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Article

Seasonal Variation and Antimicrobial Activity of *Myrcia myrtifolia* Essential Oils

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Os óleos essenciais das folhas, flores e frutos de *Myrcia myrtifolia* DC foram coletados ao longo dos anos de 2002 e 2003 e foram analisados utilizando CG-DIC e CG-EM. No total foram identificados 28 componentes sendo que o α-pineno foi a substância predominante em todas as amostras analisadas apresentando-se em concentrações que variaram entre 61.5 e 90.9%. As propriedades antimicrobianas do óleo essencial das folhas coletadas em outubro de 2002 foram avaliadas contra seis bactérias, duas leveduras e cinco fungos filamentosos sendo mais fortemente ativo contra *Microsporum canis* e *Trichophyton rubrum*, ativo contra *Staphylococcus aureus*, *Staphylococcus aureus* resistente à meticilina, *Candida albicans*, *Cryptococcus neoformans* e *Aspergillus fumigatus*. O óleo ainda mostrou uma toxicidade moderada, LC_{50} of 479,16 μg mL^{-1}, contra *Artemia salina*.

This work reports the seasonal variation of the composition of leaf volatile oils and the composition of volatile oils from flowers and fruits of *Myrcia myrtifolia* DC harvested in the sand dunes of Salvador, Bahia, northeastern region of Brazil between 2002 and 2003. The oils were analyzed by GC-FID and GC-MS so that 28 components were identified. α-Pinene was predominant in a range from 61.5 to 90.9% in all samples analyzed. The leaf oil collected in October 2002 had their antimicrobial properties tested against six bacteria, two yeasts and five filamentous fungi being active against *Staphylococcus aureus*, methicilin-resistant *Staphylococcus aureus*, *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus*, and showed strongest activity against *Microsporum canis* and *Trichophyton rubrum*. The oil displayed moderate toxicity against *Artemia salina* showing a LC_{50} of 479.16 μg mL^{-1}.

Keywords: Myrtaceae, *Myrcia myrtifolia,* essential oil, monoterpene, sesquiterpene, antimicrobial activity

Introduction

Aromatic plants have been used since ancient times as antiseptics and anti-infectious agents, as aroma in perfumes and cosmetics as well as preservative and flavour ingredients in food and beverages. Their biological properties are directly related with their chemical compositions, which can be affected by environmental, geographical, seasonal and circadian variations.

The family Myrtaceae is constituted by 140 genera with about 3000 species, which are widely distributed in America and Australia.[1] The genus *Myrcia* comprises more than 300 species that grow in all Brazilian territory. Indigenous tribes and traditional Brazilian communities have used several species of this genus as astringent, against diabetes and diarrhea, as diuretic, to stanch hemorrhages, against the hypertension and ulcers of the mouth.[2]

Some works were published regarding the chemistry and the biological properties of *Myrcia* species. From *M. citriofolia*, the occurrence of a C-methylated flavone, eucalyptin, was related,[3] from the methanolic extract of *M. multiflora* were isolated flavonol, flavanone and acetophenone glucosides along with myricitrin, mearnssitin, quercitrin, desmanthin-1 and guaijaverin, this

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extract were found to show antidiabetic properties and potent inhibitory activities on aldose reductase and α-glucosidase.[4,5] The studies of essential oils of sixteen *Myrcia* species demonstrated that sesquiterpenes are predominant for all of them but for *M. acuminatissima* and *M. bombycina* for which the monoterpenes fraction is slightly larger.[6-8] In a study with volatile oils of some *Myrtaceae* species the lack of activity against *Escherichia coli* and good activity against *Staphylococcus aureus* and *S. epidermidis* in the majority of species was related.[9] The antifungal activity of essential oils from *Myrtaceae* species against dermatophytes was attributed to its terpenes constituents.[10]

The aim of this work was the study of seasonal variation of composition of the leaf volatile oils and the composition of volatile oils from flowers and fruits of *M. myrtifolia* that was harvested in the sand dunes of Salvador, Bahia, northeastern region of Brazil between 2002 and 2003, along with the study of the antimicrobial properties of the leaf oil collected in October 2002 against six bacteria, two yeasts and five filamentous fungi. Additionally, a preliminary toxicity evaluation of the leaf oil with brine shrimp (*Artemia salina*) bioassay was performed.

Experimental

Plant material

Two specimens of *M. myrtifolia* DC, apart from each other by approximately 100 m, were selected to work. Leaves of the two specimens were collected every month from May 2002 to December 2003 always between 8:00 and 10:00 pm. Flowers and fruits were collected when they were present. The leaves, immature flowers and immature fruits were carefully separated, cleaned and frozen at –20 °C until the extraction. Voucher specimens were deposited in the herbarium Alexandre Leal Costa of the Instituto de Biologia, UFBA, under the number 52168.

Essential oil analysis

The volatile oils were obtained from leaves, fruits and flowers after 3 h hydro-distillation in a Clevenger modified apparatus. The identification of compounds was performed by comparison of their retention indices and mass spectra with those reported in the literature[11,12] and stored in the NIST libraries (Mass Spectral Library, 1998). The retention indices were calculated by co-injection with a standard saturated *n*-alkanes homologous series.

GC analyses were performed using a gas chromatograph HP 5890 equipped with capillary DB-5 column (30 m × 0.25 mm, film thickness 0.25 μm) and a FID detector. The oven temperature was programmed from 60 °C to 240 °C at rate of 3 °C min^{-1}, and isothermal at 240 °C for 10 min, using H_2 as the carrier gas (1.0 mL min^{-1}). Injector and detector temperatures were 220 °C and 300 °C, respectively.

GC-MS analyses were performed using a gas chromatograph HP 6890 interfaced with a HP 5873 Mass Selective Detector (ionisation voltage 70 eV) equipped with capillary HP-5MS column (30 m × 0.25 mm, film thickness 0.25 μm), using He as the carrier gas (1.0 mL min^{-1}). Oven and injector temperatures were as above.

Microbial strains

Thirteen microbial strains were used to access the antimicrobial properties of the test sample: four Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Salmonella choleraesuis* ATCC 10708, *Pseudomonas aeruginosa* ATCC 27853 and *Proteus mirabilis* ATCC 29906), two Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923 and methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 33591), two yeasts (*Candida albicans* ATCC 90028 and *Cryptococcus neoformans* ATCC 32045) and five filamentous fungi (*Aspergillus fumigatus* ATCC 16913, *Cladosporium herbarum* ATCC 26362, *Microsporum canis* ATCC32903, *Penicillium notatum* ATCC 9478 and *Trichophyton rubrum* ATCC 28189). Microorganisms were obtained from the culture collections of the Instituto Nacional de Controle de Qualidade em Saúde- INCQS/ Brazil. Bacteria and fungi were grown on Mueller Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) (Difco Laboratoires). Inocula were prepared in sterile saline from 24 h, 48 h and 7-14 days cultures of bacteria, yeasts and filamentous fungi respectively, and standardized against 0.5 McFarland Standard to obtain suspensions containing approximately 10^8 cfu mL^{-1} of bacteria and 10^6 cfu mL^{-1} of fungi.

Essential oils dilution

The essential oils were tested pure and at dilutions ranging from 8% to 0.125% (v/v), corresponding, respectively, to concentrations of 69.50 mg mL^{-1} to 1.08 mg mL^{-1}. The dilutions were prepared in sterile distilled water with addition of Tween 80 (Merck) to the first dilution in a concentration of 10% (v/v), each dilution being mixed on Vortex.[14] Due to the similarity of the oils

composition, only the oil of the leaves collected in October 2002 had its antimicrobial properties and toxicity tested.

Preparation of microorganism culture

To evaluate antimicrobial activity, assays were performed using the agar diffusion method.[14,15] For each microorganism, 1 mL of standardized inoculum was transferred to sterile Petri dishes and mixed with 20 mL of molten agar at 45 °C (MHA for bacteria and SDA for fungi). After solidification, 6 mm wells were made on agar (six per plate) to which 50 μL of each oil dilution was transferred. After 30 min at room temperature, plates with bacteria were incubated at 35-37 °C for 24 h, 28-30 °C for 48 h with yeasts and 10-14 days with filamentous fungi. Growth control of test strains and diluent control were simultaneously performed. Susceptibility tests using standard substances obtained from Cecon/SP were performed by applying the diffusion method with filter paper disks of 6 mm diameter Chloranphenicol (30 μg) for Gram-negative bacteria, Vancomycin (30 μg) for *Staphylococcus* species and Ketoconazol (50 μg) for fungi (NCCLS, 2000). The results were showed on Table 3. All the tests were performed in duplicate. The bioassay results were expressed in terms of mean of inhibition zone diameters: < 9 mm, inactive; 9-12 mm, partially active; 12-18 mm, active; > 18 mm, very active.

Minimum Inhibitory Concentration (MIC)

MIC assays were performed by Broth Microdilution Method as described in National Committee for Clinical Laboratory Standards with 100 mL aliquots of diluted leaf oil and standards antimicrobial agents as controls.[16,17] Bacterial suspensions were standardized with 0.5 McFarland standard and diluted to give final concentrations of 5×10^5 cfu mL^{-1} for bacteria and $0.5\text{-}2.5 \times 10^3$ cfu mL^{-1} for yeasts. The minimum bactericidal concentrations were visualized by inoculating 20 μL of sterile 0.01% sodium resazurin solution to each plate well and after 3 h incubation. The minimal inhibitory concentration, defined as the lowest concentration that inhibited bacterial growth, was determined by the emergence of a blue colour at the wells indicating absence of growth.[18] The minimum fungicidal concentrations were determined by absence of fungal growth when compared with positive and negative controls. The MIC assays were performed with microorganisms for which the oil was considered active in the agar diffusion assay (inhibition zone diameters larger than 12 mm) except to filamentous fungi.

Toxicity against Artemia salina

The brine shrimp lethality assay was performed by the method of McLaughlin.[19] Brine shrimp eggs (*Artemia salina*) were hatched in saline solution of NaCl (38 g L^{-1}).

The essential oil was tested at concentrations of 10, 100 and 1000 mg L^{-1}. Survival was measured after 24 h incubation at 10 °C. The collected data were computerized and LC_{50} values determined by Probit analysis.

Results and Discussion

The analysis of the leaf essential oil from the first collected material (May, 2002) demonstrated a larger predominance of monoterpenes (80.3%), being the main component α-pinene (62.0%), these results were in disagreement with the previous studies published about other *Myrcia* species to which sesquiterpenes were the main components.[6-8] To verify if this result it would have been caused by a seasonal condition, the essential oils were collected and analyzed monthly during the years 2002 and 2003. To minimize the effects of the variation of other such factors as soil composition, humidity, light intensity, and others, the leaves, flower and fruits were always collected from two specimens very close each other. Sixteen samples of leaves, six from flowers and two from fruits were analyzed between May 2002 and December 2003. In total, twenty-eight compounds were identified in the oils (Tables 1 and 2). The monoterpene fraction was present in higher amount in all samples, 94.1% in average, being hydrocarbons the main compounds. The most abundant component in all samples analyzed was α-pinene. In the leaves, its concentrations varied from 62.0% (May 2002) to 87.3% (October 2002). In the flowers α-pinene concentrations varied from 61.5% (November 2002) to 85.1% (December, 2002). In the immature fruits, its concentrations were 88.1% (May 2002) and 90.9% (June 2003). Although in low concentrations, limonene and β-pinene were the only compounds besides α-pinene, present in all leaves, flowers and fruits samples. *trans*-Pinocarveol which was present in all leaves samples, it was not present in flowers while in the fruits it was present in May 2002 and not in June 2003. β-Cariophyllene was present in larger concentrations in the flowers. In the fruits and leaves either it was not present or it was present in concentrations smaller than 1.0%.

In a general way, the composition of the leaf oils did not show meaningful seasonal variation, however, the leaf oils yield was found to vary during the year reaching its maximum between August and December.

Table 1. Seasonal composition of essential oils from leaves of *Myrcia myrtifolia* collected from May 2002 to December 2003

Compound	RI	5/02	7/02	8/02	10/02	12/02	01/03	2/03	3/03	4/03	6/03	7/03	8/03	9/03	10/03	11/03	12/03
E-3-hexenol	846	0.1	-	-	-	0.1	-	-	-	0.2	0.2	0.1	0.1	T	-	T	0.2
E-2-hexenal	854	1.2	0.5	1.4	0.7	0.6	0.1	-	0.3	1.3	1.3	0.9	0.2	0.2	0.2	0.1	1.5
α-pinene	938	62.0	81.4	83.9	87.3	80.6	86.8	72.1	80.4	84.6	81.5	75.7	83.4	84.3	82.7	86.7	81.2
α-fenchene	945	-	-	t	-	0.1	0.2	0.2	-	0.2	0.2	0.2	0.2	0.3	0.1	0.1	0.1
camphene	947	-	-	t	-	0.2	-	----	--	-	-	t	-	-	0.2	0.2	0.2
β-pinene	975	0.7	0.8	0.6	0.7	0.6	0.6	1.0	1.5	0.9	0.7	0.1	0.8	0.6	0.9	1.0	0.9
α-terpinene	1016	-	-	t	-	0.1	-	-	-	0.2	-	t	0.2	-	-	-	0.1
p-cimene	1023	-	-	t	-	0.2	-	-	-	2.8	2.2	0.1	-	0.1	0.1	0.1	0.1
limonene	1028	2.2	2.7	2.1	2.3	2.1	3.1	2.6	2.6	3.0	1.5	2.3	2.3	2.4	2.7	2.4	2.0
eucalyptol	1030	2.3	1.8	1.6	2.1	1.1	1.6	1.7	2.1	-	-	1.5	1.7	1.4	1.5	1.5	1.3
γ-terpinene	1062	-	-	t	-	-	-	-	-	-	-	t	0.1	0.1	0.1	0.1	0.1
p-menta-3,8-diene	1069	0.3	-	t	-	-	-	0.3	-	-	-	0.2	0.2	0.1	0.3	0.2	0.2
terpinolene	1087	05	0.9	0.7	0.5	-	0.8	0.8	0.9	0.4	0.6	0.6	0.7	0.8	0.9	0.7	0.6
linalool	1099	0.4	0.4	t	-	-	0.5	0.4	0.4	-	-	0.2	0.2	0.2	0.2	0.1	0.2
endo-fenchol	1112	0.3	0.4	t	-	0.2	0.2	0.2	0.4	0.2	0.2	0.3	0.3	0.3	0.4	0.2	0.3
trans-pinocarveol	1139	4.1	3.1	3.0	1.9	1.4	1.6	2.4	2.8	2.4	1.8	2.2	2.3	2.3	2.0	1.2	1.7
pinocarvone	1160	0.4	0.4	t	-	0.2	-	0.4	-	0.3	0.2	0.2	0.4	0.2	0.4	0.1	0.2
borneol	1163	0.6	0.5	t	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.4	-	0.4	0.6	0.2	0.3
terpin-4-ol	1175	0.3	-	t	-	0.2	-	-	-	0.2	-	0.2	0.2	0.2	0.3	0.2	0.3
α-terpineol	1192	5.8	6.1	4.9	4.2	3.3	3.4	2.8	7.0	2.4	3.2	4.7	4.1	4.4	5.1	3.5	3.9
myrtenol	1196	-	-	t	-	-		-	-	-	-	0.1	0.1	0.1	0.2	0.2	0.1
trans-carveol	1217	0.4	-	t	-	-	-	-	-	-	-	0.2	0.1	0.2	0.2	0.1	0.2
β-cariophyllene	1417	-	0.7	1.7	-	-	-	-	-	-	-	0.2	0.1	-	0.2	0.1	0.3
α-humulene	1450	-	-	t	-	-	-	0.7	0.4	-	-	t	-	-	-	-	-
α-cadinene	1535	2.1	-	t	-	0.2	-	0.4	-	-	-	-	-	-	-	0.1	0.2
hinesol	1637	0.6	-	t	-	-	0.4	-	-	0.3	0.2	-	0.1	-	-	-	-
14-OH-α-humulene	1710	-	-	t	-	-	-	-	0.6	-	-	-	-	-	-	-	0.1
Total		87.8	98.9	99.2	99.5	92.0	99.6	88.3	99.7	99.7	94.1	90.4	98.3	98.5	98.5	98.5	96.8
aliphatic compounds		1.3	0.5	1.4	0.7	0.7	0.1	-	0.3	1.5	1.5	1.0	0.3	0.2	0.2	0.1	1.7
monoterpenes hydrocarbons		65.7	84.9	86.6	90.3	83.3	91.5	77.0	85.4	92.1	86.7	78.5	87.9	88.1	88.0	90.8	85.5
oxygenated monoterpenes		14.6	12.7	9.5	8.5	6.7	7.6	8.3	13.0	5.8	5.7	10.0	9.4	9.7	9.4	7.3	8.5
sesquiterpenes hydrocarbons		2.1	0.7	1.7	-	-	-	2.8	0.4	-	-	0.2	0.1	-	0.2	0.1	0.3
oxygenated sesquiterpenes		0.6	-	-	-	-	-	0.2	0.6	-	-	-	0.1	-	-	-	0.2
yield of the oil / (%, m/m)		nm	0.08	0.13	0.20	0.14	0.11	nm	0.14	nm	0.08	0.17	0.12	0.20	0.18	0.16	0.25
mass of fresh leaves / g		41.0	295.0	407.0	210.0	164.0	79.0	89.0	126.0	155.0	242.0	218.0	312.0	201.0	280.0	280.0	396.0

t = amounts < 0.1%; nm = not measured; - = not detected.

The evaluation of antimicrobial activity by the agar diffusion method showed that the leaf volatile oil presented activity to some specific microorganisms. Table 3 summarizes the results listing only the microorganisms that had their growth inhibited. The essential oil evaluated in this screening did not present inhibition against Gram-negative bacteria (*E. coli*, *S. choleraesuis*, *P. aeruginosa*, *P. mirabilis*) and against the fungi *C. herbarum* and *P. notatum*. The pure oil was active against *S. aureus*, methicilin resistant *S. aureus* (MRSA), *C. albicans*, *C. neoformans* and *A. fumigatus* and was very active against *M. canis* and *T. rubrum*. At 8%, the oil was partially active against *S. aureus* and *C. albicans*, active against MRSA, *C. neoformans* and *A. fumigatus*, and very active against *M. canis* and *T. rubrum*. At 1% it just showed partial activity against *M. canis*.

The minimum inhibitory concentrations of leaf essential oil were determined against four microbial species (Table 4). These results indicated that the oil presented strong activity against *S. aureus*, methicilin resistant *S. aureus and C. albicans* with MIC of 0.25%, 0.25% and 0.125%, respectively and weaker activity against *C. neoformans*. The results obtained by both agar diffusion and broth microdilution methods were different. Many factors vary between assays which include differences in microbial growth, exposure of micro-organisms to plant oils, the solubility of oils or oil components, the use and quantity of emulsifier and others elements.[14,20,21] In the agar diffusion method, the hydrophobic nature of the majority of essential oil components hamper the uniform diffusion of these substances through the agar medium that may account for differences on the results obtained.

The antimicrobial properties of the oil are supposed to be associated to the high hydrocarbon monoterpene content, especially to its majority component, α-pinene. The antimicrobial activity of this compound was related to its capacity of destruction of cellular integrity, inhibition

Table 2. Composition of essential oils from flowers and fruits of *Myrcia myrtifolia* collected in different months and years

Compound	RI	10/02	11/02	12/02	01/03	03/03	10/03	05/02	06/03
		Fl	Fl	Fl	Fl	Fl	Fl	Fr	Fr
E-2-hexenal	854	0.1	-	-	-	-	-	-	-
α-pinene	938	76.2	61.5	85.1	74.5	77.4	80.8	88.1	90.9
α-fenchene	945	0.1	-	-	-	-	-	-	0.1
camphene	947	0.2	-	-	-	-	-	-	-
β-pinene	975	0.1	1.7	2.3	1.6	1.4	2.2	1.4	1.1
α-terpinene	1016	0.1	-	-	-	0.4	-	-	-
p-cimene	1023	-	-	-	-	-	-	-	1.8
limonene	1028	0.1	1.8	3.0	1.8	3.4	2.3	2.4	0.2
eucalyptol	1030	2.5	1.2	1.4	-	1.6	0.8	1.1	-
γ-terpinene	1062	0.1	-	-	-	-	0.1	-	-
p-menta-3,8-diene	1069	0.2	-	-	-	0.3	-	-	-
terpinolene	1087	1.0	-	0.5	-	1.3	0.4	-	0.2
linalool	1099	0.2	-	-	-	0.7	-	-	-
endo-fenchol	1112	0.2	-	-	-	-	-	-	-
trans-pinocarveol	1139	0.1	-	-	-	-	-	3.8	-
pinocarvone	1160	-	-	-	-	0.3	-	-	-
borneol	1163	0.2	-	-	-	-	-	-	-
terpin-4-ol	1175	0.2	-	-	-	0.2	-	-	-
α-terpineol	1192	4.4	2.4	1.1	-	2.9	0.5	-	0.5
myrtenol	1196	2.1	-	3.7	-	0.9	-	-	-
α-copaene	1373	-	3.9	0.7	-	-	0.2	-	-
β-cariophyllene	1417	1.3	6.2	1.2	8.7	2.9	5.0	-	0.3
α-humulene	1450	0.2	1.5	-	-	-	-	-	-
α-cadinene	1535	0.2	-	0.7	1.6	-	-	-	-
hinesol	1637	-	1.3	-	-	0.5	0.3	-	0.4
14-OH-α-humulene	1710	-	-	0.3	-	-	-	-	-
Total		88.8	77.6	99.3	96.6	95.7	94.7	96.8	95.8
aliphatic compounds		0.1	-	-	-	-	-	-	-
monoterpenes hydrocarbons		77.1	65.0	90.9	77.9	84.2	85.8	91.9	94.3
oxygenated monoterpenes		9.9	3.6	6.2	-	6.6	1.7	4.9	0.5
sesquiterpenes hydrocarbons		1.7	7.7	1.2	10.3	2.9	5.7	-	0.3
oxygenated sesquiterpenes		-	1.3	-	-	0.5	0.3	-	0.4
yield of the oil / (%, m/m)		0.26	nm	nm	nm	nm	nm	0.37	0.35
mass of fresh material / g		85.0	25.0	25.0	12.0	7.0	10.0	125.0	78.0

nm = not measured; - = not detected; Fl = flowers; Fr = fruits.

Table 3. Antimicrobial activity of leaf essential oil from *M. myrtifolia* collected in October 2002, showed as mean of duplicate of zones of growth inhibition (mm)

Microbial strain	Oil dilution						VA	KT
	Pure oil	8%	4%	2%	1%	0.5%	30 μg	50 μg
S. aureus	13.0	11.2	10.2	NI	NI	NI	21.0	
MRSA[a]	13.0	12.2	11.0	10.0	NI	NI	19.0	
C. albicans	12.8	11.8	10.2	NI	NI	NI		23.0
C. neoformans	16.5	14.2	12.0	9.5	NI	NI		26.0
A. fumigatus	14.0	12.5	10.8	NI	NI	NI		21.0
M. canis	24.5	22.0	17.5	15.5	11.5	NI		22.0
T. rubrum	22.0	20.5	15.5	11.5	NI	NI		20.0

[a] methicilin-resistant *Staphylococcus aureus*; NI: No inhibition; VA = Vancomycin; KT = Ketoconazol.

of respiration and ion transportation process and to increase membrane permeability of yeast cells.[22,23]

Dermatophytoses (onychomycosis, tinea or ringworm) are infections that occur in the hair, skin, nails and are caused by dermatophyte fungi, mainly *T. rubrum* and *M. canis*.[24] Some species of *Candida* have also been related as onychomicosis-causing fungi. The high activity of the essential oil of *M. myrtifolia* against *T. rubrum*, *M. Canis* and *C. albicans* and the moderate toxicity obtained in a preliminary evaluation with *Artemia salina* bioassay, LC_{50}

Table 4. Minimum inhibitory concentration of aerial parts essential oil from *M. myrtifolia* against four microbial species

Microorganism	Sample %	Controls / (mg mL^{-1})		
	(v/v)	OX	VA	KT
S. aureus	0.25	0.25	-	-
MRSA[a]	0.25	-	0.50	-
C. albicans	0.125	-	-	0.13
C. neoformans	1.0	-	-	0.25

[a] methicilin resistant *Staphylococcus aureus*; OX: Oxacilin; VA: Vancomycin; KT: Ketoconasol.

of 479.16 μg mL^{-1}, suggested that the oil could have a potential use as an alternative method to combat these microorganisms.

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