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REVIEW ARTICLE

Meta-analysis of the antifungal activities of three essential oils as alternative therapies in dermatophytosis infections

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Abstract

Aims: This work examines the available scientific evidence about the efficiency of essential oils (EO) as an alternative therapy to traditional treatment of fungal infections, including onychomycosis, assessing the effect of the three EO most frequently studied for their antifungal activity (thyme, cinnamon and tea tree EO) against three causative agents of fungal diseases in humans: *Trichophyton rubrum*, *Trichophyton mentagrophytes* complex and *Candida albicans*.

Methods and Results: The PRISMA statement protocol was followed to conduct a bibliographical search and 54 articles that met all the inclusion criteria were retrieved. Differences were observed in the MIC and MFC values depending on the micro-organism strain and the EO used. The lowest MIC were observed with *Cinnamomum zeylanicum* EO (0.013–1120 μl ml^{-1}) against the three micro-organisms. For MFC, the lowest value was found for *Thymus vulgaris* EO (4.2 μl ml^{-1}) against *Trichophyton rubrum*.

Conclusions: The antifungal effects of EO could be a very promising solution to overcome the therapeutic shortcomings of antimycotic medication. More experiments are needed to examine the properties of these oils to devise effective and non-aggressive therapies for treatment of dermatophytosis.

Significance and Impact of Study: The results indicate that EO remain good candidates for future treatments and could provide a solution for failed medications and/or adverse reactions to current pharmacological treatments.

KEYWORDS

antifungal treatment, *Candida albicans*, *Cinnamomum zeylanicum*, dermatophytosis, *Melaleuca alternifolia*, *Thymus vulgaris*, *Trichophyton mentagrophytes* complex, *Trichophyton rubrum*

INTRODUCTION

Pathogenic fungi cause infections in humans, plants and animals. Dermatophytosis is a fungal infection caused by dermatophytes. It is the main cause of superficial mycosis and presents a significant public health problem (Mahmoudvand et al., 2014). Dermatophytes are divided into three genera (*Trichophyton (T), Microsporum (M), Epidermophyton (E)*) and have an affinity for keratinized tissues, in which they are able to grow, for example, hair, skin and nails (Abd Rashed et al., 2021; Ahmadi et al., 2015). Other fungal infections of human skin and its

appendages can be caused by nondermatophyte fungi, such as yeasts (*Candida (C), Pitorosporum (P), Cryptococcus (C)*) and mould fungi (*Aspergillus, Scopulariopsis*) (Bhatia & Sharma, 2014). Onychomycosis is the dermatophytosis that affects the nails (Hoy et al., 2012; Vlahovic, 2016). Because the nail unit has no immunity mediated by effective cells, it is more susceptible than other parts of the skin to infection by these micro-organisms (Lipner & Scher, 2019). Approximately 90% of toenail onychomycoses and 75% of all nail onychomycoses are caused by dermatophytes, in particular *Trichophyton mentagrophytes* complex and *Trichophyton rubrum* (Bodman & Krishnamurthy, 2021; Gupta et al., 2015; Joyce et al., 2019; Thomas et al., 2010; Youssef et al., 2018). In the case of yeast onychomycoses, *Candida albicans* accounts for approximately 70% of all onychomycoses caused by yeast (Hoy et al., 2012).

Fungal infections are increasing due to factors including their spread among at-risk populations, population longevity, the use of immunosuppressive treatments, diabetes mellitus, obesity, hyperhidrosis and nail injury (Hoy et al., 2012; Leung et al., 2020; Papini et al., 2015; Tchernev et al., 2012; Thomas et al., 2010). However, oral treatment of these infections has disadvantages, including increased resistance due to overuse of antifungal agents, pharmacological interactions with other medicines and adverse side effects such as the risk of hepatic lesion, thus limiting the suitability of this type of treatment (Abd Rashed et al., 2021; Martín-Aragón & Benedí, 2004; Valdes & M. P., 2000; Vlahovic, 2016). Moreover, some oral treatments, such as terbinafine, are ineffective against nondermatophyte fungi (Chang et al., 2007; Hoy et al., 2012). Another key consideration is that oral antimycotic therapies can affect the pharmacokinetics of previously prescribed medications and may change their effects, ranging from reduced efficiency to increased toxicity (Gupta et al., 2018). Because of this, a more appropriate strategy for antifungal treatment could be to develop alternative therapies to conventional treatments. Alternative therapies currently under investigation include the use of essential oils (EO) as possible antifungal agents (Abd Rashed et al., 2021; Hongpattarakere et al., 2008; Lopes et al., 2016; Mahmoudvand et al., 2014; Parrish et al., 2020).

The European Pharmacopoeia Commission recently adopted the following definition of EO (Agencia Española de Medicamentos y Productos Sanitarios, 2016; European Pharmacopoeia: Essential oils, 2021):

> *Odorous product, usually of complex composition, obtained from a botanically defined plant raw material by steam distillation, dry distillation, or a suitable mechanical process without heating.*

Although numerous studies indicate the suitability of EO as an alternative treatment for fungal infections, the properties of each oil largely depend on its composition (da Cruz Cabral et al. 2013; D'agostino et al., 2019; Abd Rashed et al., 2021). As the definition of EO implies, composition can vary due to several factors, including the extraction method, the type and species of plant they are obtained from, the soil composition and the plant growth stage at harvest (European Pharmacopoeia: Essential oils, 2021). Due to the difficulty of attributing a specific effect to a particular compound or various compounds (Lopes et al., 2016), it is important to determine EO composition through chemical analyses such as gas chromatography and mass spectrometry (GC–MS) to verify and standardize the composition of EO and ensure consistency between batch lots over time (Parrish et al., 2020).

The specific mechanism of action of EO remains unclear (Abd Rashed et al., 2021; Parrish et al., 2020). Initially a process of permeabilization was proposed (Flores et al., 2015), in which the EO break down the fungal cell wall and cytoplasmic membranes. Changes in cytoplasmic membrane permeability affect electrolyte balance and may even cause a loss of cell content. Essential oils also impact the mitochondrial membranes by altering their polarity, affecting the flow of protons and the ability to produce ATP (Abd Rashed et al., 2021; Saad et al., 2013; Swamy et al., 2016).

According to some authors, certain EO components are irritating to the skin and mucous membranes and can cause significant damage at high doses (Carson et al., 2006; Lee et al., 2013). Even though the content of these components is minimal in EO, there are no guarantees that adverse reactions will be minimized.

Numerous studies have demonstrated the antifungal action of various commercial EO in studies in vitro (Córdoba et al., 2019; Gucwa et al., 2018; Michalczyk & Ostrowska, 2021; Villar Rodríguez et al., 2021; Wińska et al., 2019). The parameters most frequently used to assess their antimicrobial activity are minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC), that is, the minimum concentration of antifungal agents needed to inhibit growth of the fungus or reduce its growth by 99.9%, respectively (Abd Rashed et al., 2021; Natu & Tatke, 2019; Sharifi-Rad et al., 2017). The EO most frequently studied for their antifungal activity are thyme (*Thymus vulgaris*), cinnamon (*Cinnamomum zeylanicum*) and tea tree (*Melaleuca alternifolia*) (D'agostino et al., 2019; Scalas et al., 2018). Thyme EO is used as an antifungal agent because of its high thymol and carvacrol content (Kowalczyk et al., 2020; Pinto et al., 2006), while cinnamon EO contains eugenol and cinnamaldehyde (Khan & Ahmad, 2011) and tea tree EO contains terpenic

hydrocarbons, mainly monoterpenes and sesquiterpenes and their associated alcohols (Carson et al., 2006; Singh, 2001).

To examine the available scientific evidence on the efficiency of EO as an alternative therapy to traditional treatment of fungal infections, we analysed studies that used three EO (thyme, cinnamon and tea tree) against three of the causative agents of the most frequent fungal diseases in humans: *T. mentagrophytes complex, T. rubrum* and *C. albicans*.

MATERIAL AND METHOD

The study was conducted following the PRISMA statement protocol (Urrútia & Bonfill, 2010). A refined search of articles was carried out in September 2021 using two search engines (PubMed and WoS). The search terms were: "essential oils", "onychomycosis", "antifung*" and "fungal*", combining them with the EO "thymus vulgari*", "cinnamomum zeylanicu*", "tea tree oil", "melaleuca alternifol*" and the micro-organisms "rubru*", "mentagrop*", "candida albican*" and the boolean operator AND. The search term "mentagrop*" includes all species of the *Trichophyton mentagrophytes* complex, composed of five species: *T. mentagrophytes, T. interdigitale, T. erinacei, T. quinckeanum* and *T. benhamie*.

The following search formulas were used: "essential oils AND onychomycosis AND antifung* AND thymus vulgari*", "essential oils AND antifung* AND thymus vulgari*", "essential oils AND fungal* AND thymus vulgari*", "essential oils AND antifung* AND thymus vulgari* AND rubru*", "essential oils AND antifung* AND thymus vulgari* AND mentagrop*", "essential oils AND antifung* AND thymus vulgari* AND candida albicans*", "essential oils AND onychomycosis AND antifung* AND cinnamomum zeylanicum*", "essential oils AND antifung* AND cinnamomum zeylanicum*", "essential oils AND fungal* AND cinnamomum zeylanicum*", "essential oils AND antifung* AND cinnamomum zeylanicum* AND rubru*", "essential oils AND antifung* AND cinnamomum zeylanicum* AND mentagrop*", "essential oils AND antifung* AND cinnamomum zeylanicum* AND candida albicans*", "essential oils AND onychomycosis AND antifung* AND melaleuca alternifol*", "essential oils AND antifung* AND melaleuca alternifol*", "essential oils AND fungal* AND melaleuca alternifol*", "essential oils AND antifung* AND melaleuca alternifol* AND rubru*", "essential oils AND antifung* AND melaleuca alternifol* AND mentagrop*", "essential oils AND antifung* AND melaleuca alternifol* AND candida albicans*", *", "essential oils AND onychomycosis AND antifung* AND tea tree oil*", "essential oils AND antifung* AND tea tree oil*", "essential oils AND fungal* AND tea tree oil*", "essential oils AND antifung* AND tea tree oil* AND rubru*", "essential oils AND antifung* AND tea tree oil* AND mentagrop*", "essential oils AND antifung* AND tea tree oil* AND candida albicans*".

The articles retrieved using this search strategy are shown in the flow diagram in Figure 1.

The inclusion criteria for accepting articles for the study were: clinical studies performed in vitro and in vivo clinical trials with humans and animals with the commercial EO specified as antimycotic treatment against reference strains of the selected micro-organisms and with clinical samples of nails infected with onychomycosis by *T. rubrum* and *T. mentagrophytes complex*, following the protocols CLSI M38-A and/or EUCAST E. Def.11.0 for dermatophytes and CLSI M27-A2 and/or E. Def.7.3.2 for the yeasts; studies that determined the MIC and MFC; concentration measurement expressed as a percentage (%) and/or v/v ($\mu l\,ml^{-1}$); written in English and/or Spanish; and published from January 2001 to September 2021.

Articles were excluded when they had the search terms but addressed fungal infections elsewhere (food, crops, etc.). Studies in which the micro-organisms came from clinical samples other than those specified and articles about other types of EO were also excluded. Similarly, others articles that did not follow the protocols indicated, were not written in English and/or Spanish, or were published outside the period 2001 to 2021 were not accepted for the study.

After the initial search in the databases, 981 articles were examined (298 from PubMed and 683 from WoS) and 279 were excluded as duplicates. After the articles had been read and the exclusion criteria had been applied, only 54 were selected for analysis and included in the final review. The 54 articles were classified into three categories: 24 for *T. vulgaris* EO, 15 for *C. zeylanicum* EO and 22 for *M. alternifolia* EO. Seven articles had several EO in common (see Figure 1).

RESULTS AND DISCUSSION

The data obtained from the articles reviewed show that the three EO have antifungal activity against *C. albicans, T. mentagrophytes complex* and *T. rubrum*, each with a specific concentration, as shown in the MIC and MFC data. Table 1 shows the range of MIC and MFC values of the three EO for the three micro-organisms:

The MIC values of *T. vulgaris* EO against *C. albicans* range from 0.16 to 3120 $\mu l\,ml^{-1}$ and the MFC values range from 0.32 to 12,500 $\mu l\,ml^{-1}$ (Alves et al., 2010; Assiri et al., 2016; Baj et al., 2020; Bogavac et al., 2015; Chaftar et al., 2015; Das Neves et al., 2009; Gavanji & Larki, 2015; Giamperi et al., 2002; López et al., 2007;

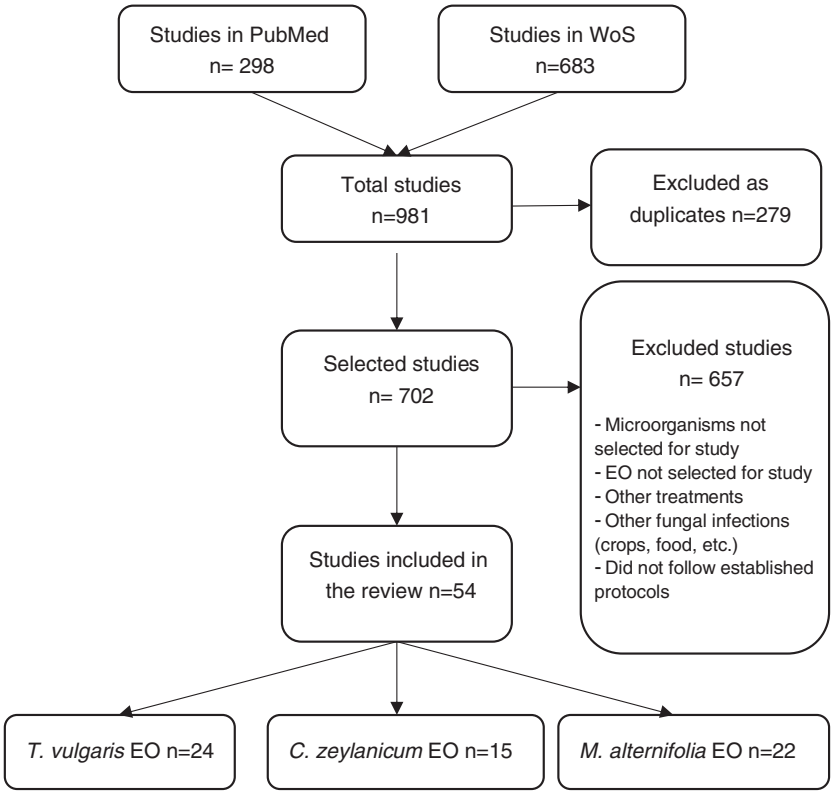

FIGURE 1 Flow diagram of the article selection process using the defined search strategy

Nzeako & Bushra, 2008; Orchard et al., 2017; Pina-Vaz et al., 2004; Pinto et al., 2006; Sacchetti et al., 2004; Sartoratto et al., 2004; Simo Kamdem et al., 2015; van Vuuren et al., 2009). Other authors, for example, Donaldson et al. (2005), Rajkowska et al. (2014, 2016) and Gucwa et al. (2018), expressed the MIC of *T. vulgaris* EO against *C. albicans* as a percentage, from 0.04 to 0.31% (Donaldson et al., 2005; Gucwa et al., 2018; Rajkowska et al., 2014, 2016). For *T. mentagrophytes* sp., the MIC values are 2.2–2000 μl ml^{-1} and the MFC values are 3–460 μl ml^{-1} (Bozin et al., 2006; Giamperi et al., 2002; Inouye et al., 2001; Orchard et al., 2017; Simo Kamdem et al., 2015). For *T. rubrum*, the MIC values are 0.32–100 μl ml^{-1} and the MFC values are 4.2 μl ml^{-1} (Bozin et al., 2006; Inouye et al., 2001; Khan et al., 2014) (see Table 1).

The MIC values of *C. zeylanicum* EO against *C. albicans* range from 0.013 to 1120 μl ml^{-1} and the MFC values range from 52 to 2500 μl ml^{-1} (Castro & Lima, 2013; Elgammal et al., 2020; Gavanji & Larki, 2015; Jantan et al., 2008; López et al., 2007; Miller et al., 2014; Orchard et al., 2017; Rangel et al., 2018; Unlu et al., 2010). In the studies by Tran et al. (2020) and Veilleux and Grenier (2019), the antifungal action is expressed as a percentage: the MIC of *C. zeylanicum* EO against *C. albicans* is <0.03–0.039% and the MFC is <0.03–0.078% (Tran et al., 2020; Veilleux & Grenier, 2019). For *T. mentagrophytes* sp., the MIC values are 0.08–250 μl ml^{-1} and the MFC values are 125–1000 μl ml^{-1} (Ayatollahi Mousavi & Kazemi, 2015; Inouye et al., 2001; Jantan et al., 2008; Makimori et al., 2020; Orchard et al., 2017). For *T. rubrum*, only the MIC values were obtained, ranging from 0.08 to 12.5 μl ml^{-1} (Inouye et al., 2001; Jantan et al., 2008) (see Table 1).

The MIC values of *M. alternifolia* EO against *C. albicans* are 9.7–8960 μl ml^{-1} and the MFC are 10,000–17,929 μl ml^{-1} (Francisconi et al., 2020; Juliano et al., 2008; Noumi et al., 2011; Orchard et al., 2017; Rosato et al., 2008; van Vuuren et al., 2009). In the studies by Haba et al. (2014), Mertas et al. (2015), Mondello et al. (2006), Hammer et al. (2003), Rajkowska et al. (2014, 2016) and Rosato et al. (2021) on *M. alternifolia* EO against *C. albicans*, the MIC value was 0.008–0.5% and the MFC value was 0.25–0.5% (Haba et al., 2014; Hammer et al., 2003; Mertas et al., 2015; Mondello et al., 2006; Rajkowska et al., 2014, 2016; Rosato et al., 2021). For *T. mentagrophytes* sp. and *T. rubrum*, only the concentration in μl ml^{-1} of the MIC was obtained, with values of 250–800 μl ml^{-1} and 400 μl ml^{-1}, respectively (Inouye et al., 2001; Orchard et al., 2017). For *T. mentagrophytes* sp. and *T. rubrum*, the MIC values are 0.3% and 0.06–0.3% and the MFC values are 0.5% and 0.06–0.25%, respectively (Hammer, 2002; Roana et al., 2021) (see Table 1).

Table 1 shows that *C. zeylanicum* EO has the lowest MIC values against the three micro-organisms and *T. vulgaris* EO has the lowest MFC values against *T. rubrum* and *T. mentagrophytes* sp.

Table 2 shows the range of MIC and MFC values of the three EO against the micro-organisms *T. rubrum* and *T.*

TABLE 1 Range of MIC and MFC values of the EO for each micro-organism

	C. albicans		*T. Mentagrophytes* sp.		*T. rubrum*	
EO	MIC	MFC	MIC	MFC	MIC	MFC
T. vulgaris	0.016–3120[b] (Alves et al., 2010; Baj et al., 2020; Bogavac et al., 2015; Chaftar et al., 2015; Das Neves et al., 2009; Gavanji & Larki, 2015; Giamperi et al., 2002; López et al., 2007; Nzeako & Bushra, 2008; Orchard et al., 2017; Pina-Vaz et al., 2004; Pinto et al., 2006; Sacchetti et al., 2004; Sartoratto et al., 2004; Simo Kamdem et al., 2015; van Vuuren et al., 2009)	0.32–12,500[b] (Alves et al., 2010; Assiri et al., 2016; Baj et al., 2020; Bogavac et al., 2015; Gavanji & Larki, 2015; Giamperi et al., 2002; Pina-Vaz et al., 2004; Pinto et al., 2006; Simo Kamdem et al., 2015)	2.2–2000[b] (Bozin et al., 2006; Giamperi et al., 2002; Inouye et al., 2001; Orchard et al., 2017; Simo Kamdem et al., 2015)	3–460[b] (Bozin et al., 2006; Giamperi et al., 2002; Simo Kamdem et al., 2015)	0.32–100[b] (Bozin et al., 2006; Inouye et al., 2001; Khan et al., 2014)	4.2[b] (Bozin et al., 2006)
	0.04–0.31[a] (Donaldson et al., 2005; Gucwa et al., 2018; Rajkowska et al., 2014, 2016)					
C. zeylanicum	0.013–1120[b] (Castro & Lima, 2013; Elgammal et al., 2020; Gavanji & Larki, 2015; Jantan et al., 2008; López et al., 2007; Miller et al., 2014; Orchard et al., 2017; Rangel et al., 2018; Unlu et al., 2010)	52–2500[b] (Castro & Lima, 2013; Gavanji & Larki, 2015; Rangel et al., 2018)	0.08–250[b] (Inouye et al., 2001; Jantan et al. 2008; Ayatollahi Mousavi & Kazemi, 2015; Orchard et al., 2017; Makimori et al., 2020)	125–1000[b] (Ayatollahi Mousavi & Kazemi, 2015; Makimori et al., 2020)	0.08–12.5[b] (Inouye et al., 2001; Jantan et al. 2008)	–
	<0.03–0.039[a] (Tran et al., 2020; Veilleux & Grenier, 2019)	<0.03–0.078[a] (Tran et al., 2020; Veilleux & Grenier, 2019)				
M. alternifolia	9.7–8960[b] (Francisconi et al., 2020; Juliano et al., 2008; Noumi et al., 2011; Orchard et al., 2017; Rosato et al., 2008; van Vuuren et al., 2009)	10,000 – 17,929[b]	250–800[b]	–	400[b] (Inouye et al., 2001)	–
	0.008–0.5[a] (Haba et al., 2014; Hammer et al., 2003; Mertas et al., 2015; Mondello et al., 2006; Rajkowska et al., 2014, 2016; Rosato et al., 2008, 2021)	0.25–0.5[a] (Hammer, 2002; Roana et al., 2021)	0.3[a] (Hammer, 2002; Roana et al., 2021)	0.5[a] (Hammer, 2002; Roana et al., 2021)	0.06–0.3[a] (Hammer, 2002; Roana et al., 2021)	0.06–0.25[a] (Hammer, 2002; Roana et al., 2021)

Abbreviations: MIC, Minimum inhibitory concentration; MFC, Minimum fungicidal concentration. Ref., reference.

[a]Concentration measurements of the oil in percentage (%).

[b]Concentration measurements of the oil in v/v ($\mu l\,ml^{-1}$).

TABLE 2 Range of MIC and MFC values of the EO against *T. rubrum* and *T. mentagrophytes* isolated from onychomycosis clinical samples

EO	*T. Mentagrophytes* sp.		*T. rubrum*		Ref.
	MIC	MFC	MIC	MFC	
T. vulgaris	2.2[b]	4[b]	2[b]	4.2[b]	(Bozin et al., 2006)
M. alternifolia	0.3[a]	0.5[a]	0.06–0.3[a]	0.06–0.25[a]	(Hammer, 2002; Roana et al., 2021)

Abbreviations: MIC: Minimum inhibitory concentration; MFC: Minimum fungicidal concentration. Ref.: reference.

[a]Concentration measurements of the oil in percentage (%).

[b]Concentration measurements of the oil in v/v ($\mu l\,ml^{-1}$).

mentagrophytes sp. isolated from onychomycosis clinical samples. It can be seen that *T. rubrum* requires a lower concentration of the two EO used to inhibit growth. No studies were found with onychomycosis clinical samples using *C. zeylanicum* EO.

The micro-organism strain of *C. albicans* used in each study and the MIC and MFC values obtained in the two units of measure identified ($\mu l\,ml^{-1}$ and %) are shown in Table 3. The final column indicates the study that reported the data for each strain used.

Tables 4 and 5 show the same data as Table 3 but refer to the micro-organism strain of *T. mentagrophytes* sp. and *T. rubrum* used in each study and the MIC and MFC values obtained in $\mu l\ ml^{-1}$.

Tables 2 to 5 show that T. *vulgaris* EO has been more frequently mentioned in studies than the other two EO chosen, as 24 articles address the activity of this EO against the micro-organisms selected. The micro-organism found in more in vitro tests is *C. albicans*, for the three EO. The most frequently used reference strain of this micro-organism is ATCC 10231 (26), followed by strain ATCC 90028 (7) (see Table 3).

Only Orchard et al. (2017) and Inouye et al. (2001) studied *M. alternifolia* EO against *T. mentagrophytes* sp. and *T. rubrum* with standard strains, and no data were obtained about their MFC except against *C. albicans* (see Tables 3, 4 and 5) (Inouye et al., 2001; Orchard et al., 2017). In contrast, Hammer (2002) and Roana et al. (2021) determined the MIC and MFC values for *T. mentagrophytes* sp. and *T. rubrum* using onychomycosis clinical samples (Hammer, 2002; Roana et al., 2021). The same occurred with *T. vulgaris* and *C. zeylanicum* EO for *T. rubrum*, for which no MFC data were obtained (Das Neves et al., 2009; Miller et al., 2014; van Vuuren et al., 2009) (see Table 5). However, Bozin et al. (2006) reported data on the MFC of *T. vulgaris* EO against *T. rubrum* using onychomycosis clinical samples (Bozin et al., 2006). Only three studies using onychomycosis clinical samples were found, with the micro-organisms *T. rubrum* and *T. mentagrophytes* sp. and *T. vulgaris* and *M. alternifolia* EO. The two EO show very similar values for the two micro-organisms, but are not comparable because the authors measured MIC and MFC with different concentration measures (see Table 2). These two micro-organisms are particularly significant for podiatrists, as 90% of toenail onychomycoses are caused by these dermatophytes (Bodman & Krishnamurthy, 2021; Gupta et al., 2015; Joyce et al., 2019; Thomas et al., 2010; Youssef et al., 2018).

With regard to in vivo studies, tests were found about the antifungal activity of EO on the selected micro-organisms. We found three in vivo clinical tests on humans reporting antifungal efficacy of EO against *T. rubrum* (Romero-Cerecero et al., 2008, 2009), but only one of the tests used *M. alternifolia* EO among its components, showing complete cure in 12 months in 78.5% ($n = 14$) of patients with onychomycosis caused by *T. rubrum* (Alessandrini et al., 2020). Another in vivo clinical test was performed on patients with palatal inflammation due to denture stomatitis (n = 27) caused by *C. albicans*, in which the inflammation in the mouth decreased (Catalán et al., 2008). Various clinical tests on animals (mice, rats and guinea pigs) were also found, as shown in Table 6.

Of the three micro-organisms studied, *C. albicans* shows the lowest MIC values with the three EO (see Table 1). Both *T. rubrum* and *T. mentagrophytes* sp. show the lowest MIC values with *C. zeylanicum*.

The studies reviewed revealed different MIC and MFC values depending on the micro-organism strain and the commercial EO used. This may depend on where the EO is from, its chemical composition and the research methodology used to obtain the values (Parrish et al., 2020), although further study is necessary to demonstrate this. The MIC and MFC values of *C. albicans* with the three EO are very broad, due to the high number of studies addressing this pathogen (see Table 1). The lowest MIC range found was for *C. zeylanicum* EO against *T. rubrum*, and the lowest MFC was for *T. vulgaris* EO against *T. rubrum*, which may be due to the few studies with these EO for this micro-organism, and therefore more studies are needed to determine the ideal concentration (see Table 1). Each specific oil needs to be studied against each specific pathogen

TABLE 3 Reference strains of *C. albicans* with their respective MIC and MFC for the three EO: *T. vulgaris*, *C. zeylanicum* and *M. alternifolia*

C. albicans	*T. vulgaris*			*C. zeylanicum*				*M. alternifolia*				Ref.
Strain	**MIC***	**MFC***	**MIC†**	**MIC***	**MFC***	**MIC†**	**MFC†**	**MIC***	**MFC***	**MIC†**	**MFC†**	
ATCC 10231			0.04									(Gucwa et al., 2018)
ATCC 10231	0.64	0.64										(Pinto et al., 2006)
ATCC 10231			0.25							0.5		(Rajkowska et al., 2014)
ATCC 10231			0.25							0.5		(Rajkowska et al., 2016)
ATCC 10231	0.16–0.32	0.32										(Pina-Vaz et al., 2004)
ATCC 10231	72											(Sacchetti et al., 2004)
ATCC 10231	86	137		32	52							(Gavanji & Larki, 2015)
ATCC 10231	200											(Sartoratto et al., 2004)
ATCC 10231	320											(Das Neves et al., 2009)
ATCC 10231		320										(Assiri et al., 2016)
ATCC 10231	3300							6000				(van Vuuren et al., 2009)
ATCC 10231	460											(Nzeako & Bushra, 2008)
ATCC 10231	1000			500				1.500				(Orchard et al., 2017)
ATCC 24433	550	550										(Simo Kamdem et al., 2015)
ATCC 10231	1250	2500										(Baj et al., 2020)
ATCC 10231	0.11	0.23										(Bogavac et al., 2015)
ATCC 10231				0.16–0.63								(Jantan et al. 2008)
ATCC 10231				3.3								(Elgammal et al., 2020)
ATCC 10231				70								(Unlu et al., 2010)
ATCC 10231						0.03–0.13	0.03–0.25					(Tran et al., 2020)
ATCC 10231								500				(Juliano et al., 2008)
ATCC 10231								3500				(Rosato et al., 2008)
ATCC 10231										0.008		(Haba et al., 2014)
ATCC 10231										0.125	0.25	(Mertas et al., 2015)
ATCC 10231										0.25		(Mondello et al., 2006)
ATCC 10231										0.5	0.5	(Hammer et al., 2003)
ATCC 10231										0.5		(Rosato et al., 2021)
ATCC 2091	525	0.60										(Giamperi et al., 2002)
ATCC 2091						<0.03–0.13	<0.03–0.25					(Tran et al., 2020)
ATCC 2091								9.7	>10,000			(Noumi et al., 2011)

(Continues)

TABLE 3 (Continued)

C. albicans	*T. vulgaris*			*C. zeylanicum*				*M. alternifolia*				Ref.
Strain	**MIC***	**MFC***	**MIC†**	**MIC***	**MFC***	**MIC†**	**MFC†**	**MIC***	**MFC***	**MIC†**	**MFC†**	
ATCC 90028			0.31									(Donaldson et al., 2005)
ATCC 90028				0.31–1.25								(Miller et al. 2015)
ATCC 90028				1120								(Unlu et al., 2010)
ATCC 90028								312	>10,000			(Noumi et al., 2011)
ATCC 90028										0.5		(Rosato et al., 2008)
ATCC 90028								8960	17,920			(Francisconi et al., 2020)
ATCC 90028										0.25	0.5	(Hammer et al., 2003)
ATCC 90029				125	125							(Rangel et al., 2018)
ATCC 90029										0.25		(Mondello et al., 2006)
ATCC 64550	0.026			0.013								(López et al., 2007)
ATCC 3153	300											(Chaftar et al., 2015)
ATCC 40277				312.5	2500							(Castro & Lima, 2013)
ATCC 60193				250	250							(Rangel et al., 2018)
ATCC 28366						0.039	0.078					(Veilleux & Grenier, 2019)
ATCC 14053								3500				(Rosato et al., 2008)
ATCC 76615										0.25		(Mondello et al., 2006)
ATCC 24433										0.25		(Mondello et al., 2006)
CBS-562	3120	12,500										(Alves et al., 2010)

Abbreviations: ATCC/CBS, strain of standard reference micro-organism; MIC*, Minimum inhibitory concentration in μl ml^{-1}; MFC†, Minimum fungicidal concentration in μl ml^{-1}; MIC†, Minimum inhibitory concentration in %; MFC‡, Minimum fungicidal concentration in % and Ref., reference.

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TABLE 4 Reference strains of *T. mentagrophytes* sp. with their respective MIC and MFC for the three EO: *T. vulgaris, C. zeylanicum* and *M. alternifolia*

T. Mentagrophytes sp.	*T. vulgaris*		*C. zeylanicum*		*M. alternifolia*		Ref.
Strain	MIC	MFC	MIC	MFC	MIC	MFC	
ATCC 9533	500		190		250		(Orchard et al., 2017)
ATCC 4808	2000	3					(Giamperi et al., 2002)
E 1425	260	460					(Simo Kamdem et al., 2015)
TIMM 1189	200		12.5		800		(Inouye et al., 2001)
T14– Australian QC			0.08				(Jantan et al., 2008)
ATCC 9533			71	125			(Ayatollahi Mousavi & Kazemi, 2015)
ATCC 200099			125	122			
ATCC 11480			250	1000			(Makimori et al., 2020)

Abbreviations: ATCC/TIMM/E, strain of standard reference micro-organism; MIC, Minimum inhibitory concentration; MFC, Minimum fungicidal concentration. Concentration measurements of the oil in v/v ($\mu l\,ml^{-1}$). Ref., reference.

TABLE 5 Reference strains of *T. rubrum* with their respective MIC and MFC ($\mu l\,ml^{-1}$) for the three EO: *T. vulgaris, C. zeylanicum* and *M. alternifolia*

T. rubrum	*Thymus vulgaris*		*C. zeylanicum*		*M. alternifolia*		Ref.
Strain	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	
TIMM 2659	100		12.5		400		(Inouye et al., 2001)
IOA9	72						(Khan et al., 2014)
T28 – Australian QC			0.08				(Jantan et al., 2008)

Note: TIMM/T28/IO‡: strain of standard reference micro-organism. MIC: Minimum inhibitory concentration; MFC: Minimum fungicidal concentration. Concentration measurements of the oil in v/v ($\mu l\,ml^{-1}$). Ref.: reference.

TABLE 6 Clinical trials with animals infected by the micro-organisms for the three EO: *T. vulgaris, C. zeylanicum* and *M. alternifolia*

Animals	n	Location	Micro-organism	EO	Action	Ref.
Male guinea pigs	55	Dermal	*T. mentagrophytes* sp.	*C. zeylanicum*	Complete cure at 9–11 days	(Ayatollahi Mousavi & Kazemi, 2015)
Mice	12	Oral	*C. albicans*	*M. alternifolia*	Protective activity	(Ninomiya et al., 2012)
Mice	6	Oral	*C. albicans*	*M. alternifolia*	Anti-inflammatory action	(Ninomiya et al., 2013)
Mice	12	Oral	*C. albicans*	*M. alternifolia*	Reduced microscopic lesions	(Campos Rasteiro et al., 2014)
Rats	10	Vaginal	*C. albicans*	*M. alternifolia*	Removed infections	(Mondello et al., 2006)
Wistar rats	20	Dermal	*T. rubrum* *T. mentagrophytes* sp.	*T. vulgaris*	Complete cure at 8–20 days Anti-inflammatory action	(Soković et al., 2008)

Abbreviations: EO, Essential Oil; n: sample size; Ref., References.

species, and it would be worthwhile studying the effect of each particular compound in the EO.

Identifying the effects of the EO, at different concentrations, on certain pathogenic micro-organisms could help to combat acquired resistance of micro-organisms to medications and may also reduce medication side effects.

Although recent studies are promising, we agree with Bogavac et al. (2015) and Mutlu-Ingok et al. (2020) that we must continue to consider the possible toxicity of these products, because EO are known to present a high risk of skin allergy and irritation. It is therefore important to continue studying EO and accurately determine the safety (harmlessness) of their use and the associated risks (Bogavac et al., 2015; Mutlu-Ingok et al., 2020).

CONCLUSION

Essential oils are a possible alternative therapy with empirical evidence of good results, and the antifungal effects

of EO may be a very promising solution to overcome the therapeutic shortcomings of antimycotic medication, which are increasing with immunosuppressive treatments and the appearance of resistant strains, among other factors. Some studies have highlighted the fungicidal action of EO against diseases caused by fungi in humans, including onychomycosis, a high prevalence condition.

Cinnamomum zeylanicum and *T. vulgaris* EO have been shown to be effective against *C. albicans*, *T. rubrum* and *T. mentagrophytes* sp. However, *M. alternifolia* EO has little scientific evidence as yet, and the MIC has been reported only for *T. rubrum* and *T. mentagrophytes* sp. in isolated strains. *Candida albicans* is the most frequently studied microorganism against EO, but more studies with EO against *T. rubrum* and *T. mentagrophytes* sp. are needed, as these are the most frequent causative agents of onychomycosis.

Despite all the progress found in studies on EO, more experiments are needed to examine the properties of the oils already studied, and other oils, to devise effective and nonaggressive therapies for treatment of dermatophytosis.

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CONFLICT OF INTEREST

No conflict of interest declared.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study

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