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ORIGINAL ARTICLE

Antifungal activity of essential oils of *Croton* species from the Brazilian *Caatinga* biome

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Abstract

Aims: To find new antifungal agents among essential oils from Brazilian *Croton* species.

Methods and Results: Plant leaves were steam distilled and the obtained essential oils were analyzed by gas chromatography/mass spectroscopy. The main constituents were estragole and anethole for *Croton zehntneri*, methyl-eugenol and bicyclogermacrene for *Croton nepetaefolius* and spathulenol and bicyclogermacrene for *Croton argyrophylloides*. The antifungal activity of essential oils was evaluated against *Candida albicans*, *Candida tropicalis* and *Microsporum canis* by the agar-well diffusion method and the minimum inhibitory concentration (MIC) by the broth microdilution method. Essential oils of *Croton* species demonstrated better activity against *M. canis.* Among the three plants *C. argyrophylloides* showed the best results, with MIC ranging from 9 to 19 μg ml^{-1}. The acute administration of the essential oil up to 3 g kg^{-1} by the oral route to mice was devoid of overt toxicity.

Conclusions: The studied essential oils are active *in vitro* against the dermatophyte *M. canis* and present relative lack of acute toxicity *in vivo*.

Significance and Impact of the Study: Because of its antifungal activity and low toxicity, the essential oils of studied *Croton species* are promising sources for new phytotherapeutic agents to treat dermatophytosis.

Introduction

During the past several years, there has been an increasing incidence of fungal infections because of growth in immunocompromised people, such as organ transplant, cancer and HIV/AIDS patients. This fact, coupled with the resistance to antibiotics and the toxicity during prolonged treatment with many antifungal drugs (Giordani *et al.* 2001), has prompted an extensive search for newer drugs to treat mycosis (Fostel and Lartey 2000).

The conventional treatment of fungal diseases is limited in comparison with antibiotic therapy for bacterial infection. Part of the reason is that fungi are eukaryote organisms, thus making it difficult to develop a drug that is selectively toxic to the fungal cell and not to the host (Harris 2002). In addition to the small number of drugs available for mycosis treatment, the emerging resistance has been encouraging the search for alternatives that are more efficient, cheaper and less toxic than traditional therapies and natural products (Cavaleiro *et al.* 2006).

The use of plant extracts and phytochemicals with known antifungal properties can be of great significance in therapeutic treatments. In recent years, a number of studies have been conducted in different countries to prove such efficiency (Prasad *et al.* 2004; Ledezma and Apitz-Castro 2006; Pyun and Shin 2006; Rasooli *et al.*

2006; Unland and Higgins 2006; Fontenelle *et al.* 2007; Matasyoh *et al.* 2007). Antimicrobial properties of plants are due to compounds synthesized in their secondary metabolism (Nascimento *et al.* 2000).

Many plants from Brazilian biomes have been used as natural medicines by local population for treatment of many diseases, including mycosis (Souza *et al.* 2002; Duarte *et al.* 2005; Cruz *et al.* 2007) and several plants have shown antimicrobial properties (Bertini *et al.* 2005; Botelho *et al.* 2007). Considering that the *Caatinga* scrublands is a biome with extreme diversity of medicinal plants, more phytochemical and pharmacological research is needed to establish the potential use of these plants as alternative treatments (Almeida *et al.* 2006).

Widespread in the flora of Northeastern Brazilian, mainly in the *Caatinga* region, *Croton* species are used for several purposes in folk medicine. Some pharmacological activities of *Croton* essential oils have been validated, such as antispasmodic, antimicrobial and antihypertensive activity for *Croton nepetaefolius* (De Albuquerque *et al.* 1974; Lahlou *et al.* 2000; Magalhães *et al.* 2004). In addition, the antinociceptive and antimicrobial effects of *Croton zehntneri* have been demonstrated (Lemos *et al.* 1990; Oliveira *et al.* 2001). Morais *et al.* (2006) have reported that the essential oils of *C. zehntneri*, *C. nepetaefolius*, *Croton argyrophylloides* and *Croton sonderianus* showed *in vitro* larvicidal effect against *Aedes aegypti*. However, the antifungal properties of the essential oil from above cited *Croton* species remain unknown.

The aims of this study were to determine the chemical constitution of the essential oils from *C. nepetaefolius*, *C. argyrophylloides* and *C. zehntneri* species, to test their essential oils *in vitro* antifungal activity against *Candida* spp. and *Microsporum canis*, and to evaluate their acute toxicological effects *in vivo*.

Materials and methods

Plant material and extraction of essential oils

Plant samples were collected in Viçosa city, Ceará State (3°33′46′ latitude S, 41°05′42′ longitude W), situated in Northeastern Brazil. The taxonomic identification was confirmed by botanists of the Prisco Bezerra Herbarium (Federal University of Ceará, Brazil), where voucher specimens were deposited with reference numbers 32 448, 32 444 and 32 446 for *C. nepetaefolius*, *C. argyrophylloides* and *C. zehntneri*, respectively. The *Croton* species' essential oils were extracted from leaves by the steam distillation method in a Clevenger type apparatus, as described by Craveiro *et al.* (1976).

Gas-chromatography (GC)/mass spectral analysis

The chemical analysis of the essential oils constituents was performed on a Shimadzu QP-2010 instrument employing the following conditions: column: DB-5ms (Agilent, part No. 122–5532) coated fused silica capillary column (30 m × 0·25 mm × 0·25 μm); carrier gas: He (1 ml min^{-1}, in constant linear velocity mode); injector temperature was 250°C, in split mode (1 : 100) and the detector temperature was 250°C. The column temperature programming was 35–180°C at 4°C min^{-1} then 180–280°C at 17°C min^{-1} and at 280°C for 10 min; mass spectra: electron impact 70 eV. The injected sample volume was 1 μl. Compounds were identified by their GC retention times relative to known compounds and by comparison of their mass spectra with those present in the computer data bank (National Institute for Standard Technology – NIST – 147 198 compounds) and published spectra (Stenhagen *et al.* 1974; Adams 2001).

Fungal strains

A total of 10 strains of *M. canis*, five strains of *Candida albicans* and three strains of *Candida tropicalis* were included in this study. Both *M. canis* and *Candida* spp. strains were isolated from symptomatic dogs and cats. The strains were stored in the fungal collection of the Specialized Medical Mycology Center – CEMM (Federal University of Ceará, Brazil), where they were maintained in saline (0·9% NaCl), at 28°C. At the time of the analysis, an aliquot of each suspension was taken and inoculated into potato dextrose agar (Difco, Detroit, MI, USA), and then incubated at 28°C for 2–10 days.

Inoculum preparation for antifungal susceptibility tests

For the agar-well diffusion method, based on Gurgel *et al.* (2005) and Fontenelle *et al.* (2007), stock inocula were prepared on day 2 and day 10 for *Candida* spp. and *M. canis*, respectively, grown on potato dextrose agar (Difco) at 28° C. Potato dextrose agar was added to the agar slant and the cultures were gently swabbed to dislodge the conidia. The suspensions with blastoconidia of *Candida* spp. or suspension of hyphal fragments of *M. canis* were transferred to a sterile tube and adjusted by turbidimetry to obtain inocula of *c.* 10^6 CFU ml^{-1} blastoconidia of *Candida* spp. and 10^5 CFU ml^{-1} hyphal fragments or conidia of *M. canis*. The optical densities of the suspensions were spectrophotometrically determined at 530 nm and then adjusted to 95% transmittance.

For the broth microdilution method, standardized inocula (2·5–5 × 10^3 CFU ml^{-1} for *Candida* spp. and 5 × 10^4 CFU ml^{-1} for *M. canis*) were also prepared by

turbidimetry. Stock inocula were prepared on day 2 and day 10 for *Candida* spp. and *M. canis* cultures, respectively, grown on potato dextrose agar at 28°C. Sterile normal saline solution (0·9%; 3 ml) was added to the agar slant and the cultures were gently swabbed to dislodge the conidia from the hyphal mat for *M. canis* (Brilhante *et al.* 2005) and the blastoconidia from *Candida* spp. (Brito *et al.* 2007). The suspensions of conidia with hyphal fragments of *M. canis* and blastoconidia suspension of *Candida* spp. were transferred to sterile tubes, and the volume of both suspensions adjusted to 4 ml with sterile saline solution. The resulting suspensions were allowed to settle for 5 min at 28°C, and their density was read at 530 nm and then adjusted to 95% transmittance. The suspensions were diluted to 1 : 2000 for *Candida* spp. and 1 : 500 for *M. canis*, both with RPMI 1640 medium (Roswell Park Memorial Institute – 1640) with L-glutamine, without sodium bicarbonate (Sigma Chemical Co., St Louis, MO, USA), buffered to pH 7·0 with 0·165 mol l^{-1} morpholinepropanesulfonic acid (Sigma), to obtain the inoculum size of *c.* 2·5–5 × 10^3 CFU ml^{-1} for *Candida* spp. and 5 × 10^4 CFU ml^{-1} for *M. canis*.

Agar-well diffusion susceptibility test

The antifungal activity of essential oils from *Croton* species were evaluated against *C. albicans* ($n = 5$), *C. tropicalis* ($n = 3$) and *M. canis* ($n = 10$), by the agar-well diffusion method according to Gurgel *et al.* (2005) and Fontenelle *et al.* (2007). Petri dishes with 15 cm diameter were prepared with potato dextrose agar (Difco). The wells (6 mm in diameter) were then cut from the agar and 0·100 ml of essential oil was delivered into them. The oil was weighed and dissolved in mineral oil to obtain the test concentrations of 25 000, 50 000, 75 000 and 100 000 $\mu g\ ml^{-1}$. Stock solutions of griseofulvin (1000 $\mu g\ ml^{-1}$; Sigma) and amphotericin B (AMB) (5 $\mu g\ ml^{-1}$; Sigma) were prepared in distilled water and tested as positive controls for *M. canis* and *Candida* spp., respectively. Each fungal suspension was inoculated on the surface of the agar. After incubation, for 3–5 days for *Candida* spp. and 5–8 days for *M. canis*, at 28°C, all dishes were examined for zones of growth inhibition and the diameters of these zones were measured in millimetres. Each experiment was repeated at least twice.

Broth microdilution method

The minimum inhibitory concentration (MIC) for *Candida* spp. was determined by the broth microdilution method, in accordance with the Clinical and Laboratory Standards Institute – CLSI (formerly NCCLS; M27-A2), (NCCLS M27A 2002). The broth microdilution assay for *M. canis* was performed as described by Jessup *et al.* (2000), Fernandez-Torres *et al.* (2002) and Brilhante *et al.* (2005), based on the M38-A document (CLSI; formerly NCCLS M38A 2002). The minimum fungicidal concentration (MFC) for both *Candida* spp. and *M. canis* were determined according Fontenelle *et al.* (2007). In addition, *Candida parapsilosis* (ATCC 22 019) and *Candida krusei* (ATCC 6528) strains were used as quality controls for broth microdilution method.

The essential oils of *Croton* species were prepared in 100% mineral oil. AMB (Sigma) and griseofulvine (Sigma) were prepared in distilled water. For the susceptibility analysis, the essential oils were tested in concentrations ranging from 4 to 5000 $\mu g\ ml^{-1}$.

The microdilution assay was performed in 96-well microdilution plates. Growth and sterile control wells were included for each essential oil tested. The microplates were incubated at 37°C and read visually after 2 days for *Candida* spp. and 5 days for *M. canis*. The assays for all essential oils were run in duplicate and repeated at least twice. The MIC was defined as the lowest oil concentration that caused 100% inhibition of visible fungal growth. The results were read visually as recommended by CLSI. The MFC was determined by subculturing 100 μl of solution from wells without turbidity, on potato dextrose, at 28°C. The MFCs were determined as the lowest concentration resulting in no growth on the subculture after 2 days for *Candida* spp. and 5 days for *M. canis*.

Animals

Swiss mice (*Mus musculus*; 25–30 g), of both sexes, were housed in temperature-controlled rooms and given water and food *ad libitum* until used. All the protocols that included animals were approved by the research ethics committee of the State University of Ceará, Fortaleza, Ceará, Brazil. The animals were used as recommended by the guide for the care and use of laboratory animals from the National Academy Press (USA; 1996) which is in line with the principles for animal use in Brazil.

Acute toxicity

For the acute toxicity analysis, the essential oil was administered to the mice ($n = 10$ mice per group) orally or intraperitoneally at doses ranging from 100 to 3000 mg kg^{-1}. The results obtained were compared with those for the control animals (3% Tween 80 in saline v/v). The lethal dose 50 (LD_{50}) was calculated by the

probit method by using SPSS 7·0 for Windows. The animals were observed for an additional period of 1 h and the general effects were noted in a modified table from a previous work (Malone and Robichaud 1962).

Statistical analysis

The antifungal activity evaluated by the agar-well diffusion method was expressed as mean ± SD of the diameter of the growth inhibition zones (mm). The antifungal activity of the essential oils was analyzed by linear correlation for individual analysis and by the one-way ANOVA followed by Tukey test to evaluate differences between the effect of different doses of each essential oil. The LD_{50} was calculated at 95% confidence intervals, using SPSS 7·0 for Windows.

Results

The chemical analyses demonstrated that *C. nepetaefolius* has methyl-eugenol (15·7%) and bicyclogermacrene (14·1%) as main constituents, while the main constituents of *C. argyrophylloides* are spathulenol (20·3%) and bicyclogermacrene (11·7%), and those of *C. zehntneri* are estragole (72·9%) and anethole (14·3%) (Table 1).

Through the agar-diffusion method, this study shows that essential oils from *C. nepetaefolius* and *C. argyrophylloides* were effective only against *M. canis* strains (n = 10). Nevertheless, the essential oil from *C. zenhtneri* was effective against both *M. canis* and *Candida* species (Tables 2 and 3). Briefly, *C. nepetaefolius* essential oil was effective against *M. canis* at concentrations of 25 000, 50 000, 75 000 and 100 000 μg ml^{-1}, but no statistically significant difference was found among the three last concentrations. *C. argyrophylloides* also presented a relevant antifungal activity against *M. canis*, showing a tendency to a dose-dependent effect, and the best activity was obtained using 100 000 μg ml^{-1} (growth inhibition zone: 31·2 ± 9·9 mm). *C. zehntneri* inhibited *M. canis* growth at all concentrations and the best effect (22 ± 7·3 mm) was obtained with the highest concentration (Table 2). The positive control, griseofulvin, induced significant growth inhibition zones (51·6 ± 12·4 mm). On the other hand, the negative control, mineral oil, was devoid of antifungal effect against all strains of *M. canis* (n = 10) and *Candida* spp. (n = 8).

The essential oils obtained from *C. nepetaefolius* and *C. argyrophylloides* were ineffective against *Candida* spp. strains (n = 8) in the agar-well diffusion susceptibility tests, even in higher concentration (100 000 μg ml^{-1}) (Table 3). On the other hand, *C. zehntneri* essential oil was effective against these yeasts. The maximal inhibition of fungal growth was obtained with 100 000 μg ml^{-1} (12·6 ± 1·1 mm), but there were no differences among the tested concentrations. The positive control, AMB, had a significant effect (10·7 ± 1·5 mm) (Table 3).

Table 1 Chemical composition of the essential oils of *Croton* species

K.I.*	Components	Composition (%**)		
		C. a.	*C. n.*	*C. z.*
939	α-Pinene	5·6	–	–
976	Sabinene	4·7	–	–
991	Myrcene	–	–	0·4
1033	1,8-Cineole	11·2	8·0	0·6
1050	E-β-Ocimene	–	–	0·4
1189	α-Terpineol	–	3·5	–
1196	Estragole	–	–	72·9
1285	Anethole	–	–	14·3
1298	Ortho-Vanillin	–	5·0	–
1339	β-Elemene	–	–	0·2
1391	β-Elemene	6·2	3·7	0·3
1404	Methyl-Eugenol	–	15·7	–
1413	E-α-Bergamotene	–	4·6	–
1419	E-Caryophyllene	5·7	11·4	1·6
1435	E-α-Bergamotene	–	9·1	0·2
1439	Aromadendrene	–	3·4	–
1455	α-Humulene	–	5·9	0·2
1461	Alloaromadendreno	–	–	0·3
1480	Germacrene-D	–	–	0·9
1485	β-Selinene	3·2	–	–
1500	Bicyclogermacrene	11·7	14·1	5·1
1505	Cupareno	–	–	0·2
1506	β-Bisabolene	–	3·0	0·2
1524	δ-Cadinene	–	–	0·2
1578	Spathulenol	20·3	4·3	0·6
1581	Caryophyllene oxide	–	4·4	–
1593	Veridiflorol	–	–	0·3

*Retention index. The identified constituents are listed in their order of elution from a nonpolar column.

**The % composition is the % peak area of the total essential oil composition.

C. a., *Croton argyrophyllloides*; *C. n.*, *Croton nepetaefolius*; *C. z.*, *Croton zenhtneri*; –, components were not detected.

Through the broth microditution method, it was seen that the MIC of *C. zehntneri* essential oil against *M. canis* strains (n = 6) ranged from 620 to 1250 μg ml^{-1} and the MFC ranged from 1250 to 2500 μg ml^{-1}. The MIC of *C. zehntneri* essential oil for *Candida* spp. strains (n = 6) was ≥2500 μg ml^{-1} and the MFC was ≥5000 μg ml^{-1}. *C. argyrophylloides* and *C. nepetaefolius* essential oils were effective only against *M. canis* strains (n = 6), with MIC ranging from 9 to 19 μg ml^{-1} and MFC from 39 to 78 μg ml^{-1} for *C. argyrophylloides* essential oil and MIC >5000 μg ml^{-1} for *C. nepetaefolius* essential oil (Table 4).

Concerning acute toxicity analysis, the oral administration of the essential oils at doses ranging from 100 to 3000 mg kg^{-1} induced no remarkable alterations in the behaviour pattern of the mice, such as: trembles,

Table 2 Antifungal activity of the essential oils of *Croton* species against *Microsporum canis* in the agar-well diffusion assay

	Growth inhibition zones (mean ± SD): *M. canis*				
	Essential oils and drug concentrations (μg ml^{-1})				
Essential oils/drug	25 000	50 000	75 000	100 000	1000
C. nepetaefolius	10·2 ± 1·7^{a}	18·6 ± 4·6^{b}	18·7 ± 4·2^{b}	19·8 ± 7·2^{b}	–
C. argyrophylloides	14·8 ± 4·0^{a}	21·1 ± 4·2^{a,b}	26·2 ± 5·5^{b}	31·2 ± 9·9^{c}	–
C. zehntneri	11·8 ± 3·0^{a}	16·8 ± 5·8^{a,b}	18·7 ± 4·4^{b}	22 ± 7·3^{b}	–
Griseofulvin	–	–	–	–	51·6 ± 6·7

Small letters mean significant differences in the rows at $P < 0{\cdot}05$.
Each experiment was performed in duplicate.
($n = 10$).

Table 3 Antifungal activity of the essential oils of *Croton* species against *Candida* spp. in the agar-well diffusion assay

	Growth inhibition zones (mean ± SD): *Candida* spp.				
	Essential oil and drug concentrations (μg ml^{-1})				
Essential oils/drug	25 000	50 000	75 000	100 000	5
C. nepetaefolius	NI	NI	NI	NI	–
C. argyrophylloides	NI	NI	NI	NI	–
C. zehntneri	7·4 ± 1·1^{a}	9·0 ± 1·4^{a}	10·0 ± 1·5^{a}	12·6 ± 1·1^{a}	–
Amphotericin B	–	–	–	–	10·75 ± 1·5

NI, no inhibition of fungal growth.
Small letters mean significant differences in the rows at $P < 0{\cdot}05$.
Each experiment was done in duplicate.
($n = 8$).

Table 4 Minimum inhibitory and fungicidal concentrations of essential oils of *Croton* species against *Microsporum canis* and *Candida* spp.

	Essential oil *C. nepetaefolius*		Essential oil *C. argyrophylloides*		Essential oil *C. zenhtneri*	
Strains	MIC	MFC	MIC	MFC	MIC	MFC
C. albicans						
CEMM 01-3-075	NI	NI	NI	NI	>5000	–
CEMM 01-3-069	NI	NI	NI	NI	>5000	–
CEMM 01-3-077	NI	NI	NI	NI	>5000	–
CEMM 01-3-074	NI	NI	NI	NI	>5000	–
C. tropicalis						–
CEMM 01-2-078	NI	NI	NI	NI	2500	5000
CEMM 01-2-063	NI	NI	NI	NI	2500	5000
(Geometric range)	–	–	–	–	2500	5000
M. canis						
CEMM 01-3-188	>5000	–	9	39	620	1250
CEMM 01-5-190	>5000	–	9	39	620	1250
CEMM 01-4-104	>5000	–	9	39	1250	2500
CEMM 01-5-189	>5000	–	19	78	620	1250
CEMM 01-4-097	>5000	–	19	78	1250	2500
CEMM 01-3-165	>5000	–	19	78	1250	2500
(Geometric range)	–	–	13·57	55·15	880·34	1767·77

MIC, minimum inhibitory concentration expressed in μg ml^{-1}; MFC: minimum fungicidal concentration μg ml^{-1}; CEMM: specialized medical mycology center; NI, no inhibition.
Each experiment was repeated at least twice.
Broth microdilution method.

convulsions, dyspnea and ataxia. After the intraperitoneal administration, the calculated LD_{50} for *C. nepetaefolius* and *C. argyrophylloides* were 163·8 mg kg^{-1} (155·3–172·3) and 168·6 mg kg^{-1} (160·5–176·7), respectively. *C. zehntneri* was devoid of any overt toxicity after intraperitoneal administration. None of the essential oils tested presented

remarkable signs of toxicity after oral administration up to 3 g kg^{-1}.

Discussion

The data from this study provide evidence that the essential oils from *C. nepetaefolius*, *C. argyrophylloides* and *C. zehntneri* could be an alternative natural source to treat dermatophytosis. However, a specific study of the safety and *in vivo* efficacy of them and clinical trials are still required to evaluate the practical relevance of the *in vitro* results. The acute toxicity analysis of these *Croton* essential oils revealed that the oral administration at doses ranging from 100 to 3000 mg kg^{-1} induced no remarkable alterations in the mice behaviour pattern. *C. zehntneri* was devoid of any overt toxicity after intraperitoneal administration. Although additional tests must be performed, the results show that the essential oils of these *Croton* species have no acute toxicity. These preliminary toxicity data will be helpful in specific studies to establish the safe profile of these essential oils.

Among natural products, essential oils are one of the most promising groups from which a new prototype of antifungal agents can be developed (Pyun and Shin 2006). The ease of use and relative lack of toxicity of many essential oils leads to numerous possible formulations of topical application for dermatomycoses (Harris 2002). Corroborating these opinions, different authors have evidenced that plant essential oils are effective against some pathogenic fungi responsible for these diseases (Harris 2002; Duarte *et al.* 2005; Pyun and Shin 2006; Fontenelle *et al.* 2007; Tullio *et al.* 2007). In particular, we have demonstrated that essential oil of *Lippia sidoides* Cham. is devoid of overt *in vivo* toxicity and has *in vitro* activity against *M. canis*, *Candida* species and *Malassezia pachydermatis* (Fontenelle *et al.* 2007), and now we have also shown that essential oils from *C. nepetaefolius*, *C. argyrophylloides* and *C. zehntneri* posses *in vitro* antifungal properties with no acute toxicity.

Several previous studies have demonstrated the activity of essential oils against dermatophytes and *Candida* spp. (Tepe *et al.* 2005; Cavaleiro *et al.* 2006; Magwa *et al.* 2006; Fontenelle *et al.* 2007; Matasyoh *et al.* 2007). However, there is no specific study of the antifungal activity of essential oils from *C. zehntneri*, *C. nepetaefolius* and *C. argyrophylloides*. In the present study using agar-well diffusion and broth microdilution methods, we have demonstrated that *C. zehntneri* essential oils have *in vitro* antifungal activity against both *M. canis* and *Candida* spp. However, *C. nepetaefolius* and *C. argyrophylloides* were efficient only for *M. canis*. *C. nepetaefolius* essential oil was effective against *M. canis* at concentrations of 25 000, 50 000, 75 000 and 100 000 $\mu g\ ml^{-1}$, but no statistically significant difference was found among the three last concentrations. There is no difference among the higher doses, because the interval lies in the saturation area of the dose-response curve. So, we have differences only for the extremities.

Among these three plants *C. argyrophylloides* showed the best results against the dermatophyte, with MIC ranging from 9 to 19 $\mu g\ ml^{-1}$ and MFC from 39 to 78 $\mu g\ ml^{-1}$. The results obtained here are very important to establish the essential oils of these *Croton* species, particularly from *C. argyrophylloides*, as promising plants from which a new prototype of phytotherapeutic agent against dermatophyte can be developed.

The antifungal activity of the essential oil of *C. zenhteneri* may be attributed to its major constituent estragole (methyl-chavicol) and/or anethole, which have shown antifungal properties against *Aspergillus parasiticus* (Karapinar 1990; Singh *et al.* 2006). The major constituents of *C. nepetaefolius* were methyl-eugenol (15·7%) and bicyclogermacrene (14·1%). Methyl-eugenol was isolated and identified as the antifungal constituent of the oil from *Artemisia dracunculus* L. var. *dracunculus* (Meepagala *et al.* 2002). *C. argyrophylloides* presented as main constituents spathulenol (20·3%) and bicyclogermacrene (11·7%), which were identified as antifungal constituents of the essential oil of *Hyptis suaveolens* (L.) Point from Tanzania, and supporting the hypothesis that these constituents have the capacity for inhibition the tested fungal strains as they showed activity against other fungal species (Malele *et al.* 2003).

Probably there is a correlation between the antifungal activity of the studied essential oils and their main components. Corroborating this hypothesis, previous studies have demonstrated that essential oils in which spathulenol and caryophyllene oxide are the main compounds have inhibitory activity on filamentous fungi species (Farag *et al.* 2004; Wenqiang *et al.* 2006). Although the antifungal activity of bicyclogermacrene from essential oils is unknown, the antimicrobial potential of terpenoid compounds has already been described (Cavaleiro *et al.* 2006; Cavin *et al.* 2006). The anti-*Candida* activity of *C. zehntneri* essential oil might be related to its main compound, estragole. The antifungal potential of estragole alone (Shin and Kang 2003) or in combination with ketoconazole (Shin and Pyun 2004), has been previously described, particularly against *Candida* species. Additionally, the effect of anethole, a compound with a well-known antifungal potential (Kordali *et al.* 2005; Fujita *et al.* 2007) might contribute to this anti-*Candida* effect.

In brief, because of their antifungal activity and its low toxicity, *Croton* species essential oils are promising sources of new phytotherapeutic agents to treat mycoses,

especially dermatophytosis. However, *in vivo* studies must be performed to confirm this efficacy *in vitro*.

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