CHEMICAL COMPOSITION AND CHOLINESTERASE INHIBITION OF ESSENTIAL OILS OF THREE CHEMOTYPES FROM *Croton zehntneri*

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ABSTRACT

Croton zehntneri Pax. & K. Hoffm. (Euphorbiaceae) is an aromatic plant native of the northeaster region of Brazil; and is popularly known as "canela de cunhã". The chemical composition of the essential oils from leaves, stalks and roots from three chemotypes of *C. zehntneri* obtained by hydrodistillation were analyzes by GC-MS. *E*-anethole, was the main component in the essential oils of all plants parts of chemotype 1. While the phenylpropanoids stragole, eugenol, Z-methyl isoeugenol and *E*-methyl isoeugenol were the major component in the essential oils of chemotypes 2 and 3, respectively. On TLC the essential oils and the major compounds showed an acetylcholine esterase inhibitory effect.

Key words: *Croton zehntneri*, Euphorbiaceae, essential oils, phenylpropanoids, chemotypes, cholinesterase inhibition.

RESUMEN

Croton zehntneri Pax. & K. Hoffm. (Euphorbiaceae) es una planta aromática nativa de la región nororiental de Brasil, y es popularmente conocido como "canela de cunhã". La composición química de los aceites esenciales de hojas, tallos y raíces de três quimiotipos de *C. zehntneri* obtenido mediante hidrodestilación fueron examinados por GC-MS. *E*-anetol, fue principal componente en los aceites esenciales de todas las partes de la planta de quimiotipo 1. Si bien la fenilpropanóides estragol, eugenol, Z-metil isoeugenol and *E*-metil isoeugenol fueron el componente principal en los aceites esenciales de quimiotipos 2 e 3, respectivamente. El TLC estos aceites esenciales y los principales compuestos mostraron una acetilcolina esterase efecto inhibitorio.

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INTRODUCTION

Croton is an extensive genus comprising around 1,300 species from Euphorbiaceae family. This genus with wide range of bioactive compounds have been found to exert vasorelaxant activity (Baccelli *et al.*, 2007). Popular uses include treatment of cancer, constipation, diabetes, digestive problems, dysentery, external wounds, fever, hypercholesterolemia, hypertension, inflammation, intestinal worms, malaria, pain, ulcers, and weight-loss (Salatino *et al.*, 2007). Previous phytochemical investigations show that this genus possesses alkaloids (Murillo *et al.*, 2001; Araujo-Junior *et al.*, 2004), flavonoids (Peres *et al.*, 1997; Maciel *et al.*, 2000; Graikou *et al.,* 2004), triterpenoids and steroids (Peres *et al.*, 1998; Guadarrama *et al.*, 2004), and a large number of diterpenoids (McChesney and Silveira, 1990; El Mekkawy *et al.*, 2000; Barbosa *et al.*, 2003; Giang *et al.*, 2004; Santos *et al.*, 2008; Santos *et al.*, 2009). *C. zehntneri* (Euphorbiaceae) is an aromatic plant native in northeastern Brazil; and popularly known as "canela de cunhã". The specie is used in traditional medicine as sedative, appetite stimulating, antianorexigen and for the relief of gastrointestinal disturbances (Oliveira *et al.*, 2001). The essential oil also acts as intestinal muscle relaxant (Coelho-de Souza *et al.*, 1997, 1998), depressor central effect (Lazarini e*t al.*, 2000) and antinociceptive (Oliveira *et al.*, 2001). The literature reports chemical composition and larvicidal activity of the essential oil of leaves, stalk and inflorescences of chemotype 1 from *C. zehntneri* (Santos *et al.*, 2007). Alzheimer's disease (AD) is a neurodegenerative disease characterized by cognite impairment and personality changes. Inhibition of acetylcholinesterase (AchE) serves as a strategy for the treatment of AD, senile dementia and Parknson's disease (Anonymous, 2000). Synthetic medicines as tacrine, donepezil, and the natural product-based rivastigmine used for treatment of cognitive dysfunction and memory loss associated with AD have their adverse effects including gastrointestinal disturbances and problems associated with bioavailability (Schulz, 2003), which necessitates finding better AChE inhibitors from natural resources. In recent years, essential oils and their monoterpene constituents have received much attention for their effects on AChE, e.g. essential oils from *Melissa offcinalis, Rosmarinus officinalis* and *Salvia lavandulaefolia* and monoterpenes such as geraniol, 3-carene, α-caryophyllene, limonene, sabinene, 1,8-cineole, α and β-pinene, γ-terpinene, bornyl acetate, geraniol, linalool, camphor, borneol, have been reported to inhibit AChE *in vitro* (Howes *et al.*, 2003; Perry *et al.*, 2003). The literature reports the antioxidant activities of the essential oil of leaves of chemotype 1 from *C. zehntneri* (Morais *et al.*, 2006), which is associated with the activation of lipoxygenases, which catalyse the formation of hydroperoxides of polyunsaturated fatty acids (PUFAs); a hydroperoxide radical may react with fatty acids to produce dioxoenes, which are regarded as plant defence compounds (Spiteller, 1993). The antioxidant effects in essential oil may therefore have relevance in mammals, particularly in disorders involving oxidative stress such as AD. Besides the fact that phenylpropanoid *E*-anethole, the main component in all oils of chemotype 1 from *C. zehntneri* have been reported to inhibition AChE (Menichini *et al.*, 2009). This work we report an evaluation of the cholinesterase inhibition effect, as well the chemical composition of essential oils from three chemotypes of *C. zehntneri.*

MATERIALS AND METHODS

Plant material

Leaves, stalks and roots of three chemotypes from *C. zehntneri* were collected in april and may 2008, in Ubajara and Croatá

da Serra, Ceará State, Brazil. The plant material was identified by Dr. Edson Paula Nunes at the Herbário Prisco Bezerra (EAC), Departamento de Biologia, Universidade Federal do Ceará, Fortaleza, CE, Brazil, where the vouchers specimens (No. 42389, 42774 and 43048) was deposited.

Extraction of the essential oils

The fresh leaves (954 g, 327 g and 1000 g), stalks (1500 g, 1015 g and 700 g) and roots (198 g, 174 g and 186 g) of chemotypes 1, 2 and 3 from *C. zehntneri* were subjected to hydrodistillation in a Clevenger-type apparatus for 2 hours to afford leaves (0.80%, 0.90% and 0.84%), stalks (0.30%, 0.27% and 0.29%) and roots (0.60%, 0.06% and 0.14%) of pale yellow oils, respectively. The yields (w/w) were calculated based on the fresh weight of the plant materials. The isolated oils, after drying over anhydrous sodium sulfate and filtered, were stored in sealed glass vials and maintained under refrigeration before analysis.

Gas Chromatography-Mass Spectrometry

GC-MS for the analysis of the volatile constituents was carried out on a Hewlett-Packard Model 5971 GC/MS using a non-polar DB-5 fused silica capillary column (30 m x 0.25 mm i.d., 0.25 μm film thickness); carrier gas helium, flow rate 1 ml/min and with split mode. The injector temperature and detector temperature were 250 °C and 200 °C, respectively. The column temperature was programmed from 35 °C to 180 °C at 4 °C/min and then 180 °C to 250 °C at 10 °C/min. Mass spectra were recorded from 30 - 450 m/z. Individual components were identified by matching their 70 eV mass spectra with those of the spectrometer data base using the Wiley L-built library and two other computer libraries MS searches using retention indices as a preselection routine (Alencar *et al.*, 1990), well as by visual comparison of the fragmentation pattern with those reported in the literature (Adams, 2001).

Measure the activity of acetylcholinesterase Solutions patterns

The following solutions were prepared: (1) 50 mM Tris-HCl, pH 8; (2) 1 mM 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB or Ellman's reagent) and (3) 1 mM acetylthiocoline iodide (ACTI). Lyophilized enzyme AChE was dissolved in buffer solution (1) to make 1000 U / mL stock solution, and further diluted with buffer solution (1) to get 3 U / mL enzyme (Rhee *et al.*, 2001).

Test on Layer Chromatography (TLC)

Samples (1.5 - 2.5 μL) were applied in CCD, DC-Alufolien, Silicagel 60 F254, 0.2 mm Merck. Spatter to the plate with solutions (2) + (3), leaving over 3 min. After drying, spraying is the enzyme 3 U / mL and in 10 minutes, appeared to be yellow. Where there was inhibition of the enzyme, there is a white halos. In about 20 to 30 minutes, the color disappeared (Rhee *et al.*, 2001).

RESULTS AND DISCUSSION

The essential oils extracted from leaves, stalks and roots of three chemotypes from *C. zehntneri* were analysed by CG/MS and the constituents identified and quantified are summarized in Table 1. A total of 32 compounds were identified in the six sample oils and they are arranged in order of elution on a DB-5 column. As can be seen, the oils analysed were characterized by a high amount of phenylpropanoids (Figure 1). Sixteen constituents were identified in the oil from leaves (98.7%), stalks (94.4%) and roots (99.8%) from chemotype 1, the phenylpropanoid *E*-anethole **1** was the main component in all these oils. In the essential oils from chemotype **2** were identified twenty two constituents were identified in the oil from leaves (99.1%), stalks (97,4%) and roots (99.7%) the main component in the oil from leaves and stalks was eugenol **2**, while Z-methyl isoeugenol **4** was the major constituent in the roots. In

Table 1: Chemical composition of essential oil from leaves, stalk and roots of three chemotypes from *C. zehntneri*

		Chemotype 1			Chemotype 2			Chemotype 3		
Compounds	RI[a]	leaves (%)	stalks (%)	roots (%)	leaves (%)	stalks (%)	roots (%)	leaves (%)	stalks (%)	roots (%)
Camphene	954					0.2				
β-pinene	979	2.3			0.7	0.5				
1,8-cineole	1031	1.1	5.2		1.5	11.0				
Z-ocimene	1037					0.6				
E-ocimene	1050				0.7	1.3				
Camphor	1146		2.5	2.7		1.3				0.4
Borneol	1169		2.6	3.7		0.3				0.7
α-terpineol	1189				0.3	2.4				
Stragole	1196	3.7	1.2			2.0	12.6	90.2		2.9
p-anisaldehyde	1250		1.8							
Z-anethole	1253	0.3								
E-anethole	1285	89.1	70.5	33.7					14.7	
δ-elemene	1338								0.7	0.07
Eugenol	1359				84.2	49.1	1.2			
Isoledene	1376									0.5
α-copaene	1377		1.5						6.0	
β-elemene	1391		2.1			1.3			11.4	1.1
Methyl eugenol	1404			28.4						
α-gurjunene	1410					0.4				
E-caryophyllene	1419	0.4			1.2	6.8	4.4		4.5	
Z-bergamotene	1435				0.1	1.9				
Z-Methyl isoeugenol	1454		6.3	1.7	0.2	2.6	81.5	0.3	53.4	2.5
α-humulene	1455					0.7				
Germacrene D	1485	0.4	0.7		0.3					
E-Methyl isoeugenol	1492			29.6		12.2				91.5
Bicyclogermacrene	1500	1.4			4.2			1.7	1.6	
δ-cadinene	1523					0.2			0.8	
Eugenol acetate	1523				5.7					
Germacrene B	1561								0.9	
Caryophyllene oxide	1583					2.6				
TOTAL		98.7	94.4	99.8	99.1	97.4	99.7	92.2	94.0	99.6

[a] Retention indices

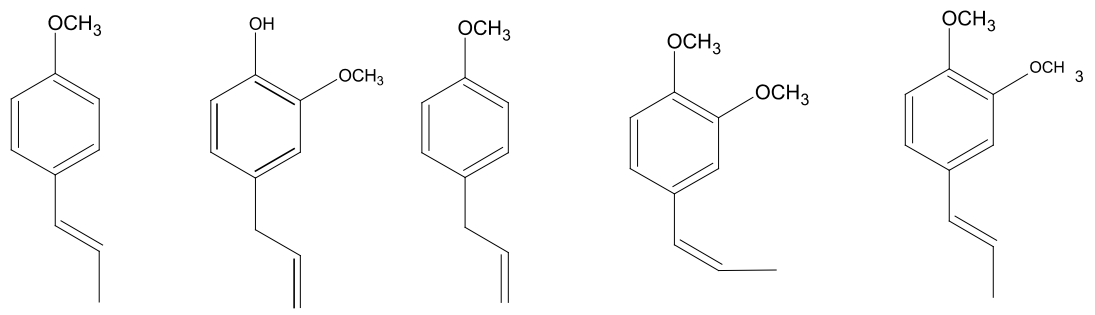

Figure 1: Structures of major constituents from the essential oils of three chemotypes from *C. zehntneri*

addition, fourteen constituents from leaves (92.2%), stalks (94.0%) and roots (99.6%) from chemotype 3, the main component in leaves was stragole **3**, while *Z*-methyl isoeugenol **4** and *E*-methyl isoeugenol **5** were the major in stalks and roots, respectively. The finding of inhibition of AChE is possible following the methodology of Elmann, adapted by Rhee for thin layer chromatography (TLC). In this test, is used the reagent 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) and acetylthiocholine iodide (ATCI) in buffer, and subsequently applies to AChE enzyme (3 U / ml). A preview of inhibition is made through observation of white halos. The essential oils of leaves, stalks and roots from three chemotypes of *C. zehntneri* was guided by bio-enzyme inhibition. The presence of essential oils downloads is confirmed by the appearance of white halos (Elmann *et al.*, 1961, Rhee *et al.*, 2001). On TLC the essential oils from leaves, stalks and roots and major constituent *E*-Anethtole of chemotype 1 from *C. zehntneri* showed an acetylcholine esterase inhibitory effect (Table 2). This result is in agreement with the literature, since *E*-anethole has been reported to inhibition AChE (Menichini *et al.*, 2009). The essential oils from stalks and roots of chemotypes 2 and 3 showed an acetylcholine esterase inhibitory effect, respectively. The major constituent eugenol of the essential oils from leaves and stalks of chemotype 2 showed an anti-cholinesterase activity. While stragole the major constituent of the essential oils from leaves of chemotype 3 was not active (Table 2). These results can be explained by the presence in these oils of constituents β-pinene, 1,8-cineole, camphor and borneol, which have been reported to inhibit AChE *in vitro* (Howes *et al.*, 2003; Perry *et al.*, 2003), besides the fact that the monoterpenoids and phenylpropanoids presence in these oils may act synergistically to inhibit AChE (Savelev *et al.*, 2003). The results clearly indicate that some structural features are important for biological activity. The presence of a conjugated double bond and a hydroxyl group seem to be important for the activity test, since the *E*-anethole and eugenol containing these structural features have shown activity, while the stragole which has no such characteristics was not active. The anti-cholinesterase activity of this compounds can be explained by hydrophobic interactions between hydrophobic active site of AChE and hydrocarbon skeleton of the phenylpropanoids (Mukherjee *et al.,* 2007).

Table 2: Cholinesterase inhibition of essential oils of three chemotypes from *C. zehntneri*

Chemotypes	Essential oils[a]	Zone of inhibition (mm)
1	leaves	6
	stalks	6
	roots	7
2	leaves	N[b]
	stalks	5
	roots	6
3	leaves	N[b]
	stalks	5
	roots	6
	E-anethole	7
	stragole	N[b]
	eugenol	8
	physostigmine [a,c]	9

[a]Concentration = 2 mg/mL
[b]N = No effect
[c]Positive control

CONCLUSION

Acetylcholinesterase (AchE) inhibitors have therapeutic applications in Alzheimer's disease (AD). On TLC the essential oils from three chemotypes of *C. zehntneri.* showed an anti-cholinesterase effect. The results from the present study demonstrate these essential oils are the promising source of cholinesterase inhibitors natural agents.

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