8 ± 5.0		37.3 ± 4.2		39.4 ± 6.3	

* Statistically significant difference from the control (Anova, $P < 0.05$)
** IZD: Inhibition zone diameter (Mean ± SE in mm) by a disk diffusion method for two separate experiments in triplicate.

Table 4 Antifungal activity of *M. recutita* flower essential oil (15.62 to 1000 μg/mL) against opportunistic saprophytes.
Activité antifongique de l'huile essentielle de fleurs de M. recutita *(15,62 à 1000 μg/mL) contre des saprophytes opportunistes.*

EO concentration (μg/mL)	Fungal growth (total dry weight; mg)									
	A. flavus		*A. fumigatus*		*A. niger*		*T. harzianum*		*F. oxysporum*	
	Mean ± SD	Inhibition (%)	Mean ± SD	Inhibition (%)	Mean ± SD	Inhibition (%)	Mean ± SD	Inhibition (%)	Mean ± SD	Inhibition (%)
0.0	42.0 ± 1.0	0.0	31.3 ± 1.1	0.0	42.7 ± 1.1	0.0	31.3 ± 0.6	0.0	22.0 ± 1.0	0.0
15.62	40.3 ± 0.6	4.0	29.3 ± 1.1	6.4	41.2 ± 0.8	3.5	29.2 ± 0.6	6.4	18.2 ± 0.8*	17.4
31.25	38.3 ± 2.1	8.7	28.3 ± 1.1	9.6	40.3 ± 0.6	5.5	27.0 ± 1.7	13.8	15.0 ± 1.0*	31.8
62.5	30.3 ± 4.0*	27.8	25.0 ± 1.0*	20.2	37.2 ± 0.6*	12.5	26.3 ± 1.1*	16.0	9.7 ± 0.6*	56.0
125	23.0 ±1.0*	45.2	10.5 ± 1.8*	66.5	21.3 ± 0.6*	50.0	22.3 ± 2.1*	28.7	5.5 ± 1.8*	75.0
250	19.7 ± 1.5*	53.2	8.0 ± 1.0*	74.5	17.0 ± 4.0*	60.2	21.2 ± 0.6*	31.9	4.0 ± 0.0*	81.8
500	16.7 ± 2.3*	60.3	6.3 ± 1.1*	79.8	4.7 ± 0.5*	89.1	18.3 ± 3.1*	41.5	3.3 ± 0.6*	84.9
1000	15.0 ± 1.0*	64.3	2.0 ± 0.5*	93.6	4.5 ± 0.9*	89.4	7.0 ± 1.0*	77.7	2.3 ± 0.5*	89.4
EC_{50} (μg/mL)	200.8		103.2		125.0		638.8		53.7	
EC_{90} (μg/mL)	> 1000		862.3		> 1000		> 1000		> 1000	
Ketoconazole (10 μg/disk)**	20.5 ± 4.0		18.3 ± 3.8		36.9 ± 3.7		21.7 ± 5.3		29.7 ± 7.4	

* Statistically significant difference from the control (Anova, $P < 0.05$).
** IZD: Inhibition zone diameter (Mean ± SE in mm) by a disk diffusion method for two separate experiments in triplicate.

dermatophytes exposed to ketoconazole (10 μg/disk) was reported in the range of 16.2 to 39.4 mm.

Inhibitory effects on the growth of opportunistic saprophytes

Table 4 shows the percent of dose-dependent growth inhibition of opportunistic saprophytes exposed to serial two-fold concentrations of *M. recutita* oil (15.62 to 1000 μg/mL). *M. recutita* oil inhibited the growth of opportunistic saprophytes in the total range of 4.0 to 93.6%. This inhibition was calculated in the ranges of 4.0 to 64.3%, 6.4 to 93.6%, 3.5 to 89.4%, 6.4 to 77.7% and 17.4 to 89.4% for serial two-fold concentration of 15.62 to 1000 μg/mL for *A. flavus*, *A. fumigatus*, *A. niger*, *T. harzianum* and *F. oxysporum*, respectively. Differences were significant comparing the control groups for *F. oxysporum* in all concentrations and for other saprophytes in concentrations above 31.25 μg/mL (Anova, $P < 0.05$). EC_{50} values of plant oil were calculated as 53.7, 103.2, 125.0, 200.8, and 638.8 μg/mL for *F. oxysporum*, *A. fumigatus*, *A. niger*, *A. flavus* and *T. harzianum*, respectively (Table 4).

As shown in Table 4, inhibition zone diameter for opportunistic saprophytes exposed to ketoconazole (10 μg/disk) as a control was reported in the range of 18.3 to 36.9 mm.

On the basis of our results, methanolic and *n*-hexane extracts of eight *Hypericum* species and essential oil of *F. vulgare* roots listed in Table 1 did not show any significant inhibition on the growth of dermatophytes and opportunistic saprophytes tested (results are not shown in details).

Discussion

In the present study, the flower essential oil of *M. recutita* was reported as a strong inhibitor of fungal growth for all 10 fungal species tested including major pathogenic dermatophytes and opportunistic saprophytes. We used bioassay in liquid culture as a well-known method for in vitro antimicrobial tests, which enable the calculating of dry weight as an index of fungal growth. This method provides reliable data on dose-dependent inhibition of a fungus exposed to plant extracts and other sources of antifungals enable the researchers to calculate EC values in different extents. The plant oil showed fungistatic and fungicidal activities toward the fungal isolates at various concentrations used which was significant for the concentrations from 15.62 to 1000 μg/mL for opportunistic saprophytes and from 2.5 to 80 μg/mL for pathogenic dermatophytes.

Despite the large data have now been existing about antimicrobial effects of medicinal plants, little had been conducted in the area of antifungal medicinal plants [1,36]. Naeini et al. [27] reported the antifungal activity of *Zataria multiflora* and some plants of Apiaceae family against medically important as well as plant pathogenic *Fusarium* species. Antifungal activity of tea tree oil against *T. equinum* has been reported by Nardoni et al. [28]. More interestingly, in a recent report by Nardoni et al. [29], *in vivo* effectiveness of EOs of some Mediterranean autochthonous plants was established against zoophilic dermatophytes including *M. canis*, *T. mentagrophytes* and *T. equinum*. These authors concluded that tested EOs with remarkable antifungal properties may be attractive as new natural antifungal drugs in the management of superficial mycoses of animals.

The genus *Matricaria* comprises different species grown all over the world and particular interest has been taken in *M. recutita* as a species with a wide range of useful biological activities [4]. It is known for a diverse range of pharmacological actions including antimicrobial, anti-inflammatory, antioxidant, antispasmodic, antiviral, carminative, sedative and antiseptic properties [33,44]. Despite a lots of data are

now exist about antimicrobial activity on *M. recutita*, the subject has been a matter of controversy at least for fungi for many years because of contrary reports exist in the literature. Some researchers reported antifungal activity of the plant oil against different phytopathogenic and medically important fungi [23,43,44], while the others showed weak or no growth inhibition in this regard [5,13,14,22,35].

In the present study, it was shown that the antifungal activity of *M. recutita* flower EO is not only dose-dependent, but also it depends on the type of fungus examined. As an interesting result, dermatophytes from the both genera *Microsporum* and *Trichophyton* were inhibited by a higher percentage than that of opportunistic saprophytes from the genera *Aspergillus*, *Fusarium* and *Trichoderma*. Among dermatophytes tested, *T. tonsurans* was the most sensitive species to antifungal effects of plant oil, while *M. gypseum* showed the lowest sensitivity in this regard. Although growth of all tested opportunistic fungi was inhibited considerably by the plant EO, no fungicidal effect was reported even at the highest EO concentration of 1000 μg/mL.

In a comprehensive study by Pauli and Schilcher [33], therapeutic effects of *M. recutita* essential oil and its constituents such as farnesol and α-bisabolol in treatment of dermatophytosis in mouse and rat models have been established by oral administration. The indicated that the results for α-bisabolol were comparable with known antifungal drugs including nystatin, griseofulvin, fluconazole, itraconazole and ketoconazole.

In the present study, chamazulene was identified as the main constituent of *M. recutita* oil comprising about 61.3% of the total oil. This compound has shown to possess wound-healing, anti-inflammatory and antimicrobial properties. So, strong antifungal properties of *M. recutita* oil against dermatophytes and opportunistic saprophytes reported here may be attributed to chamazulene. An interesting issue is that chamazulene as a pronounced anti-inflammatory compound may be responsible for skin healing effects of *M. recutita* and in a narrower extent, for therapeutic effects of the plant oil on psoriasis as an important skin disorder with unknown etiology [3].

We did not find any antifungal activity for the roots essential oil of *F. vulgare* as well as methanolic and *n*-hexane extracts of eight *Hypericum* species tested. Dillapiol was the main constituent of *F. vulgare* oil in GC/MS analysis comprising 87.5% of the total oil. This compound is shown to affect aflatoxins production by *A. parasiticus* without any obvious effect on fungal growth [37]. It may be considered as a good reason for no antifungal activity of *F. vulgare* oil against tested fungi in the present study. The genus *Hypericum* is well known for its antitumor, antiviral and antidepressant properties [21]. Very limited data has been documented about antimicrobial activity of *Hypericum* species [7,8]. All eight plant species tested by us did not show antifungal activities. It may be due to the fact that hypericin, the most important bioactive metabolite of *Hypericum* is a photodynamic agent needs to light and oxygen for bioactivation, the situation which provided manually in photodynamic therapy of cancers.

In total, with respect to the strong antifungal activity against medically important fungi from pathogenic dermatophytes to opportunistic saprophytes evidenced in the present study, as well as proven biosafety and *in vivo* antifungal activity of plant materials, *M. recutita* could be considered as a potential candidate for producing commercial antifungals suitable for treatment of dermatophytosis and other important fungal infections.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgments

This work was supported financially by the Pasteur Institute of Iran (Grant No. 528).

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