Antidermatophytic activity of essential oils

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The increasing impact of dermatophytic infections, the limitations encountered in their treatments (e.g. resistance, side-effects, and high toxicity), the rising overprescription and overuse of conventional antifungals and high treatment costs all have stimulated the research for alternative natural drugs, such as essential oils. This paper reviews the *in vitro*/*in vivo* activity of essential oils and their major compounds against dermatophyte strains. A state of knowledge of the chemical composition of the oils, *in vitro* susceptibility tests and *in vivo* models used in antidermatophytic assays as well as the mechanism of action involved are referred under the perspective of the potential use of essential oils as antifungal agents in the clinical treatment of dermatophytosis.

Keywords antifungal activity; dermatophytosis; volatile oils

1. Introduction

Dermatophytes are responsible for serious human pathogenic infections that have increased during the last decades, particularly among high risk patients [1, 2]. These infections are a major cause of morbidity-associated superficial mycoses, with frequent relapses and often refractory to therapy [3]. Although conventional antifungal drugs are available (azoles, allylamines, and morpholine derivates), an increasing resistance to these conventional compounds can result in treatment failure. Moreover, the effective life span of classical antifungals is in fact limited due to their frequent use as antifungals and immunosuppressive drugs as well as in organ transplantation, lymphomas and HIV secondary infections.

In the last years, research in aromatic and medicinal plants, and particularly their essential oils (EO), has attracted many investigators. EO have traditionally been used during centuries for their antifungal properties [4]. More recently, several studies have shown evidence of the huge potential of these natural products as antifungal agents [e.g. 5-10] justifying their current use in a number of pharmaceutical, food, and cosmetic products. Therefore, it is not surprising that EO are one of the most promising groups of natural products, for the development of broad-spectrum, safer and cheaper antifungal agents.

Several methodologies are available to evaluate the *in vitro* antidermatophytic activity of EO. The agar-based disk diffusion, broth dilution, and vapor phase tests are the mostly used. Also *in vivo* models have been developed in order to access effectiveness of *in vitro* results. Despite the widespread use of EO by humans and the large evidence, in recent studies, of their potential as complementary or alternative options for prophylaxis and treatments of dermatophytosis, their exact mechanism of action, remains poorly understood.

This paper reviews the current knowledge concerning the antidermatophytic activity of EO.

2. Dermatophytes and dermatophytosis

Dermatophytes are classified as geophilic, zoophilic and antropophilic species according to their main habitat or host. The first group is abundant in soils and is normally associated with decomposing keratinous structures such as hair, feathers, fur, and horns. On the other hand, zoophilic and antropophilic fungi have a more specific distribution and infect animals and humans, respectively [11]. Species of the genera *Trichophyton*, *Microsporum* and *Epidermophyton* (Fig. 1) are responsible for common infections in the skin and appendages, generally called dermatophytosis or ringworm infections [11, 12]. These fungi colonize keratinized human or animal tissues causing an infection that may vary from mild to very intense. The range of severity depends upon several factors including the host reaction to the metabolic products produced by fungi, the virulence of the infecting strain, the site of infection (tissue keratinization), and also environmental factors [13, 14]. The infections are highly prevalent due to the large number of reservoirs (skin, hair, nails), the readiness of transmission from one host to another, and to the high resistance of the strains to adverse environmental conditions. Antropophilic dermatophytes generally coexist in equilibrium on the skin and normally cause only mild irritation. Thus, dermatophytic infections are usually noninvasive, but in immunocompromised patients they can rapidly progress to life-threatening disseminated infections. Moreover, some zoophilic and geophilic dermatophytes are responsible for quite severe inflammatory reactions due mainly to delayed hypersensitivity responses to fungi proteases. In addition, in recent years, infections have increased considerably among pediatric and geriatric populations [15, 16].

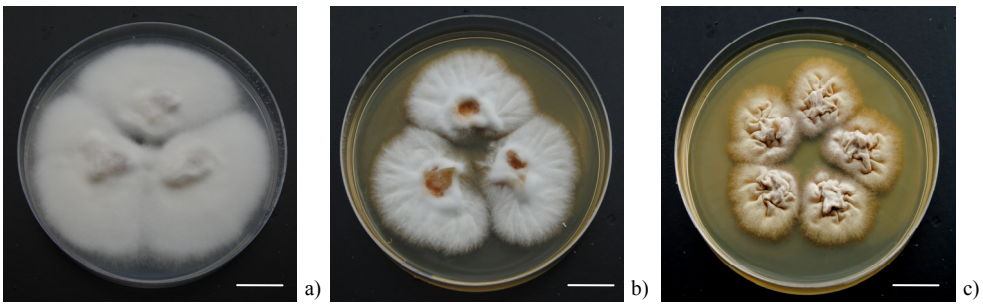

Fig. 1 Morphology of *Trichophyton* sp., *Microsporum* sp. and *Epidermophyton* sp. colonies on Sabouraud dextrose agar after 7 days of culture at 30ºC; Bars = 2cm

Cutaneous dermatophytosis are usually recognized by their scaly patches, with central clearing and sharply demarcated, annular, erythematous, advancing margins, sometimes presenting vesicles, blisters and pustules [11]. However, in many cases, its diagnosis is not clinically obvious, requiring mycological analyses, such as direct microscopic observations, fungi isolation and culture, biochemical and physiological tests and/or molecular approaches [17]. Furthermore, in some cases, it is very difficult to distinguish dermatophytosis from other clinical conditions that also exhibit similar symptoms. For example, *Tinea corporis* may mimic other infections such as nummular eczema, subacute cutaneous lupus erythematosus, pustular psoriasis, subcorneal pustular dermatosis, photoallergic/phototoxic dermatitis, herpes simplex and varicella zoster virus infections [18]. Taking into account treatment costs, duration and side effects of conventional antifungals, an accurate diagnosis is crucial to define which treatments must be applied [17]. Table 1 shows a classification of human dermatophytosis based on tissue keratinisation at the site of infection, as suggested by Degreef [19]. The sites of infection, as well as the common name of the infection, and the species involved are also indicated.

Dermatophytosis is also very common among pets and livestock, yet uncommon in wild animals. Besides the high contagiousness among animal communities and the difficulties in applying effective control measures, long duration of the disease and high costs of treatments are other factors impairing a successful fight against this disease. Also of great concern is the ability of zoophilic dermatophytes to be transmitted to humans (zootonic transmission), hence causing serious public health problems. Among the zoophilic species most commonly involved in these infections are *Microsporum canis*, *Trichophyton mentagrophytes*, *T. equinum* and *T. verrucosum*, as well as geophilic *M. gypseum* [20].

Treatment of dermatophytosis includes both oral and topical formulations mainly from two antifungal drug families: azoles and allylamines [3]. More recently, echinocandins have also been used but only for systemic *Candida* and *Aspergillus* infections [21, 22]. Superficial mycosis (e.g. *Tinea pedis, T. mannum*, *T. corporis and T. cruris*) usually respond to topical antifungals [3, 11, 23]. The most common agents are azoles (eg. clotrimazole, miconazole, econazole, oxiconazole, tioconazole) and allylamines, (e.g. terbinafine and naftifine). Morpholine derivates such as amorolfine and butenafine have been also used [3]. With topical medication, primarily mild skin reactions may occur at the site of application [24]. For patients displaying wide areas of infection or in cases of severe or persistent infections, oral therapy should be considered and the same is true for infections caused by *T. unguium* and *T. capitis*, where terbinafine, itraconazole, fluconazole, griseofulvin, and ketoconazole are the indicated antifungals to be used [3,23]. However, oral formulations may be responsible for major side-effects including hepatotoxicity, neurotoxicity, nephrotoxicity, hematologic reactions and rare skin problems like Stevens-Johnson syndrome. Drug interactions and the consequent reduction of their effectiveness are other causes that must also be evaluated [3, 25, 26]. Some antifungals are inhibitors of enzymes involved in the metabolism of other drugs. For example, it is well known that itraconazole inhibits cytochrome (CYP) 3A4 and, therefore, should not be administrated to patients receiving triazolam, oral midazolam, lovastatin, simvastatin, quinidine or pimozide. Other example is fluconazole which inhibits both CYP 2C9 and CYP 3A4. Thus, cautions should be taken in patients receiving phenytoin, warfarin, cyclosporine, and oral sulfonylurea hypoglycemic agents. A third example is terbinafine a drug that interacts with CYP 1A2 and must not be administered to patients treated with warfarin, nortriptyline or theophylline [26-28]. Also, gastrointestinal interactions may occur with drugs or substances that affect gastric acidity. For example, antacids, histamine-2 receptor blockers, proton pump inhibitors and oral didanosine decrease the absorption of capsule formulations of itraconazole while coca-cola may significantly increase it [28]. Considering the aforementioned statements it can be concluded that the success of dermatophytosis treatments depends not only of the knowledge of the disease but also of other factors such as clinical pattern and severity of the infection, causative agent, and possible drug interactions with concomitant medications as well as patient's preference [29].

Concerning veterinary, only a reduced number of antifungals are readily available and licensed. The same is true for livestock in which the use of systemic drugs is limited due to the use of these animals and their by-products for human consumption [20].

Although conventional antifungals have proved to be effective against many infections, dermatophytosis remains difficult to eradicate due to frequent recurrence, fungi resistance, and side-effects of most antifungal drugs. In order to

improve cure rates it is absolutely necessary to increase the efficiency of treatments. For this purpose either a combination of antifungal therapies such as the use of several oral antifungals with different mechanisms of action, e.g. terbinafine and itraconazole [30-32], or the use of both oral and topical formulations [33-35] may help in the fight against these diseases. More recently a new approach combining conventional antifungals with EO has shown promising results [36].

Table 1 Human dermatophytosis

Type of skin/keratinisation	Site of infection	Disease	Common dermatophyte species
Glabrous skin	Exposed skin	*Tinea corporis* (ringworm of the body)	*Trichophyton rubrum, T. verrucosum, Microsporum canis*
	Inguinal region	*T. cruris* ("Jock itch")	*Tricophyton rubrum*, *T. mentagrophytes* var. *interdigitale*, *Epidermophyton floccosum*
	Face	*T. faciei*	Zoophilic *Trichophyton* species
Highly keratinised skin	Feet	*T. pedis* ("Athlete's foot")	*Tricophyton rubrum*, *T. mentagrophytes* var. *interdigitale*, *Epidermophyton floccosum*
	Hands	*T. manuum*	*Tricophyton rubrum*
Skin rich in terminal hair follicles	Scalp, eyebrows, eyelashes	*T. capitis* (scalp ringworm)	*Tricophyton* spp., *Microsporum* spp.
	Beard, mustache (adult man)	*T. barbae*	*T. mentagrophytes, T. verrucosum*
Nails	Toenails, fingernails	*T. unguium* (onychomycosis)	*Tricophyton rubrum, T. mentagrophytes* var. *interdigitale*

3. Essential oils

EO are natural complex mixtures of terpenic and non-terpenic compounds. In general monoterpenes and sesquiterpenes as well as their oxygenated derivatives are the predominant constituents but phenylpropanoids, fatty acids and their esters may also occur [37]. These secondary metabolites can be found in various plant organs (flowers, fruits, seeds, leaves, stems, and roots) being produced and stored in different secretory structures. The type of structure (secretory cells, osmophores, secretory cavities, secretory ducts, glandular trichomes or epidermal cells) is closely related to the plant family [38]. Anatomical details of these structures are also very relevant to the market value of aromatic plants since they allow the verification of authenticity, detection of substitutions and/or adulterations [39]. In nature EO play important roles as signaling agents namely in the protection of plants against microorganisms, insects, and herbivores, as attractants of pollinators, and in allelopathic interactions [37, 40].

Aromatic plants and their EO have traditionally been used since antiquity for their biological properties (bactericidal, fungicidal, virucidal, antiparasitical, insecticidal), as well as for cosmetic and medicinal applications [37, 41]. In recent years, research on aromatic plants has attracted many researchers and *in vitro* screening programs, based on ethnobotanical approaches, proved to be very efficient in validating traditional uses and providing new ways in the search for active compounds [42]. Nowadays many EO are commercially valued in the pharmaceutical, agronomic, food, sanitary, cosmetic, and perfume industries [37]. The largest global markets for medicinal and aromatic plants are China, France, Germany, Italy, Japan, Spain, United Kingdom and United States of America [43]. In table 2 a summary of the main EO with commercial value is represented.

Several methodologies can be employed to extract EO from plants. However, concerning the International Standard Organization on Essential Oils [44] they must be obtained exclusively by distillation of plant material using water, steam or dry distillation or by expression, this last method being exclusively used to extract compounds from *Citrus* spp. fruits. The EO obtained are characterized as volatile liquids, presenting a strong odour, rarely colored, soluble in organic solvents and insoluble in water. Both wild field-growing and cultivated plants can be used to extract EO or other secondary metabolites. However, for ecological reasons, the gathering of large amounts of plants growing in the wild must be avoided since this can threat the species and reduce biodiversity. Therefore, attention should be shifted towards the development of effective protocols for plant propagation in order to produce a large quantity of plants from which chemicals of interest can be extracted thus preventing the exploitation of wild populations [45]. These approaches allow large-scale propagation in controlled conditions in any time of the year, hence avoiding damage of natural populations [45, 46-48]. The production of EO and other secondary metabolites in plants is under diverse physiological, biochemical, metabolic and genetic regulation [49] and usually shows a variable chemical composition due to both intrinsic (sexual, seasonal, ontogenetic, and genetic variations) and extrinsic (ecological and environmental variations) factors [50, 51]. Therefore, EO quality strongly depends upon all these factors that may interfere and limit plant biomass and oil yield. Furthermore, the common occurrence of chemotypes and ecotypes are major drawbacks which often impair the production of high standard and uniform EO that can compete in global markets. To evaluate EO

quality several procedures are known, namely sensory evaluations, physicochemical tests and chromatrospectral techniques [52]. The latter allow a detailed qualitative and quantitative characterization of the EO, being capillary gas chromatography and mass spectrometry the main techniques employed [53, 54]. Analytical guidelines published by several institutions such as European Pharmacopoeia, ISO, WHO are available and must be followed to assure the good quality of the commercialized EO and of the plants from which they are obtained.

Table 2 Main essential oils produced worldwide (adapted from Lawrence [55])

Plant family	Essential Oils	Species	Top 20
Apiaceae	Ajowan	*Trachyspermum copticum* (L.) Link	
	Anise	*Pimpinella anisum* L.	
	Bitter fennel	*Foeniculum vulgare* Mill. var. *vulgare*	
	Caraway	*Carum carvi* L.	
	Celery seed	*Apium graveolens* L.	
	Coriander	*Coriandrum sativum* L.	18
	Cumin	*Cuminum cyminum* L.	
	Dill weed	*Anethum graveolens* L.	
	European dill seed	*Anethum graveolens* L.	
	Indian dill seed	*Anethum sowa* Roxb. ex Flem.	
	Sweet fennel	*Foeniculum vulgare* Mill. var. *dulce*	
Asteraceae	Armoise	*Artemisia herba-alba* Asso	
	Blue chamomile	*Chamomilla recutita* (L.) Rauschert	
	Davana	*Artemisia pallens* Wall. ex DC	
	Muhuhu	*Brachylaena hutchinsii* Hutch.	
	Roman chamomile	*Anthemis nobilis* L.	
	Sea wormwood	*Artemisia maritima* L.	
	Tarragon	*Artemisia dracunculus* L.	
	Tagetes	*Tagetes minuta* L.	
	Wild chamomile	*Ormenis mixta* Dumort. and *O. multicaulis* Braun-Blanq & Maire	
	Wormwood	*Artemisia absinthum* L.	
Cupressaceae	Cedarwood (Chinese)	*Chamaecyparis funebris* (Endl.) Franco	14
	Cedarwood (USA)	*Juniperus virginiana* L. and *J. ashei* Buchholz	9
Poaceae	Citronella	*Cymbopogon winterianus* Jowitt and *C. nardus* (L.) Rendle	4
Lamiaceae	Basil	*Ocimum basilicum* L.	
	Clary sage	*Salvia sclarea* L.	
	Cornmint	*Mentha arvensis* L. f. *piperascens* Malinv. ex Holmes	2
	Lavandin	*Lavandula intermedia* Emeric ex Loisel	15
	Lavender	*Lavandula angustifolia* Mill.	
	Marjoram	*Origanum majorana* L.	
	Native spearmint	*Mentha spicata* L.	13
	Ocimum	*Ocimum gratissimum* L. *gratissimum*	
	Patchouli	*Pogostemon cablin* (Blanco) Benth.	20
	Peppermint	*Mentha* x *piperita* L.	5
	Rosemary	*Rosmarinus officinalis* L.	
	Sage	*Salvia officinalis* L.	
	Scotch spearmint	*Mentha gracilis* Sole	
	Spike lavender	*Lavandula latifolia* Medik.	
	Thyme	*Thymus zygis* L. and *T. vulgaris* L.	
Lauraceae	Camphor	*Cinnamomum camphora* (L.) J. Presl.	17
	Litsea cubeba	*Litsea cubeba* (Lour.) Pers.	10
	Sassafras (Brazil)	*Ocotea pretiosa* (Nees) Benth.	11
	Sassafras (Chinese)	*Cinnamomum micranthum* (Hayata) Hayata	16
Myrtaceae	Eucalyptus cineole-type	*Eucalyptus globulus* Labill., *E. polybractea* R.T. Baker and other *Eucalyptus* species	3
	Eucalyptus citronellal-type	*Eucalyptus citriodora* Hook.	7
	Clove leaf	*Syzygium aromaticum* (L.) Merr. and L.M. Perry	8
Rutaceae	Grapefruit	*Citrus paradisi* Macfady	19
	Lemon	*Citrus limon* (L.) N.L. Burm.	6
	Lime distilled	*Citrus aurantifolia* (Christm. & Panz.) Swingle	12
	Orange	*Citrus sinensis (L.) Osbeck*	1

4. *In vitro/in vivo* antifungal susceptibility testing in dermatophytes

Dermatophytic infections can be disfiguring, recurrent, and chronic. Besides, they usually require long term treatments [56]. Thus, the development of standard antifungal susceptibility tests for dermatophytes is very useful due to the increase incidence of systemic fungal infections and to the growing number of new antifungal agents used in therapy. According to Espinel-Ingroff [57] an *in vitro* susceptibility test should be able to provide a reliable measure of two or more antifungal agents, correlate the *in vitro* data with *in vivo* activity, predict the therapy outcomes, monitor the development of resistance in a normal susceptible population, and forecast the therapeutic potential of new antifungals. However, antifungal susceptibility testing in filamentous fungi, such as dermatophytes, is far from straightforward due to slow growth rates and dimorphism of certain strains. Furthermore, several characteristics of antifungals such as solubility, stability, modes of action, and partial inhibition ability, may also interfere in the methodology adopted [57]. In 2008, the Clinical and Laboratory Standards Institute (CLSI) approved a reference method (M38-A) for broth dilution antifungal susceptibility testing of filamentous fungi (moulds) responsible for invasive (*Aspergillus* spp., *Fusarium* spp., *Rhizopus* spp., *Pseudallescheria boydii* [*Scedosporium apiospermum*], *Sporothrix schenckii* and other opportunistic moulds) and cutaneous (*Epidermophyton* spp., *Microsporum* spp., *Trichophyton* spp.) infections [58]. This method has been applied to evaluate several antifungal drugs [56, 59] allowing the evaluation of minimal inhibitory concentrations (MIC) and minimal lethal concentrations (MLC). More recently, in 2010, the CLSI developed an alternative reference disk diffusion method (M51-A) to determine antifungal susceptibility of filamentous fungi, but this approach was not applied to dermatophytes [60]. Nevertheless, in the same year, Nweze *et al.* [61] optimized an agar-based disk diffusion method to determine the susceptibility of dermatophytes to antifungal agents. This method seems to be advantageous due to its simplicity [62] and low-cost [63]. Its main limitation is the lack of reproducibility due to differences in diffusion properties of EO components what can result in irregular inhibition zones [64]. Etest (AB Biodisk, Sweden) is another commercially available agar diffusion system and has been used by several researchers [e.g. 65-67]. More recently, the colorimetric-based assay (2,3-bis(2-methoxy-4-nitro-5 [(sulfenylamino)carbonyl]-2*H*-tetrazolium hydroxide, commonly called XTT assay), was also applied to dermatophyte strains [56].

The standard assays, aforementioned and currently used for the evaluation of classical antifungal drugs, can also be used as experimental systems to study the antifungal activity of natural compounds such as EO. However, certain modifications must be considered due to EO complex chemical composition, insolubility in water, and volatility [53]. The most common tests used to assess the antidermatophytic activity of EO are: agar-based disk diffusion [61], agar dilution [68], broth dilution [58], vapor phase activity test [6], poisoned food technique [69] and, more rarely, the overlay bioautographic method [70]. Although several methodologies are available to evaluate the antidermatophytic activity of EO, the lack of standardization in several criteria makes it difficult to compare results obtained by different laboratories. Hadacek and Greger [71] suggested the standardized broth microdilution methodology to meet the demands of all researchers involved in antifungal susceptibility testing of filamentous fungi. The main variables in these kind of assays are the plant material used, the methods used to extract the EO, the role of solvents, the type of strains used (collection or clinical) and their growing conditions, the type of culture medium and pH value, incubation time and temperature, as well as the test assay chosen [72,73]. Also MIC and MLC values may have different definitions according to the test used and the data used to represent them may also differ [e.g. 7, 74].

Some *in vivo* tests regarding EO efficiency, toxicity and applicability have also been performed. The efficiency of antifungal agents on human nails (onychomycosis treatment), has been tested on keratinous structures of animals, namely pigskin [75], ovine [76] and sheep hooves [77, 78]. EO acute toxicity has been evaluated in mice regarding behavior alterations such as trembles, convulsions, dyspnea and ataxia. Lethal doses of EO have also been determined for these animals [79]. Moreover, ointment formulations with oils were assayed for their applicability in guinea-pigs, previously infected with ringworm [80-82]. *In vivo* studies in humans have also been performed using the Patch test method [83]. Ointment formulations, tested on volunteers, were used to determine the maximum tolerable concentrations as well as long-term toxicity for irritant activity of EO [84]. These approaches are required whether a future commercial exploitation of the oils is envisaged.

5. Essential's oil antidermatophytic activity

Several *in vitro* studies have been published confirming the effect of EO and their major compounds on dermatophytic fungi (*Trichophyton*, *Microsporum* and *Epidermophyton*). Some of the most recent studies (last 5 years) on the antidermatophytic activity of EO are represented in Table 3. Other significant screening assays comprising numerous species and, therefore, not reported in Table 3, are summarized below.

A screening assay of 72 EO against *T. mentagrophytes*, using vapour phase test, was carried out by Inouye *et al.* [5]. The most active oils were *Origanum vulgare*, *Thymus serpyllum*, *Eugenia caryophyllata*, *Cymbopogon nardus*, *Pelargonium roseum*, *Lindera umbellata*, *Aniba rosaeodora*, *Thymus vulgaris*, *Lavandula latifolia, L. angustifolia* and *Melaleuca alternifolia*. Since this method is performed in sealed conditions and due to difficulties in applying this *in vivo*, the authors suggested that both potent vapor activity and potent contact activity of the oils would be necessary for anti-infectious therapy [5]. The *in vitro* antifungal activity of several commercial EO against clinical strains isolated

from onychomycosis was also studied by Tullio et al. [6]. For most strains, lower MIC's were obtained using the vapor phase method. *Thymus vulgaris* and *Eugenia caryophyllata* (*Syzigium aromaticum*) oils were the best fungi inhibitors due to the presence of phenolic compounds, namely thymol, carvacrol and eugenol [6]. Moreover, the antidermatophytic activity of seven species of *Artemisia* from Canada was evaluated using the agar diffusion method. *A. bienis* EO, rich in (E)-*β*-farnesene (40%) was the most active [85]. Also, a screening assay on seven Malaysian *Cinnamomum* oils and their main compounds was performed using a microdilution broth method against several dermatophytes. The authors were able to establish a correlation between chemical composition and antifungal activity, showing that the strong antifungal activity of the bark and leaf oils of *C. zeylanicum* was related to the high levels of cinnamaldehyde (44.2%) and eugenol (90.2%) while high amounts of benzyl benzoate (>50%) in the leaf oils of *C. rhynchophyllum*, *C. microphyllum*, *C. pubescens*, *C. impressicostatum*, and *C. mollissimum* were responsible for selective toxicity against dermatophytes [7]. More recently, several EO from Argentina were assayed for their antidermatophytic activity, using a microdilution broth test. The EO of *Acantholippia seriphioides, Gymnophyton polycephalum and Satureja parvifolia* proved to be promising sources for treating dermatophyte-related infections [10].

Combination therapy of available antifungal drugs with EO has also been assessed [86, 87]. However, regarding dermatophytes, very few studies are known. Shin and Lim [88] evaluated the combination of *Pelargonium graveolens* EO and its main compounds (citronellal and geraniol) with ketoconazole, against *Tricophyton* spp. The antifungal activity of ketoconazole was significantly enhanced with the natural compounds and its minimal effective dose was also reduced, hence minimizing possible side-effects. Prun and Shin [89] evaluated the synergism between *Allium* spp. oils and ketoconazole using both a checkerboard titer and disk diffusion tests. A significant synergism between *A. sativum* oil and also allicin with this antifungal drug was demonstrated. Khan and Amhad [90] explored the combinational effect of several active EO and their main compounds with fluconazole against a clinical isolate of *T. rubrum.* The maximum level of synergism was found between cinnamaldehyde and fluconazole. This compound was able to reduce MIC of fluconazole up to 8-fold and reduce its own MIC up to 32-fold. Also the essential oil of *Syzigium aromaticum* showed the highest reduction of MIC (up to 128-fold) in combination with fluconazole.

Several factors may interfere with the amount of biologically active compounds in plants. The main aspects to be considered as well as examples of studies where they have been evidenced are summarized below:

1. Plant organ: EO from *Juniperus oxycedrus* subsp. *oxycedrus* leaves were more effective than those from berries [91]; *Cupressus lusitana* oils obtained from leaves were significantly more active than those from fruits [92]; leaf oils of *Vitex-agnus castus* showed a higher antifungal activity than flower and fruit oils [93].
2. Plant developmental stage: seeds of *Daucus carota* subsp. *halophilus* with high amounts of elemecin provided a more active oil by comparison with oils obtained from flowers [94]; EO from flowering umbels of *Daucus carota* subsp. *carota* from Sardinia were more effective than those from ripe umbels and, on the contrary, ripe umbels from Portugal were more active than flowering ones [95].
3. Plant origin: essential oils of *Crithmum maritimum* from Portugal were rich in monoterpene hydrocarbons, while oils from Sardinia were rich in phenylpropanoids. The oil from Sardinia with high amounts of dillapiole was the most active [96]; essential oils of *Calamintha nepeta* from Sardinia were more active than those from Portugal [97].
4. Activity of main compounds: carvacrol and tymol proved to be very active compounds and may be responsible for the antifungal activity of *Thymus pulegioides* [98] and *T.* x *viciosoi* EO [9]. The importance of phenolic compounds in antimicrobial activity has been described by several authors [e.g. 99, 100]; In *Crithmum maritimum,* dillapiole was associated to the high activity of the oils from Sardinia [96]; in *Ferula hermonis*, the oil fraction rich in jaeschkeanadiol (73%) was the most active [70]; in *Thymus capitellatus* the higher antifungal activity of chemotype 1,8-cineole/linalyl acetate/linalool, was due to linalyl acetate [101].
5. Activity of minor compounds: δ-3-carene, an exclusive compound of *Juniperus oxycedrus* subsp. *oxycedrus* leaf oils, proved to be fundamental for the higher antifungal activity, although it occurred in low quantities [91].
6. Chemotypes: camphor chemotype in *L. pedunculata* [8], 1,8-cineole/linalyl acetate/linalool chemotype in *Thymus capitellatus* [101] and carvacrol type essential oil of *Thymus zygis* subsp *zygis* [74] were the most active. Cultures of the most interesting chemical varieties are encouraged in order to secure high quality and homogeneity in EO.
7. Presence of toxic compounds: the presence of high amounts of toxic compounds in the oils may limit their commercialization. For example, sage EO have restriction uses in some countries due to thujones potential hepatotoxicity and neurotoxicity [102]. Also, *Mentha cervina* and *Calamintha nepeta* should not be used in aromatherapy, due to the presence of pulegone, a toxic compound for the liver [103]. Pinto *et al.* [104] showed that a oil of *Salvia officinalis* with lower contents of thujone was the most effective against dermatophytes, suggesting an alternative use as an antifungal agent; Also, *Mentha cervina* EO with low amounts of pulegone, can be obtained during the vegetative phase of the plant and used in therapeutic approaches for dermatophytosis caused by *Epidermophytum floccosum* [105].
8. *In vitro* test used: EO are complex mixtures of several compounds with various degrees of lipophilicity and relative hydrophilicity due to compounds with polar functional groups [106]. Therefore, EO with compounds with low water solubility dissolve poorly in aqueous medium, and consequently show a weak activity. Vapor phase method normally allows best results due to EO high volatility [107]. Tullio *et al*. [6] obtained lower MIC values using the vapor assay in comparison to broth dilution.

Table 3 Recent studies on the antidermatophytic activity of essential oils

Plant family	Species	Main compounds in the EO	*In vitro* test	Compounds tested	Reference
Amaranthaceae	*Chenopodium ambrosioides*	m-cymene, myrtenol	Poisoned food technique		[82]
Apiaceae	*Crithmum maritimum*	dillapiole, γ-terpinene, sabinene, thymol methyl eter, *β*-phellandrene	Broth macrodilution	dillapiole	[96]
	Daucus carota subsp. *carota*	Sardinia: *β*-bisabolene, 11-*α*-(H)-himachal-4-en-1-β-ol Portugal: geranyl acetate, *α*-pinene	Broth macrodilution		[95]
	Daucus carota subsp. *halophilus*	Flowering umbels: sabinene, *α*-pinene, limonene; Ripe umbels elemicin, sabinene	Broth macrodilution		[94]
	Distichoselinum tenuifolium	myrcene, limonene	Broth macrodilution	myrcene	[108]
	Eryngium duriaei subsp. *juresianum*	α-neocallitropsene, isocaryophyllen-14-al, 14-hydroxy-*β*-caryophyllen, caryophyllene oxide, *E*-*β*-caryophyllene	Broth macrodilution		[109]
	Ferula hermonis	*α*-pinene, *α*-bisabolol, 3,5-nonadiyne	Disk diffusion, bioautographic overlay, broth dilution	active fractions	[70]
Cupressaceae	*Metasequoia glyptostroboides*	-	Disk diffusion, broth dilution, spore germination, growth kinetics		[110]
Hypericaceae	*Hypericum perforatum*	terpinen-4-ol	Broth microdilution, time killing assay		[111]
Lamiaceae	*Calamintha nepeta subsp. nepeta*	Sardinia: pulegone Portugal: isomenthone, 1,8-cineole	Broth macrodilution		[97]
	Lavandula pedunculata	1,8-cineole, fenchone, camphor	Broth macrodilution	1,8-cineole, fenchone, camphor	[8]
	Lavandula viridis	1,8-cineole, camphor, *α*-pinene, linalool	Broth macrodilution	1,8-cineole camphor, *α*-pinene, linalool	[112]
	Mentha cervina	pulegone, isomenthone	Broth macrodilution		[105]
	Salvia officinalis	*cis*-thujone, *β*-pinene, 1,8-cineole, *α*-humulene	Broth macrodilution		[104]
	Thymus x *viciosoi*	carvacrol, *p*-cymene, thymol	Broth macrodilution	carvacrol, thymol, *p*-cymene,	[9]
	Thymus zygis subsp. *sylvestris*	chemotypes: carvacrol, thymol, geranyl acetate/geraniol, linalool	Broth macrodilution		[74]
Moringaceae	*Moringa oleifera*	pentacosane, hexacosane	Broth microdilution		[113]
Poaceae	*Cymbopogon martini*	*trans* geraniol, *β*-elemene	Poisoned food technique		[82]
	Cymbopogon winterianus	-	Disk diffusion, broth microdilution, mycelium growth and morphology		[114]
Verbenaceae	*Vitex agnus-castus*	Leaves: bicyclogermacrene , (E)-*β*-farnesene, 1,8-cineole flowers: bicyclogermacrene, manool, fruits: (E)-*β*-farnesene, bicyclogermacrene, 1,8-cineole	Broth macrodilution		[93]
	Vitex rivularis	germacrene D , *γ*-curcumene, ar-curcume, *α*-copaene, *β*-caryophyllene	Broth macrodilution		[115]
Zingiberaceae	*Curcuma longa*	terpinolene, *α*-phellendren, terpinen-4-ol	Poisoned food technique, time killing assay		[84]

6. Essential's oil mechanism of action on dermatophytes

The knowledge of both the mode and mechanism of action of EO is crucial to ensure their usefulness in therapeutic practices [53]. While EO have been extensively screened for their antifungal activity, interaction between the oils and microorganisms, which is lately responsible for its activity, is poorly understood. Regarding the few studies on this matter, *Candida* spp. and *Aspergillus* spp. have been the species mostly used [116-118] and, therefore, very little information is available for dermatophytes. The main studies on the evaluation of EO mode and mechanism of action on dermatophytes are summarized below. Pinto *et al.* [98] evaluated the ergosterol content of *T. rubrum* and showed that 0.08 µL/mL of *Thymus pulegioides* oil was able to reduce ergosterol content around 70%. A mechanism of action based on impairment of the biosynthesis of ergosterol was suggested as also occurs with conventional azole antifungal drugs [119]. Inouye *et al.* [120] through scanning electron microscopic observations showed that oregano EO were able to damage the cell membrane and cell wall in a dose and time dependent manner. Park *et al.* [121] analysed the mechanism of action of eugenol, a main compound in *Syzygium aromaticum* EO. Modifications in *T. mentagrophytes* hiphae ultrastructure were observed, namely destruction of inner mitochondrial membranes and cell wall as well as expansion of endoplasmic reticulum near cell membranes, suggesting a mechanism of action through changes in fungal cell structure, particularly at the membrane level. Bajpai *et al.* [122] performed a spore germination assay using several *T. rubrum* and *M. canis* strains as well as one strain of *T. mentagrophytes*. *Nandina domestica* oil was used and showed a strong detrimental effect on all the strains tested. Also a kinetic study of the oil was performed on *T. rubrum* KCTC 6375, showing a time-dependent kinetic inhibition of this fungus. Khan and Amhad [30] performed a time-killing dependent assay on *T. rubrum* IOA-9 to compare the ability of potent EO and active compounds with fluconazole. Cellular toxicity was assayed using red blood cell lines from sheep. No haemolysis was recorded at minimal fungicidal concentrations of the oils. Finally, electron transmission microscopy was used to detect ultastructural changes in the presence of cinnamaldehyde. Alterations included lysis of cell wall and plasma membranes, endoplasmic reticulum expansion near cell membrane, excessive vacuolization, disintegration of mitochondria, plasma membranes, cell walls, and nuclear and cytoplasmic contents, abnormal distribution of polysaccharides and leakage of cytoplasmic contents.
In general, the most active antifungal compounds of EO are mainly phenolic terpenes such as carvacrol and thymol. These compounds proved to be able to attack cell walls and membranes, affecting the permeability and release of intracellular constituents, as well as several invasive targets, allowing all together inhibition of fungal infection [122]. Many EO have also shown fungicidal activity against dermatophyte strains (MIC values equivalent to MLC values) [e.g. 8, 97, 104, 105, 108, 112]. Overall it seems that the antifungal activity of EO is not due to a single mechanism of action but may result from the effect of different compounds on several cell targets.

7. Conclusions

EO have proved, in several *in vitro* assays, to be useful alternatives to conventional antifungals for the treatment of dermatophytosis. Moreover, it seems unlikely that resistance may occur with their use since multiple mutations are required to overcome all the distinct antifungal actions of each and all of the oils constituents [118]. However, to guarantee their safety, further toxicity studies need to be performed as well as assays to clarify the mechanism of action and possible interactions with antibiotics or other compounds. The optimization of formulations, the establishment of optimal concentrations for clinical applications and the search for possible side-effects are together research lines that need to be highlighted.

Acknowledgements The support by CEF/POCI2010/FEDER and a PhD fellowship to Mónica Zuzarte (SFRH/BD/40218/2007) is gratefully acknowledged.

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