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Article

Chemical composition and antimicrobial activity of *Hedyosmum brasiliense* Miq., Chloranthaceae, essential oil

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RESUMO: Óleo essencial de *Hedyosmum brasiliense* Miq., Chloranthaceae: composição e atividade antimicrobiana. *Hedyosmum brasiliense* Miq., Chloranthaceae, é uma espécie endêmica no Brasil, conhecida como "cidrão". Embora *H. brasiliense* seja utilizada como calmante na medicina popular, não foi ainda caracterizada quanto aos constituintes químicos. Este trabalho apresenta a composição do óleo essencial, obtido pela destilação de folhas frescas e de amostra de óleo essencial armazenada por três meses, analisadas por GC-FID e CG-MS. Os efeitos inibitórios do óleo essencial foram testados pelo método da diluição em agar, contra seis espécies de bactérias (*Bacillus subtilis, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus* e *S. saprophyticus*). Adicionalmente, seis espécies de fungos (*Candida albicans, C. parapsilosis, Microsporum canis, M. gypseum, Trichophyton rubrum* e *T. mentagrophytes*) foram incluídas. Dentre os principais constituintes, α-terpineol (10,2%), curzereno (8,9%), pinocarvona (8,4%) e β-tujeno (7,1%) foram os encontrados em maior quantidade. O óleo essencial apresentou baixa atividade contra microorganismos Gram-negativos. Entretanto, a atividade é extraordinária contra bactérias Gram-positivas e fungos, com valores de MIC entre 0,125 a 2,5% (v/v).

Unitermos: *Hedyosmum brasiliense*, Chloranthaceae, óleo essencial, atividade antimicrobiana.

ABSTRACT: *Hedyosmum brasiliense* Miq., Chloranthaceae, is an endemic species of Brazil, locally known as "cidrão". Although *H. brasiliense* is popularly used as sedative, chemical constituents of this species remains uncharacterized. This work presents the essential oil composition, obtained by the distillation of fresh leaves and a sample stored for three months, analysed by GC-FID and GC-MS. The inhibitory effects of the essential oil were tested by the agar dilution method against six bacterial species (*Bacillus subtilis, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus* and *S. saprophyticus*). In addition, six fungal species (*Candida albicans, C. parapsilosis, Microsporum canis, M. gypseum, Trichophyton rubrum* and *T. mentagrophytes*) were included. Among the determined constituents, α-terpineol (10.2%), curzerene (8.9%), pinocarvone (8.4%) and β-thujene (7.1%) were found as the main components. The essential oil has low activity only against Gram-negative microorganisms. However, it is a remarkable active against Gram-positive bacteria and fungi with MIC values ranging from 0.125 to 2.5% (v/v).

Keywords: *Hedyosmum brasiliense*, Chloranthaceae, essential oil, antimicrobial activity.

INTRODUCTION

Hedyosmum brasiliense Miq., Chloranthaceae, is an aromatic shrub and its leaves are widely used as sedative, febrifuge and as a substitute of green tea. It is an abundant and endemic species of Brazil (Reitz, 1965). Despite of these facts, the phytochemistry has not been thoroughly studied and only a single publication concerning the isolation of a lindenanolide sesquiterpene lactone and its antinociceptive activity has appeared (Trentin et al., 1999).

The Chloranthaceae form a small family

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composed of four genera and about 75 species. This family, considered primitive among Angiospermae (Metcalfe & Chalk, 1950; Souza & Lorenzi, 2005) shares the occurrence of secretory cells in the leaves and stems as common feature. Some species (especially from *Chloranthus* and *Hedyosmum*) are used as ornamental or medicinal plants and for alimentary purposes, as beverages (Kawabata et al., 1981). *Hedyosmum* (from the Greek: sweet smell) is the most abundant genus only occurring in the Americas, encompassing about 46 tropical and subtropical species (Souza & Lorenzi, 2005). Gas chromatography coupled to mass spectrometry (GC-MS) analysis of essential oil has thus far only been performed for five species of Hedyosmum: *H. angustifolium, H. scabrum* (Lorenzo et al., 2003), *H. mexicanum, H. bonplandianum* and of *H. costaricensis* (Mundina et al., 2000).

In the present paper, we describe the chemical composition of the essential oil of *Hedyosmum brasiliense* in two forms of preparation: freshly extracted and after three months of storage at room temperature. The methodologies used to analyse both samples were GC with flame ionization detection (FID) and GC-MS. In addition, antimicrobial effects against a panel of human pathogens bacteria and fungi were determined.

MATERIAL AND METHODS

Plant material and extraction

Aerial parts of *Hedyosmum brasiliense* Miq., Chloranthaceae, (5 kg) were collected at the botanical park of Morro do Baú, localized in Luis Alves, state of Santa Catarina, Brazil. The specimen was identified by Dr. Ana Claudia Araújo and deposited at the Herbarium Lyman Bradford Smith (UNIVALI, Itajaí, Brazil), under catalog number 2031.

Fresh leaves (300 g) were hydrodistillated in 4.5 L of boiling distilled water using an apparatus constructed according to specifications in the Japanese Pharmacopoeia (2001). After 2 h of distillation, followed by decanting, a volume of 1.2 mL of essential oil was obtained. The sample was dried over 0.5 g anhydrous sodium sulphate then centrifuged at 3,000x g for 15 min. The resulting supernatant product was kept at 4 °C until bioactivity investigation, with exception of a small aliquot of fresh material which was split into two samples: sample A, analysed immediately after extraction and sample B, stored at room temperature for three months. Prior to analysis, the essential oil was stored in Eppendorf tube. The methodologies used to analyse both samples were GC with flame ionization detection (FID) and GC-MS.

GC-FID and GC-MS Analyses

Analytical gas chromatography was carried out using a Varian CP-3800 gas chromatograph equipped with a flame ionization detector and a CP-Sil 8 CB Low Bleed capillary column (30 m × 0.25 mm, film thickness 0.25 μm). The oven temperature was programmed as follows: 50 °C for 1 min, then rising to 240 °C at 3 °C/min, ending with 1 min at 240 °C; carrier gas He at a flow rate of 1.2 mL/min; injector and detector temperature, 250 and 280 °C respectively; samples were injected (0.2 μL) with split ratio of 1:50. GC-MS analysis was performed on a Varian CP-3800 gas chromatograph interfaced with Saturn 2000 mass spectrometer, operating in electron impact mode at 70 eV with a transfer line temperature of 240 °C, manifold 80 °C and ion trap 175 °C, scan mass range *m/z* 40-400, at a sampling rate of 3 μScan/s. The operating conditions were: 60 °C for 3 min, then rising to 220 °C at 5 °C/min, ending with 15 min at 220 °C; carrier gas He at a flow rate of 1.0 mL/min; injector temperature of 250 °C; samples were injected with split ratio of 1:100.

The oil components were identified by comparison of their retention indices (relative to n-alkanes containing 9 to 25 carbon atoms) and mass spectra with those found in literature (Adams, 1995) and/or stored on the spectrometer database (NIST 1998). The retention indices were validated using isolated standards of several natural compounds (Table 1) under the same chromatographic conditions. The relative amounts of the individual components found in the oil were based on the peak areas obtained from the FID without further correction.

Table 1. Retention indices validated with isolated natural compounds.

Compound	Retention time (min)	Theoretical retention indices	Calculated retention indices
Limonene	12.913	1024	1030
Guaiacol	15.510	1087	1089
Camphor	18.301	1141	1150
Trans-anethole	24.563	1282	1290
Safrole	24.563	1285	1290
Thymol	24.710	1289	1293
Eugenol	27.378	1356	1355

Strains

The antibacterial and antifungal activities of the essential oil were determined against the following strains: *Bacillus subtilis* (ATCC 23858), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus mirabilis* (ATCC 25933), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus saprophyticus* (ATCC 35552), *Candida albicans* (ATCC 10231), *Candida parapsilosis* (ATCC 22019), *Microsporum canis* (C112), *Microsporum gypseum* (C115), *Trichophyton mentagrophytes* (ATCC 9972), and *Trichophyton rubrum* (C137). Sources of the strains were the American Type Culture Collection for the

ATCC numbered strains (Rockville, MD, U.S.A.), and the Centro de Referencia Micológica (CEREMIC, Facultad de Ciencias Bioquímicas y Farmacéuticas, Rosario, Argentina) for clinical isolates with the C numbers.

Antimicrobial testing (Quantitative antimicrobial evaluation)

The Minimum Inhibitory Concentrations (MIC) were determined using the agar dilution method recommended by the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) M7-A6 document (NCCLS, 2003), with the following modification: a final concentration of 0.5% (v/v) Tween-20 (Sigma) was incorporated into the agar medium to enhance oil solubility. A two fold dilution series of oil, ranging from 2.5 to 0.078 % (v/v), was prepared in 1 mL Mueller-Hinton agar medium (Difco) for the bacteria, and Sabouraud dextrose agar medium (Difco) for the fungi, both at 48 °C. Slants were dried at room temperature for 30 min prior to spot inoculation with 1 µL aliquots of culture containing approximately 10^5 CFU of each bacterial species, and 10^3 CFU of each fungal species (Espinel-Ingroff & Pfaller, 1995). The slants were incubated at 35 oC for the bacteria and fungi, with exception of the dermatophytes, which were cultivated at 28-30 oC. The MIC were visually recorded after 24 h for the bacteria, 24-48 h for the yeasts, and after satisfactory fungal growth of the control for the rest of the fungi. The controls were made for each microorganism with the standard antimicrobial tetracycline and vancomycin for bacteria, and ketoconazole for fungi. Inhibition of bacterial and yeast growth in the slants containing test oil was judged by comparison with growth in blank control slants. Experiments were carried out in triplicate.The MIC were determined as the lowest concentration of oil at which a visible inhibitory growth effect on the agar slant was observed.

RESULTS AND DISCUSSION

In this study we report the chemical constituents and antimicrobial activity of the essential oil obtained from leaves of *Hedyosmum brasiliense* Miq., Chloranthaceae, which according to our knowledge have never been studied before. A greenish-yellow oil was obtained from the fresh leaves of *H. brasiliense* after 2 h of distillation, and yield 0.5% (volume/mass of leaves). A typical pleasant-smelling, herbal odour was immediately observed. The chemical composition of the oil was analyzed and is presented in Table 2.

Table 2. Composition of the essential oil of *H. brasiliense* Miq., Chloranthaceae.

Compound	Percentage		
	Sample A	Sample B	RI
α-Thujene	0.22	ND	892
α-Pinene	0.25	ND	908
β-Thujene	7.09	1.14	972
β-Pinene	0.77	ND	978
Myrcene	1.70	0.75	989
o-Cymene	2.21	0.27	1026
Limonene *	0.87	0.35	1030
1,8-Cineole (eucalyptol)	0.92	0.63	1034
β-*trans*-ocimene	0.65	ND	1036
β-*cis*-ocimene	2.29	0.46	1046
τ-Terpinene	0.54	0.35	1059
Terpinolene	0.18	0.14	1087
β-Linalool	1.87	2.35	1101
2,6-Dimethyl-3,5-heptadien-2-ol	0.62	0.67	1103
Chrysanthenone	0.74	1.12	1124
cis-4-Isopropyl-1-methyl-2-cyclohexen-1-ol	0.35	0.32	1127
cis-β-Terpineol	0.38	0.27	1145
Camphor *	0.81	0.40	1150
Pinocarvone	8.39	11.29	1166
3-Pinanone (isopinocamphone)	0.23	0.28	1180
α-Terpineol	10.22	5.11	1184
γ-Terpineol	0.72	0.54	1198

Methyl chavicol	0.59	0.52	1201
trans-Piperitol	0.35	0.17	1212
3,7-Dimethyl-2-octen-1-ol	3.3	2.13	1229
cis-Myrtanol	0.21	1.04	1243
trans-Mirtanol	1.45	2.05	1259
Bornyl acetate	0.28	0.20	1286
Thymol	0.53	0.89	1294
trans-Pinocarvyl acetate	0.66	0.83	1311
Myrtenyl acetate	0.28	ND	1325
δ-Elemene	0.53	0.68	1333
Elixene	0.19	ND	1339
Thymol acetate	1.53	1.88	1348
Citronellyl acetate	1.05	0.87	1350
Copaene	0.29	0.40	1377
1,5-Dimethyl-8-(1-methylethenyl)-(1*E*,5*Z*,8*S*)-1,5-cyclodecadiene	1.25	1.05	1391
Eugenol methyl ether	1.10	1.06	1403
Caryophyllene<(*E*)>	0.32	0.22	1421
Humulene	0.20	ND	1442
Aromadendrene<*allo*>	0.30	0.30	1462
Germacrene D	1.63	1.83	1483
Curzerene	8.93	15.95	1498
α-Farnesene	0.23	ND	1505
Eugenol acetate	0.28	0.75	1518
δ-Cadinene	0.70	0.85	1520
Elemol	0.46	0.70	1551
Eremophilene	0.60	0.65	1562
τ-Eudesmol	0.36	ND	1573
Spathulenol	5.26	4.96	1580
Globulol	1.16	0.45	1589
Viridiflorol	0.92	0.19	1598
Guaiol	0.20	ND	1600
Carotol	6.06	18.35	1606
1-*epi*-Cubenol	0.23	0.19	1631
Ledene oxide (II)	1.86	1.15	1637
τ-Muurolol	0.62	0.48	1647
Himachalol	0.44	0.30	1650
Dihydroeudesmol	3.90	1.51	1659
(2*Z*,6*Z*)-Farnesol	0.24	0.27	1698
Longifolenaldehyde	0.19	ND	1744
Eicosahydrodibenzo(a.i)fluorene	0.55	ND	1907
8,9-Dehydro-9-vinyl-cycloisolongifolene	0.32	ND	1918
Androst-1-ene-3.11-dione (5.alpha.)-	0.30	ND	2194

Identification confirmed by retention time similarity with isolated standard compounds (denoted by asterisks). RI = Retention indices on a CP-Sil 8 CB Low Bleed column. Sample A: essential oil analysed immediately after extraction. Sample B: essential oil analysed three months after extraction.

Among the seventy compounds identified, 90% are terpenes: twenty oxygenated monoterpenes (28.6%), nineteen oxygenated sesquiterpenes (27.1%), thirteen non-oxygenated sesquiterpenes (18.6%) and eleven non-oxygenated monoterpenes (15.7%). The minor compounds included three phenylpropanoids (4.3%) and four compounds of other classes.

The most abundant single compound was the monoterpene alcohol α-terpineol (10.2%), followed by the furan-containing sesquiterpenoid curzerene (8.9%), and the ketones pinocarvone (8.4%) and β-thujene (7.1%). We analyzed the effect of storage of the essential oil by comparison of the composition of the samples A and B (Figure 1). Three months after the extraction, the majority of the peaks were still present. Some peaks, mainly those related to compounds present at a very low abundance in the fresh starting material (like α-thujene, α- and β-pinene, myrtenyl acetate, elixene and humulene) were not detectable after three months. However, the percentage of the following oxygenated compounds increased: the sesquiterpene alcohol carotol (from 6.1% to 18.4%), curzerene (from 8.9% to 15.9%) and pinocarvone (from 8.4% to 11.3%). Despite these observations the odour of the two samples remained essentially unaltered. Apparently, the key components responsible for the odour did not significantly alter upon storage.

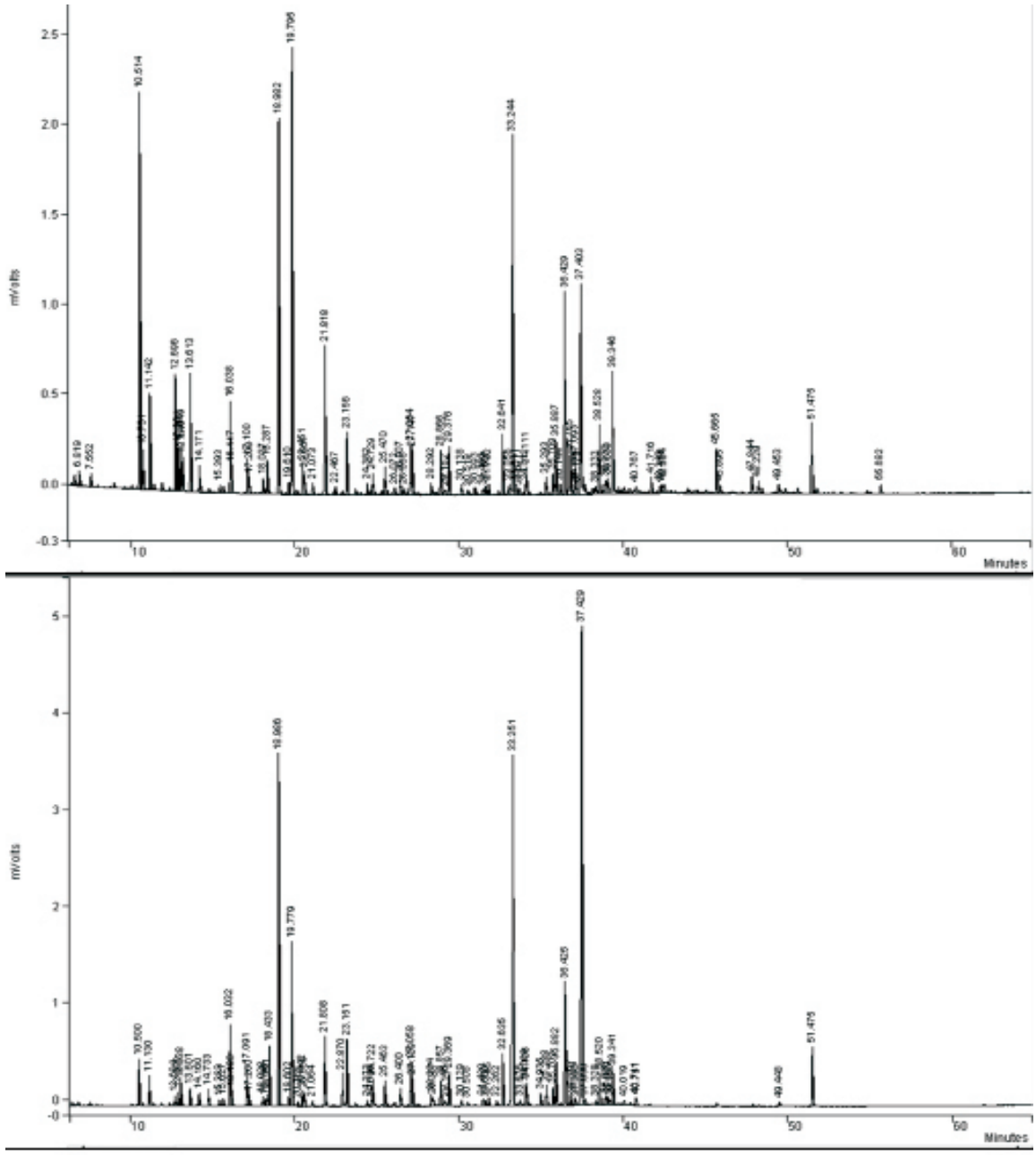

Figure 1. Chromatographic profile of the essential oil of *H. brasiliense* Miq., Chloranthaceae. A Sample A, analysed immediately after extraction and sample B, stored at room temperature for three months.

The composition of the essential oil of *H. brasiliense* was significantly different from that of other *Hedyosmum* species. Mundina et al. (2007) described the contents of three species from Costa Rica. *H. mexicanum* Cordemoy contains two abundant components: the monoterpene sabinene (28%) and the oxygen-containing sesquiterpene furanodiene (20%). In the related species H. costaricencis C. E. Wood the sesquiterpenes germacrene-D (32%), (*E,E*)-α-farnesene (8%), β-caryophyllene (6%) and β-bourbonene (6%) and the monoterpene (*E*)-β-o-cimene (2%) occur. In the third species, *H. bonplandianum* Kunth, the predominant components are the monoterpenes sabinene (15%) and (*E*)-β-ocimene (11%), the monoterpenoids linalool (3.0%) and terpoin-4-ol (7%) and the sesquiterpenes germacrene D (32%), α-bisabolene (10%) and β-caryophyllene (6.1%).

A second article (Lorenzo et al., 2003) describes the essential oil from two bolivian *Hedyosmum* species: *H. angustifolium* (Solms-Laubach) and *H. scabrum* (Solms-Laubach). Monoterpene hydrocarbons, especially α-and β-pinene (24 and 23.5% respectively), and oxygen containing monoterpenes, mainly 1,8-cineole (3.7%) and linalool (6.1%), were the principal constituents found in the *H. angustifolium* oil. The oil of *H. scabrum* was mainly made up of monoterpenes, in particular δ-3-carene, the sesquiterpenes α-gurjunene (6.6%) and germacrene-D (13%), and 3´,4´-dimethoxypropiophenone (6.6%).

Essential oils have been largely used against several diseases, occasionally based on folk medicine observations (Daniel et al., 2009; Leite et al., 2009). Thus, approximately 300 characterized essential oils are economically important because of their extensive use in the pharmaceutical, cosmetic, and perfume industries (Bakkali et al., 2008). Although we did not clinically assess the therapeutic effect of the essential oil of *H. brasiliense*, based on the composition here described, we can anticipate a potential pharmaceutical and/or cosmetic use. Two of the major compounds, α-terpineol and β-thujene, have been described to be important for the antioxidant and antimicrobial activity of several plants and or essential oils (Pengelly, 2004). Additionally, α-terpineol is also used worldwide in fine fragrances, shampoos, as well as in non-cosmetic products such household cleaners and detergents (Bathia et al., 2008).

Our results also indicate that *H. brasiliense* essential oil has antimicrobial activity against pathogenic bacteria and fungi in the agar dilution. This method is most suitable for such experiments because the relative potency of both polar and non-polar substances as well interactions in complex extracts can be measured (Ríos & Recio, 2005). As summarized in Table 3 this essential oil presented appreciable antibacterial activity against the Gram-positive bacteria *S. aureus, S. saprophyticus* and *B. subtilis*, with a MIC value of 0.312%. This points out that the essential oil could be used as topic agent to combat *Staphylococcus aureus*, one of the major causes of community-acquired and hospital-acquired infections (Deurenberg & Stobberingh, 2009). Similarly, *Staphylococcus saprophyticus*, a coagulase-negative species of *Staphylococcus* often participating in urinary tract infections is also susceptible to this essential oil.

No activity against the Gram-negative bacteria was found up to the highest concentration tested (2.5%). Thus, the use as antibacterial agent is limited to Gram-positive microorganisms. Susceptibility of Gram-positive bacteria and relative tolerance of Gram-negative bacteria to essential oils has been correlated with the presence of a hydrophilic outer layer (Kalemba & Kunicka, 2003). It is presumed that penetration of hydrophobic components in Gram-negative microorganisms is more difficult due to the presence of a second physical barrier formed by the outer membrane (Mann et al., 2000; Duffy & Power, 2001).

H. brasiliense essential oil also presented antifungal properties against a panel of human opportunistic pathogenic fungi, and the results showed significant activity against fungal dermatophytes (*M. canis, M. gypseum, T. mentagrophytes* and *T. rubrum*), that are responsible for the invasion of the keratinized portion of the skin, nails, and hair causing infection (Woodfolk, 2005). These fungi are considered to be the main organisms responsible for onychomycosis (Sarifakioglu et al., 2007), and despite new systemic antifungal therapies, is still difficult to eradicate, exemplified by the 25 to 40% recurrence in nail infections (Hay, 2001). It is hoped that novel substances, including essential oils, could form an economically sustainable alternative to treat nail infections. The worldwide spread of HIV infection increased the occurrence of opportunistic fungi, including *Candida* species (Pfaller & Diekema, 2007). Our finding of a significant antifungal activity of essential oil against *C. albicans* and *C. parapsilosis* (MIC values ranging from 0.125 to 0.312%) is therefore of clinical relevance.

Also in Table 3, it is shown the results of standard antibiotics, that it was added only to assess the susceptibility of tested strains, whereas a comparison of the antimicrobial activity of essential oils and antibiotics cannot be made from this manner (Janssen et al., 1987; Kalemba & Kunicka, 2003), since was described above, the essential oil of *H. brasiliense* it is a complex mixture of compounds, like all essential oils, where the active principle can be in small proportion. Also we stand out that it is not pretension of this study to compare the results with antibiotics used in the clinic, but that they can serve to add value if the cited essential oil as an ingredient of a pharmaceutical formulation.

The antimicrobial activity of *H. brasiliense* essential oil is suspected to be associated with one of the major components. α-terpineol is an important candidate for such a biologically active component (Kotan et al., 2007; Park et al., 2009; But some studies report that whole essential oils have a greater antimicrobial activity than the major components mixed (Gill et al., 2002; Mourey

& Canillac, 2002). This finding suggests that minor components like carotol, spathulenol, eudesmol, and *cis*-ocymene, present in our samples, could also affect the antimicrobial properties (Jasicka-Misiak et al., 2004; Razavi et al., 2009; Ugur et al., 2009).

Table 3. Antimicrobial activity of the essential oil of *H. brasiliense* Miq., Chloranthaceae.

Microorganism	MIC	
	% (vol/vol)	% (wt/vol) / µg/ml
Bacillus subtilis	0.312	$7x10^{-5}$/0.7 *
Staphylococcus aureus	0.312	$2x10^{-4}$/2 *
Staphylococcus saprophyticus	0.312	$2x10^{-4}$/2 *
Escherichia coli	>2.5	$4x10^{-4}$/4 **
Proteus mirabilis	>2.5	$2x10^{-4}$/2 **
Pseudomonas aeruginosa	>2.5	$1.6x10^{-3}$/16 **
Candida albicans	0.312	$2.5x10^{-5}$/0.25 ***
Candida parapsilosis	0.312	$1.25x10^{-5}$/0.125 ***
Microsporum canis	0.125	$1.25x10^{-5}$/0.125 ***
Microsporum gypseum	0.312	$5x10^{-5}$/0.5 ***
Trichophyton rubrum	0.125	$1.25x10^{-5}$/0.125 ***
Trichophyton mentagrophytes	0.312	$2.5x10^{-5}$/0.25 ***

MIC = Minimal inhibitory concentration, (*) vancomycin, (**) = tetracycline, (***) = ketoconazole.

In conclusion, this investigation characterized the components of *H. brasiliense* essential oil and investigated its antimicrobial properties. Our tests shown that *H. brasiliense* essential oil is in vitro active against Gram-positive bacteria, including the pathogen *S. aureus*, dermatophytes and opportunistic fungi.

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REFERENCES

Adams RP 1995. *Identification of essential oil components by gas chromatography/mass spectroscopy.* Illinois: Allured Publishing Corporation.

Bakkali F, Averbeck S, Averbeck D, Idaomar M 2008. Biological effects of essential oils - a review. *Food Chem Toxicol 46:* 446-475.

Daniel NA, Sartoretto SM, Schmidt G, Caparroz-Assef SM, Bersani-Amado CA, Kenji RKN. 2009. Anti-inflammatory and antinociceptive activities A of eugenol essential oil in experimental animal models. *Rev Bras Farmacogn 19:* 212-217.

Bathia SP, Letizia CS, Api AP 2008. Fragrance material review on (-)-α-terpineol. *Food Chem Toxicol 46*: S280-S285.

Deurenberg RH, Stobberingh EE 2009. The molecular evolution of hospital and community-associated methicillin-resistant *Staphylococcus aureus*. *Curr Mol Med 9*: 100-115.

Duffy CF, Power RF 2001. Antioxidant and antimicrobial properties of some Chinese plant extracts. *Int J Antimicrob Agents 17*: 527-529.

Espinel-Ingroff A, Pfaller MA 1995. Antifungal agents and susceptibility testing. In: Murray PR Baron EJ, Pfaller MA, Tenover FC, Yolken RH. *Manual of clinical microbiology.* 6th ed. Washington DC: American Society for Microbiology. p. 1405-1414.

Gill AO, Delaquis P, Russo P, Holley RA 2002. Evaluation of antilisterial action of cilantro oil on vacuum packed ham. *Int J Food Microbiol 73*: 83-92.

Hay RJ 2001. The future of onychomycosis therapy may involve a combination of approaches. *Br J Dermatol 145*: 3-8.

Janssen AM, Scheffer JJC, Baerheim Svenden A 1987. Antimicrobial activity of essential oils: a 1976-1986 literature review. Aspects of the test methods. *Planta Med 53*: 395-398.

Jasicka-Misiak I, Lipok J, Nowakowska EM, Wieczorek PP, Młynarz P, Kafarski P 2004. Antifungal activity of the carrot seed oil and its major sesquiterpene compounds. *Z Naturforsch C 59*: 791-796.

Kalemba D, Kunicka A 2003. Antibacterial and antifungal properties of essential oils. *Curr Med Chem 10*: 813-829

Kawabata J, Tahara S, Mizutani J 1981. Isolation and structural elucidation of four sesquiterpenes from *Chloranthus japonicus* (Chloranthaceae). *Agric Biol Chem 45*: 1447-1453.

Kotan R, Kordali S, Cakir A 2007. Screening of antibacterial activities of twenty-one oxygenated monoterpenes. *Z. Naturforsch C 62*: 507-513.

Leite AM, Lima EO, Souza EL, Diniz MFFM, Leite SP, Xavier AL, Medeiros IA 2009. Preliminary study of the molluscicidal and larvicidal properties of some essential oils and phytochemicals from medicinal plants. *Rev Bras Farmacogn 19*: 842-846.

Lorenzo D, Loayza I, Dellacassa E 2003. Composition of the essential oils from leaves of two Hedyosmum spp. from Bolivia. Flavour Fragr J 18: 32-35.

Mann CM, Cox SD, Markham JL 2000. The outer membrane of *Pseudomonas aeruginosa* NCTC6749 contributes to its tolerance to the essential oil of *Melaleuca alternifolia* (tea tree oil). *Lett Appl Microbiol 30*: 294-297

Metcalfe CR, Chalk L 1950 *Anatomy of the Dicotyledons* Vol. 1, Claredon Press, Oxford.

Ministry of Health and Welfare 2001. Crude drugs. In: *The Japanese Pharmacopoeia*, 14th ed., Tokyo: Ministry of Health and Welfare, p. 41.

Mourey A, Canillac N 2002. Anti-*Listeria monocytogenes* activity of essential oils components of conifers. *Food Contr 13*: 289-292.

Mundina M, Vila R, Tomi F, Cicció JF, Ibañez C, Adzet T, Casanova J, Cañigueral S 2000. Essential oil composition from leaves and fruits of three *Hedyosmum* species from Costa Rica. *Flavour Fragr J 15*: 201-205.

National Committee for Clinical Laboratory Standards 2003. *Methods for dilution antimicrobial susceptibility tests of bacteria that grow aerobically*, NCCLS: Wayne. P.A.

Park MJ, Gwak KS, Yang I, Kim KW, Jeung EB, Chang JW, Choi IG 2009. Effect of citral, eugenol, nerolidol and alpha-terpineol on the ultrastructural changes of *Trichophyton mentagrophytes. Fitoterapia 80*: 290-296.

Pengelly A 2004. Essential oils and resins. In: Pengelly A. The *Constituents of Medicinal Plants: An introduction to the chemistry and therapeutics of herbal* medicine. 2.ed. Cambridge: CABI Publishing. p. 85-109.

Pfaller MA, Diekema DJ 2007. Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev 20*: 133-163.

Razavi SM, Nazemiyeh H, Zarrini G, Asna-Asharii S, Dehghan G 2009. Chemical composition and antimicrobial activity of essential oil of *Prangos ferulaceae* (L.) Lindl from Iran. *Nat Prod Res 18*: 1-4.

Reitz R 1965. *Clorantaceas - Flora Ilustrada Catarinense.* Itajaí: Herbário "Barbosa Rodrigues", p. 4-10.

Ríos JL, Recio MC 2005. Medicinal plants and antimicrobial activity. *J Ethnopharmacol 100*: 80-84.

Sarifakioglu E, Seçkin D, Demirbilek M, Can F 2007. *In vitro* antifungal susceptibility patterns of dermatophyte strains causing *tinea unguium. Clin Exp Dermatol 32*: 675-679.

Souza VC, Lorenzi, H 2005. *Botânica sistemática*. Nova Odessa: Instituto Plantarum.

Trentin AP, Santos AR, Guedes A, Pizzolatti MG, Yunes R A, Calixto JB 1999. Antinociception caused by the extract of *Hedyosmum brasiliense* and its active principle, the sesquiterpene lactone 13-hydroxy-8,9-dehydroshizukanolide. *Planta Med 65*: 517-521.

Ugur A, Duru ME, Ceylan O, Sarac N, Varol O, Kivrak I 2009. Chemical composition, antimicrobial and antioxidant activities of *Centaurea ensiformis* Hub.-Mor. (Asteraceae), a species endemic to Mugla (Turkey). *Nat Prod Res 23*: 149-167.

Woodfolk JA 2005. Allergy and dermatophytes. *Clin Microbiol Rev 18*: 30-43.