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Artigo

Chemical composition and acetylcholinesterase inhibitory activity of essential oils of *Myrceugenia myrcioides* (Cambess.) O. Berg and *Eugenia riedeliana* O. Berg, Myrtaceae

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RESUMO: "Composição química e atividade inibidora de acetilcolinesterase de óleos voláteis de *Myrceugenia myrcioides* (Cambess.) O. Berg and *Eugenia riedeliana* O. Berg, Myrtaceae". A composição química dos óleos voláteis de duas espécies de Myrtaceae, *Myrceugenia myrcioides* e *Eugenia riedeliana*, ambas nativas da Mata Atlântica, foi analisada por CG-EM. A atividade inibidora de acetilcolinesterase foi determinada colorimetricamente para estes óleos. Em *M. myrcioides*, hidrocarbonetos monoterpênicos representaram a classe majoritária de compostos presentes no óleo volátil, sendo α-pineno o componente mais abundante e a atividade inibidora de acetilcolinesterase foi baixa, enquanto para *E. riedeliana* os sesquiterpenos foram observados em maiores concentrações, sendo o valerianol o componente majoritário, e este óleo apresentou uma forte atividade inibidora da enzima.

Unitermos: acetilcolinesterase, óleos voláteis, Myrtaceae, *Myrceugenia myrcioides, Eugenia riedeliana.*

ABSTRACT: The chemical composition of volatile oils from two Myrtaceae species, *Myrceugenia myrcioides* and *Eugenia riedeliana*, both native from the Brazilian Atlantic Rain Forest, was analyzed by GC-MS. Acetylcholinesterase inhibitory activity was colorimetrically evaluated for these oils. For *M. myrcioides*, monoterpene hydrocarbons represented the major class in the volatile oil, with α-pinene as the most abundant component and a weak inhibitory activity was observed, whilst for *E. riedeliana* sesquiterpenes were found in higher amounts, being valerianol the major compound, and this oil presented a strong acetylcholinesterase inhibition.

Keywords: acetylcholinesterase, essential oil, Myrtaceae *Myrceugenia myrcioides, Eugenia riedeliana.*

INTRODUCTION

Essential oils are a complex mixture of low molecular weight substances, mainly mono- and sesquiterpenes extracted from a plant, usually by hydrodistillation. Several pharmacological properties have already been attributed to these compounds by scientific research, such as analgesic, antimicrobial, antimalarial, anticarcinogenic, anti-inflammatory, anticonvulsant, antifungal, antioxidant and gastro-protector activities (Lahlou, 2004; Edris, 2007 and references therein).

Volatile compounds have also been tested for their activity in the inhibition of acetylcholinesterase (AChE) (Barbosa-Filho et al., 2006). The volatile oil of *Salvia lavandulaefoilia* has been shown to inhibit AChE both *in vitro* and *in vivo* (Perry et al., 2000; 2002). Additionally, some isolated bicyclic monoterpenes, especially α-pinene and (+)-3-carene, also presented a strong inhibition of this enzyme (Miyazawa & Yamafuji, 2005), as well as some sesquiterpenes, such as α- and β-ionone and nootkatone (Miyazawa et al., 1998a; 2001). The application of AChE inhibitors is currently the only approved therapy for enhancement of central cholinergic function in patients with Alzheimer's disease, in order to compensate their deficiency in the central nervous system functions (Rollinger et al., 2005). Therefore, the search of natural

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products with AChE inhibitory activity has become a very important issue.

The ability of synthesizing volatile oils is found almost exclusively in Angiosperm families, especially Lauraceae, Myrtaceae, Rutaceae, Asteraceae, Rosaceae, Pinaceae, Apiaceae, Myristicaceae, Lamiaceae and Verbenaceae. Among them, Myrtaceae can be considered an important source of volatile compounds in the Brazilian Atlantic Rain Forest, since it is the dominant family in this biome, in terms of number of species and individuals and total basal area (Mori et al., 1983; Tabarelli & Mantovani, 1999). There are a great number of volatile oils from species of this family that demonstrated biological activities, mainly from *Eucaliptus* and *Eugenia species*, with anti-inflammatory, analgesic, antifungal and antimicrobial activities (Pattnaik et al., 1996; Limberger et al., 1998; Costa et al., 2000.; Silva et al., 2003). However, there is little scientific information about the chemical composition and biological activities of volatile oils from native Myrtaceae species from Brazil. Moreover, the AChE inhibitory activity of Myrtaceae volatile oils is yet to be investigated. Thus, the aim of the present work was to analyze the chemical composition and to evaluate the AChE inhibitory activity of the volatile oils from *Myrceugenia myrcioides* (Cambess.) O. Berg and *Eugenia riedeliana* O. Berg, native from the Brazilian Atlantic Rain Forest.

MATERIAL AND METHODS

Plant material

Myrceugenia myrcioides (Cambess.) O. Berg and *Eugenia riedeliana* O. Berg, Myrtaceae, leaves were collected in State Park Serra do Mar, in Caraguatatuba, São Paulo, Brazil and dried at room temperature. The botanical identification was performed by Dr. Inês Cordeiro and Dr. Marcos E. G. Sobral. Voucher specimens (Cordeiro 2820 and Cordeiro 2415, respectively) were deposited in the Herbarium of the Instituto de Botânica of São Paulo.

Essential oil extraction

The essential oils were obtained by hydrodistillation for 3 h in a Clevenger-type apparatus. The oils were collected, dried over anhydrous sodium sulfate, weighted and then stored at -25 °C until testing.

CG/MS analysis

For component identification, the essential oils were submitted to Gas Chromatography and Mass Spectrometry (GC/MS) analysis, performed using an Agilent GC (6890 Series) - quadrupole MS system (5973), with a fused silica capillary column (30 m x 0.25 mm x 0.25 µm, coated with DB-5), EI operating at 70 eV. Injector and detector temperatures were set at 250 °C. The oven temperature program was 40° for 1 min, 40-240 °C at 3 °C/min and helium was employed as carrier gas (1 mL/min). The compound identification was performed by comparing retention indices (Kóvats Index (KI), determined relatively to the retention times of a series of *n*-alkanes) and mass spectra with literature data (Adams, 2007).

Acetylcholinesterase activity by microplate assay

Acetylcholinesterase activity was measured using a 96-well microplate reader (Rhee et al., 2001) based on Ellman's method (Ellman et al., 1961). In this method the enzyme hydrolyzes the substrate acetylthiocholine resulting in the production of thiocholine which reacts with 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB, Sigma Chemical Co.) to produce 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate which can be detected at 405 nm. In the 96-well plates, 25 mL of 15 mM acetylthiocholine iodide (ATCI, Sigma Chemical Co.) in water, 125 mL of 3 mM DTNB in buffer C, 50 mL of buffer B, 25 mL of essential oil sample (30 mg/mL of essential oil diluted in MeOH was dissolved with buffer A to give a concentration effect of 600 µg/mL to 4.68 µg/mL/well microplate) were added and the absorbance was measured at 405 nm every 30 s for three times. Then 25 mL of 0.22 U/mL of the enzyme acetylcholinesterase (AChE, Sigma Chemical Co.) were added and the absorbance was again read every 10 min for two times. Any increase in absorbance due to the spontaneous hydrolysis of the substrate was corrected by subtracting the rate of the reaction before the addition of the enzyme from the rate of the enzyme reaction. The percentage of inhibition was calculated by comparison with the rates for the sample to a blank (10% MeOH in Buffer A). The following buffers were used. Buffer A: 50 mM Tris-HCl, pH 8; buffer B: 50 mM Tris-HCl, pH 8, containing 0.1% bovine serum albumin V fraction (BSA, Sigma Chemical Co.); buffer C: 50 mM Tris-HCl, pH 8, containing 0.1 M NaCl and 0.02 M $MgCl_2.6H_2O$. The quantitative results of AChE inhibition were represented as means of one typical experiment performed in triplicate. The values were analyzed with the program GraphPad Prism Software, San Diego CA, version 3.0.

RESULTS AND DISCUSSION

The volatile oil yield was 0.27% for *E. riedeliana* and 0.38% for *M. myrcioides.*

The chemical composition of the volatile oils of *E. riedeliana* and *M. myrcioides* is presented in Table 1. For *M. myrcioides*, monoterpene hydrocarbons represented the major compound class in the volatile oil (58.3%), with α-pinene (**1**) as the most abundant component (23.2%), followed by limonene (**2**) (18.3%). In a previous work (Limberger et al., 2002), a predominance of sesquiterpenes, mainly those from caryophyllene and

Table 1. Results of GC/MS analysis of the volatile oil of *Myrceugenia myrcioides* and *Eugenia riedeliana*. KI: Kováts indices calculated from injection of C_5 to C_{30} alkanes on a DB-5 stationary phase.

Compound	*KI*	Area (%)	
		M. myrcioides	*E. riedeliana*
α-pinene	920	23.2	3.2
camphene	928	0.3	
β-pinene	954	4.2	
β-myrcene	971	0.5	
α-phellandrene	981	6.9	
o-cimene	996	4.2	
limonene	1007	18.3	
γ-terpinene	1034	0.4	
α-terpinolene	1063	0.3	
l-terpineol	1141	0.7	
α-terpineol	1152	0.5	
α-phellandrene epoxide	1161	0.4	
bornyl acetate	1226	0.5	
2-undecanone	1232	0.3	
carvacrol	1235	0.4	
α-cubebene	1345		2.3
1-hydroxyundecan-3-one	1350	5.9	
β-caryophyllene	1367		10.9
α-humulene	1463	1.5	1.8
allo-aromadendrene	1478	0.3	0.8
aristolochene	1484		1.5
β-selinene	1494		5.2
2-tridecanone	1498	2.5	
α-selinene	1501		6.1
γ-cadinene	1505		1.2
δ-cadinene	1510		5.7
β-sesquiphellandrene	1518		1.8
α-calacorene	1520		3.8
selina-3,7(11)-diene	1523		1.5
caryophyllene alcohol	1557		1.1
trans- sesquisabinene hydrate	1565		0.9
spathulenol	1567	6.2	
caryophyllene oxide	1567	2.3	2.6
viridiflorol	1569	0.4	
(*E*)-2-decenal	1573	2.3	
ledol	1574	0.5	
5-epi-7-epi-α-eudesmol	1579		0.9
10-epi-γ-eudesmol	1587		12.6
caryophylla-4(12),8(13)-dien-5-β-ol	1590	0.3	
α-muurolol	1591		0.9
cubenol	1592	1.0	1.4
β-eudesmol	1593		0.8
α-eudesmol	1594	0.2	

valerianol	1610		28.1
selin-7(11)-en-4-ol	1651		2.3
(*Z*)-2-decenal	1694	6.6	
6,10,14-trimetil-2-pentadecanone	1801		0.5
Total identified		90.7	97.7
Monoterpene hydrocarbons		58.3	3.2
Oxygenated monoterpenes		2.5	0.0
Sesquiterpene hydrocarbons		1.7	42.6
Oxygenated sesquiterpenes		10.9	51.6
Aliphatic compounds		17.6	0.5

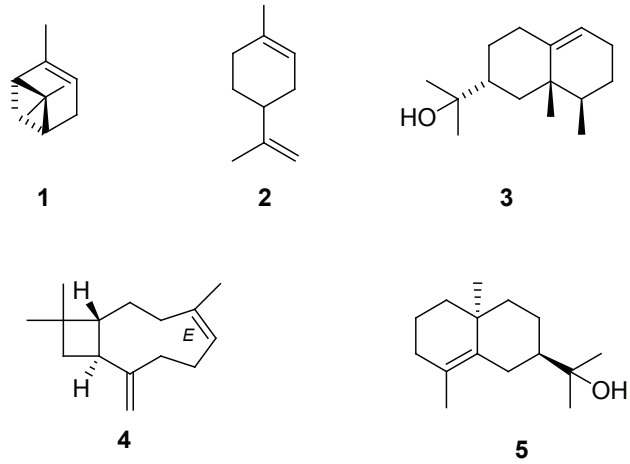
germacrene cyclization pathway, was observed in *M. myrcioides*. Theses differences might be explained by regional, populational or seasonal variations.

On the other hand, the *E. riedeliana* oil presented higher amounts of sesquiterpenes: 51.6% oxygenated and 42.6% hydrocarbons. The major compound was valerianol (**3**) (28.1%). The monoterpene concentration was very low for this species (3.2 %).

There is no previous information available on biological activities for essential oils or any other class of compounds for those species. In the present study, differences between the analyzed oils were also observed in their AChE inhibitory activity (Figure 1). The monoterpene-rich oil of *M. myrcioides* presented very low effect, with maximum inhibition of 28.9% in the concentration of 600.0 $\mu g.mL^{-1}$. Some monorterpenes have been demonstrated as potent AChE inhibitors, such as the biclyclic α-pinene and (+)-3-carene (Perry et al., 2000; Miyazawa & Yamafuji, 2005). However, the high levels of α-pinene in *M. myrcioides* volatile oil did not lead to a considerable enzyme inhibition in the present analysis. This could be due to an antagonistic effect along with the other components. Interaction among the essential oil constituent was also observed in the inhibition of AChE by *Melaleuca alternifolia* oils (Mills et al., 2004) and *Mentha* species (Myazawa et al. 1998b). Analyzing the inhibition of AChE by monoterpenoids with *p*-menthane skeleton, Myazawa et al. (1997), observed that both (+)- and (-)-limonene exhibited low inhibitory effect when compared with other monoterpenes. It is possible that limonene, present in high amounts in *M. myrcioides* oil, plays an antagonistic role in the AChE inhibition.

The sesquiterpene-rich essential oil of *E. riedeliana* was able to inhibit the enzyme activity up to 88.9% in the concentration of 600.0 $\mu g.mL^{-1}$ (Figure 1), presenting an IC_{50} of 67.3 $\mu g.mL^{-1}$. Up to now, there is very little information available on the AChE inhibitory activity related to sesquiterpenes or volatile oils rich in these components.

The structure-activity relationship for AChE inhibition has already been studied for some monoterpenoid compounds. Among those with *p*-menthane skeleton, the ketones showed a stronger inhibition than alcohols and hydrocarbons. On the other hand, for bicyclic monoterpenes, the presence of oxygenated functional groups decreased the AChE inhibition (Miyazawa & Yamafuji, 2005). In the case of sesquiterpenes, inhibitory effect has been observed for oxygenated compounds, especially ketones (Miyazawa et al., 1998a; 2001; Ryu et al., 2003). The sesquiterpene alcohols viridiflorol and elemol have also been considered as strong AChE inhibitors, with IC_{50} values of 25 and 34 $\mu g.mL^{-1}$, respectively (Miyazawa et al., 1998a). The high levels of oxygenated sesquiterpenes (51.6%) in the *E. riedeliana* oil might be related to the high AChE inhibition observed. There is no information available about the inhibitory effect of the three major constituents of *E. riedeliana* oil valerianol (**3**), (E)-β-caryophyllene (**4**) and 10-epi-γ-eudesmol (**5**). Therefore, considering the results obtained in the present study, these compounds must be considered for further studies in order to evaluate their inhibitory activity in their isolated form.



1 **2** **3** **4** **5**

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