

Chemical composition and antimycotic potential of basil, rosemary, clove and cinnamon essential oils against *Trichophyton rubrum* and *Trichophyton mentagrophytes*

Patrícia Abreu Pereira^a, Jade André de Souza^a, Luiza Bernardes Chagas^a, Juliana Butzge^a, Alexandre M. Fuentefria^a, Adelina Mezzari^{b†}, Miriam Anders Apel^{a*}

^aPrograma de Pós-Graduação em Ciências Farmacêuticas-PPGCF, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

^bFaculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

* Corresponding author: miriam.apel@ufrgs.br

Onychomycosis is a disease caused by fungi that affects the nails whose incidence is becoming increasingly frequent. The present study aimed the evaluation of the antifungal activity of essential oils from clove (*Syzygium aromaticum*), Chinese cinnamon (*Cinnamomum cassia*), rosemary (*Rosmarinus officinalis*) and basil (*Ocimum basilicum*), against the most prevalent dermatophytes implicated in onychomycosis: *Trichophyton rubrum* and *Trichophyton mentagrophytes*. The chemical composition of the oils was determined by GC-MS. The volatile oil of clove and Chinese cinnamon and basil presented, respectively, the phenylpropanoids eugenol (80.2%), *E*-cinnamaldehyde (99.4%) and methyl chavicol (68.81%) as the main components. The volatile oil of rosemary exhibited 1,8-cineole and camphor as the major compounds, representing 47% and 15% of the total content, respectively. The results obtained in broth microdilution tests indicate that basil oil presented the best antifungal potential against the tested strains of *Trichophyton rubrum* and *Trichophyton mentagrophytes* at concentrations that ranged from 8 mg/mL to 2 mg/mL. These findings point to the potential prospecting of *Ocimum basilicum* essential oil as a promising source of natural antimycotics to be subjected for further investigations.

Keywords: Onychomycosis; essential oils; antifungal; *Trichophyton*

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Introduction

Dermatomycoses are diseases caused by keratinophilic fungi, which feed on the keratin present in tissues such as skin, nails and hair of both humans and animals-(1). These diseases are among the most prevalent in the world, affecting individuals of all ages and genders, being more incident in adults and immunocompromised patients. The species more frequently found in superficial mycoses are *Trichophyton rubrum* and *Trichophyton mentagrophytes*. Onychomycosis, a fungal infection identified by the appearance of spots and deformations on the nails, is the most widespread dermatophytosis. This disease, in addition to causing physical discomfort, also affects the patient's self-esteem due to the appearance of the injury (2). Currently, onychomycosis has become increasingly common, especially in immunocompromised patients or those with some associated risk factors such as *diabetes mellitus*, psoriasis and vascular diseases. Besides that, it was demonstrated that patients with chronic renal failure who undergo hemodialysis are also more vulnerable to having fungal infections such as onychomycosis (3). Diagnosis is most often made by direct culture examination. When the diagnosis is confirmed, the treatment of choice is oral terbinafine or itraconazole, and and therapy may be supplemented with topical formulations. Oral therapy is effective, but the adverse effects and toxicity of the drugs, as well as the treatment

time make medication adherence difficult. Therefore, safe and effective alternative therapies are extremely necessary for successful treatment (4).

Fungi can have their growth inhibited by several essential oils (5). In some cases, essential oils in association with antifungal drugs was able to reduce the effective dose of oral antifungals. The use of alternative topical therapies for the treatment of this disease is extremely important for the effectiveness of the therapy, in addition to providing less toxicity, reducing treatment time and reducing adverse effects. Moreover, the discovery of new alternative therapies can help patients to better adhere to treatment, increasing the chances of a cure (6).

The application of products formulated with essential oils is an alternative that can be effective, economical and with a lower degree of toxicity. The use of essential oils obtained from plants traditionally used by the population can increase acceptance of the treatment and consequently, adherence and therapeutic success. Thus, the objective of this study was to evaluate the antifungal activity of essential oils of rosemary (*Rosmarinus officinalis*), basil (*Ocimum basilicum*), clove (*Syzygium aromaticum*) and Chinese cinnamon (*Cinnamomum cassia*) against *Trichophyton rubrum* and *Trichophyton mentagrophytes*.

Experimental section

Materials

Plant essential oils

The essential oils of rosemary (*Rosmarinus officinalis*) and basil (*Ocimum basilicum*) were purchased from local stores in the Porto Alegre city. The essential oils of Chinese cinnamon (*Cinnamomum cassia*) and clove (*Syzygium aromaticum*) were obtained by hydrodistillation from the bark and flower buds, respectively, purchased commercially.

Obtention of Chinese cinnamon (*Cinnamomum cassia*) and clove (*Syzygium aromaticum*) essential oils

To obtain the essential oil from cinnamon barks and clove flower buds, 101.2 g and 111.6 g, respectively, were grinded and subjected, separately, to hydrodistillation by using in a Clevenger-type apparatus, for 4 hours. Afterwards, the oils obtained were stored under refrigeration at -4 °C and protected from light until analysis. The yield was determined by reading the volume of oil collected based on the weight of each sample (w/v).

Gas chromatography–mass spectrometric analysis of essential oils

Chemical analysis was performed on a gas chromatograph coupled to a mass detector (GC-MS), model Shimadzu QP5000, equipped with a Durabond-DB-5 fused silica capillary column (John Wiley & Sons Scientific, US, with 30 m x 0.25 mm and filled with a 0.25 µm thick film of polydimethyldiphenylsiloxane containing 5% phenyl groups) to separate the constituents. Column temperature was programmed with a heating ramp from 60 °C (held for 3 min) to 300 °C with a variation of 3 °C/min. Helium was used as gas carrier, at 80 kPa and flow of 1.0 mL/min. Injector and detector temperatures were adjusted to 220 °C and 250 °C, respectively. Split ratio of 1:50 and injection volume of 1 µL was used. Mass selective detector was operated in the electron impact mode, EI, at the ionization energy of 70 eV, with scan range 40–550 amu. For chromatographic analysis, the oils were diluted to 2% in ethyl ether (v/v) (Synth®).

Components were identified by comparing their relative retention index, calculated by relative linear interpolation to the retention time of a series of *n*-alkanes (C₈–C₂₂) and their mass spectrum with data obtained in the literature (ADAMS, 2017) (7), in the database of the laboratory's research group or by comparison with mass spectra recorded in databases such as NIST 62 and NIST 12 (National Institute of Standards and Technology). The relative amounts of each compound were calculated from the peak areas by normalization.

Isolated from *Trichophyton rubrum* and *Trichophyton mentagrophytes*

Fungal samples from the species *Trichophyton rubrum* and *Trichophyton mentagrophytes* were obtained from the mycothèque of Microbiology Laboratory Applied from the Faculty of Pharmacy of the Federal University of Rio Grande do Sul. The chosen strains are clinical isolates from patients affected by onychomycosis, whose etiological agents are *T. rubrum* and *T. mentagrophytes*.

The strains were kept inoculated in tubes containing Sabouraud dextrose agar with chloramphenicol and incubated at 32 °C for 48 hours. These strains were replicated weekly to keep the samples in an ideal state for the study.

The RPMI solution, prepared according to the manufacturer's instructions, was autoclaved and filtered. The culture medium was also prepared conforming to the manufacturer's instructions, in tubes and plates.

To prepare the inoculum for the susceptibility test, 2 mL of sterile 0.85% saline solution was added to the tube containing the isolate and the surface of the colony was scraped with the microbiological loop. This fungal suspension was poured into the sterile tube and decanted. The transmittance reading was taken, which should be 80 to 82%. After adjusting the fungal suspension in the spectrophotometer, a 1:50 dilution was made in RPMI 1640 broth.

Assessment of antifungal activity

The MIC (minimum inhibitory concentration) is defined as the lowest concentration capable of producing inhibition of the growth of *Trichophyton rubrum* and *Trichophyton mentagrophytes* compared to the positive control. Susceptibility testing was performed in sterile 96-well microplates, in triplicate. The RPMI 1640 medium was pipetted into the microplates in an organized manner to maintain the positive control and negative controls. The tested substances were pipetted and microdiluted in broth followed by pipetting of the fungal inoculum into all wells, except the negative control. After completing the preparation of the material on the plates, they were incubated at a temperature of 32 °C. The plates were read within 120 hours. The oils were dissolved in dimethyl sulfoxide - DMSO (Nuclear®). The concentration range used for essential oils was 8 to 2 mg/mL.

Results

Yield and chemical characterization of essential oils by GC-MS

The volatile oil from clove and Chinese cinnamon was obtained by using the hydrodistillation technique in a Clevenger-type apparatus yielding 11.2% and 0.4%, respectively. Rosemary and basil essential oils were commercially obtained and, therefore, their yields are not available.

The chemical characterization of volatile oils from *Syzygium aromaticum* (clove), *Cinnamomum cassia* (Chinese cinnamon), *Rosmarinus officinalis* (rosemary) and *Ocimum basilicum* (basil) was carried out by GC-MS and, altogether, 24 compounds were identified (Table 1).

Table 1: Percentual composition of the essential oils from *Syzygium aromaticum* (clove), *Cinnamomum cassia* (Chinese cinnamon), *Rosmarinus officinalis* (rosemary) and *Ocimum basilicum* (basil) obtained by GC-MS.

Constituents	IR*	Clove (%)	Chinese cinnamon (%)	Rosemary (%)	Basil (%)
Monoterpene hydrocarbons		0	0	30.3	0
Tricyclene	914			0.8	
α -pinene	926			12.8	
Camphene	939			5.9	
β -pinene	967			6.4	
Myrcene	984			2.0	
<i>p</i> -cymene	1018			2.0	
γ -terpinene	1050			0.4	
Oxygenated monoterpenes		0	0	67.3	27.9
1,8-cineole (=eucalyptol)	1026			47.3	
Linalool	1101				27.9
Camphor	1136			15.1	
Isoborneol	1149			0.5	
Borneol	1159			1.9	
α -terpineol	1185			1.5	
Isobornyl acetate	1277			1.0	
Sesquiterpenes hydrocarbons		1.1	0.2	2.4	3.2
β -caryophyllene	1406	1.1		2.4	0.4
α - <i>trans</i> -bergamotene	1424				0.7
α -muurolene	1489		0.2		
α -humulene	1534				2.1
Oxygenated sesquiterpene		0	0.3	0	0
Cubenol	1639		0.3		
Phenylpropanoids		98.9	99.7	0	68.8
Methyl chavicol (= estragole)	1200				68.8
<i>E</i> -cinnamaldehyde	1291		99.4		
Eugenol	1382	80.2			
<i>E</i> -allyl cinnamate	1536		0.3		
<i>Z</i> -isoeugenol acetate	1539	18.7			
Total		100	100	100	99.9

* RI: retention index based in a DB-5 capillary column.

The qualitative and quantitative analyze of the essential oils revealed that the chemical composition of all the samples was characteristic for each plant species. Clove, Chinese cinnamon and basil essential oils were characterized by the predominance of phenylpropanoids, while for rosemary, monoterpenes were the main compounds (Table 1).

The chromatographic analysis of clove revealed that eugenol and *Z*-isoeugenol acetate were the principal components of the oil, being responsible for 80.2% and 18.7% of the total content, respectively. For Chinese cinnamon, the chromatographic analysis indicated *E*-cinnamaldehyde as the main component, accounting for

99.4% of the total oil content. Rosemary essential oil was characterized by 1,8-cineole, camphor and α -pinene as the major compounds, representing 47.3%, 15.1% and 12.8% of the total oil content, respectively. In the basil essential oil, the chromatographic analysis demonstrated that methyl-chavicol and linalool were the main components, being responsible for 68.8% and 27.9% of the identified substances, respectively.

Anti-*Trichophyton* activity

Broth microdilution

The anti-*Trichophyton* investigation was carried out with volatile oils in order to evaluate which of them would present a good response against the different strains of the tested *Trichophyton* species. Thus, the test was conducted by using broth microdilution assay in 96-well ELISA plates, and three strains of *Trichophyton rubrum* (TRU 90, TRU 94 and TRU 47) and three strains of *Trichophyton mentagrophytes* (TME 16, TME 32 and TME ATCC 9533) were used. The MIC of clove, Chinese cinnamon, basil and rosemary essential oils are summarized in Table 2. The

greatest antifungal activity against all tested strains was exhibited by *Ocimum basilicum* essential oil, which had MICs of 8 mg/mL to 2 mg/mL.

Table 2: Minimum inhibitory concentration (MIC) of the volatile oils tested against strains of *Trichophyton rubrum* and *Trichophyton mentagrophytes*, at concentrations of 8 mg/mL to 0.0625 mg/mL.

Species	<i>Cinnamomum cassia</i> (mg/mL)	<i>Syzygium aromaticum</i> (mg/mL)	<i>Rosmarinus officinalis</i> (mg/mL)	<i>Ocimum basilicum</i> (mg/mL)
TRU 90	>8	>8	>8	2
TRU 94	>8	>8	>8	2
TRU 47	>8	>8	>8	2
TME 16	>8	>8	>8	8
TME 32	>8	>8	>8	8
TME ATCC 9533	>8	>8	>8	2

TRU: *Trichophyton rubrum*; TME: *Trichophyton mentagrophytes*.

DISCUSSION

Essential oils are complex mixtures of organic compounds, including terpenes and phenylpropanoids. Much of the antimicrobial activity is attributed to the presence of oxygenated terpenes (such as monoterpene alcohols and aldehydes), however there are reports of hydrocarbons exhibiting antimicrobial effects. Oils characterized by the presence of aldehydes or phenols, such as thymol, carvacrol and cinnamaldehyde, generally show greater effectiveness, followed by those that contain terpene alcohols (8).

Rosmarinus officinalis (Rosemary)

The essential oil of *Rosmarinus officinalis* contains monoterpenes such as camphor, 1,8-cineole, α -pinene, verbenone, borneol and piperitone, which are responsible for the antioxidant and antimicrobial activity (9). It has been demonstrated that the essential oil of *Rosmarinus officinalis* is well characterized by the predominance of α -pinene (11.6-24.6%), 1,8-cineole (3.3-28.3%) and camphor (1.6-25.%) (10, 11, 12), although a large variation can be observed, especially for 1,8-cineole and camphor. Comparing the chemical profile obtained in all studies with the results of the present research, it can be observed that

the majoritary constituents are quite the same. According to the literature, the variation in the chemical composition of essential oils verified in different studies can be attributed to differences in crops, geographic origin, harvest time, environmental conditions, sampling and extraction methods (13, 14). There is a study in which the rosemary oils are classified into two chemotypes by the ratio of major components; one with more than 40% of 1,8-cineole and the other with almost the same percentage of 1,8-cineole, α -pinene and camphor (15). Taking this in account, the essential oil analyzed in this study belongs to the first chemotype, with more than 40% of 1,8-cineole.

For *Rosmarinus officinalis*, few studies have reported the effect of volatile oil against *Trichophyton* species. A study, using α -pinene-type *R. officinalis* oil (50.8% of α -pinene), tested the antimicrobial activity of this oil against two unknown strains of *Trichophyton*. For this, the authors used agar diffusion and agar dilution tests, and *Rosmarinus officinalis* essential oil presented moderated activity against *Trichophyton* spp. (16). In another study using box vapor assay and agar diffusion assay, the essential oil of *Rosmarinus officinalis* (1,8-cineole (32%), α -pinene (23%), camphor (9%)) and two of its main constituents, camphor and 1,8-cineole, were tested against *T.*

mentagrophytes, demonstrating moderate activity against this dermatophyte (17). This result was also observed in another study using the box vapor assay, in which *R. officinalis* oil was tested against *T. mentagrophytes* (2.5 mg/mL) and *T. rubrum* (4 mg/mL) (18).

***Syzygium aromaticum* (clove)**

Clove is a rich source of essential oil, which represents 15 to 21% of the bud. Eugenol is the main component, representing 70 to 90% of the oil content, followed by eugenol acetate, which can reach 17%, and beta-caryophyllene, in amounts between 5 and 12% (19, 20, 21). Considering the specialized literature and the results obtained, a chemical constancy can be observed in the clove essential oil.

***Cinnamomum cassia* (Chinese cinnamon)**

The results of the analysis carried out corroborate data found in the literature. Several studies have reported the chemical characterization of Chinese cinnamon essential oil, and all are very similar, demonstrating *E*-cinnamaldehyde as the major component with at least 78.49% of the oil content (22, 23, 24, 25).

Gu and collaborators (2024) (26) analyzed the composition of 20 essential oils extracted from *Cinnamomum cassia* cultivated in different geographic regions and the main compounds identified were terpenoids (34.04%), aromatic compounds (4.52%), aliphatic compounds (0.9%) among others. The hierarchical grouping analysis of the compositions showed a wide diversity of chemical composition, with four main chemotypes divided into groups A, B, C and D. All present trans-cinnamaldehyde as the majority, but with very different levels. Group A is the typical trans-cinnamaldehyde chemotype (68.75-91.23%). Group B was dominated by trans-cinnamaldehyde (42.37-6.36%) and trans-cinnamyl acetate (0.65-3.97%). Group C was dominated by trans-cinnamaldehyde (22.36-29.53%), α -calacorene (0.93-2.71%), α -cadinol (0.26-2.93%), while group D by trans-cinnamaldehyde (28.48%) and benzaldehyde (17.2%). The composition of the oil in the study was equated to chemotype A due to the high content of trans-cinnamaldehyde (26).

***Ocimum basilicum* (Basil)**

Linalool, one of the main monoterpenes of *O. basilicum*, is found in the essential oils of several medicinal plants, especially those belonging to the Lamiaceae family. Methyl chavicol (sin. estragole) is a phenylpropanoid present also in several plants and these both compounds are considered the predominant constituents in this species. Methyl chavicol and its biotransformation products have toxic potential because they are genotoxic, mutagenic and carcinogenic. However, it has been considered safe by FEMA (27), as it does not present a risk to human health in small quantities (0.6 mg/kg/day).

Most investigation carried out with essential oils of *O. basilicum* indicate monoterpene (such as camphor, limonene, 1,8-cineol, linalool, geraniol) and phenylpropanoid derivatives (including eugenol, methyl

eugenol, chavicol, estragole, methyl cinnamate) as the main compounds (28, 29). According to the predominant components in the essential oil, different chemotypes of *O. basilicum* have been proposed (such as those with high levels of linalool, methyl chavicol, methyl cinnamate, methyl ester, or eugenol) (30, 31). In this way, a study found linalool (35.1%) and eugenol (20.7%) as the major compounds in the essential oil of *O. basilicum* (32). On the other hand, another research (33) identified estragole (64.47%) and linalool (16.88%) as the main compounds of the essential oil. In another study, the major compounds in aerial parts of *O. basilicum* obtained from Iran and Turkey was investigated and linalool and methyl chavicol were also found to be the main strong aroma-active compounds (34). Moreover, methyl-chavicol (86%) was found as predominant in the essential oil of *O. basilicum* (35). The chemical compounds found in the essential oil of *O. basilicum* undergo various qualitative transformations based on different factors such as soil, altitude, temperature, period of exposure to the sun, cultivation, drying and storage conditions have an influence on its composition, which explains the variations in the amount of these compounds (36, 37).

For *Ocimum basilicum*, two studies report the mechanism of action of the volatile oil on *Trichophyton mentagrophytes* and *Trichophyton rubrum*. The first describes the action of the volatile oil, whose main constituents are linalool (35.1%), eugenol (20.7%) and 1,8-cineole (9.9%), by inhibiting germ tube formation, at values eight times lower than the MIC (MIC 0.64 μ L/mL). Furthermore, the activity of the main compounds, linalool (MIC 1.25 μ L/mL) and eugenol (MIC 0.16 μ L/mL), was also determined, and their activity was generally similar to that of the essential oil, thus suggesting that these are the main active compounds (33). The other study aimed to investigate the activity of linalool against clinical isolates of *Trichophyton rubrum*. The MIC value was determined (256 μ g/mL to 512 μ g/mL) and demonstrated that linalool exerts its action against *T. rubrum* by interfering with both mycelial growth, conidiogenesis, conidial germination, fungal morphogenesis and membrane cell phone (38). This study contributes to clarifying the action of one of the main compounds present in the essential oil of *O. basilicum*.

Cinnamomum verum, *Syzygium aromaticum*, *Cymbopogon citratus*, *Cymbopogon martini*, and their main components cinnamaldehyde, eugenol and geraniol respectively, were tested on the growth and hyphal ultrastructure of *Trichophyton rubrum*, at concentrations ranging from 0.005% to 0.32%, v/v. The antifungal activity of essential oils and their major constituents was found for the most active for cinnamaldehyde, followed by eugenol, geraniol, *C. verum* and *S. aromaticum*. Furthermore, for eugenol and cinnamaldehyde, both compounds demonstrated inhibitory action against ungerminated and germinated conidia of *T. rubrum*, with cinnamaldehyde being the most active. Furthermore, they found that the mechanism of action involves multiple sites of cinnamaldehyde action, mainly in cell membranes and endomembranous structures of the

fungus cell (39). Considering the use as a fumigation agent, the essential oils of *S. aromaticum* (eugenol 87%) and *C. zeylanicum* (cinnamaldehyde 54%) were tested against *Trichophyton mentagrophytes*, using the closed box assay. It was found that oils containing phenol as the majority component were most active, followed by oils whose main constituent is an aldehyde and then, by those with alcohol as the major compound (17).

Conclusions

The current study presents results through the application of research methodologies to highlight the potential antifungal effect and the use of essential oils in the treatment of onychomycosis. The results obtained in broth microdilution tests carried out with clove, Chinese cinnamon, rosemary and basil essential oils pointed to the potential activity of basil essential oil (*Ocimum basilicum*), inhibiting the growth of *Trichophyton rubrum* and *Trichophyton mentagrophytes* strains at concentrations that ranged from 8 mg/mL to 2 mg/mL, demonstrating potential activity for the treatment of onychomycosis.

Conflict of interest

None to declare.

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