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Full Paper

Inhibition of Acetylcholinesterase and Fatty Acid Composition in *Theobroma grandiflorum* Seeds

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Abstract: *Theobroma grandiflorum* is an important fruit tree from Sterculiaceae family, native to the Brazilian Amazon, known in the region as *cupuaçu*. The seeds have a high fat content (24%) with characteristics that resemble those of cocoa (*Theobroma cacao*) butter with potential applications in the cosmetic, pharmaceutical and food industries. The main objective of this work was to explore the seed fats from *T. grandiflorum* that were analyzed for fatty acid composition by Gas Chromatography with Flame Ionization Detector (GC-FID) and to analyze their activity for acetylcholinesterase inhibition. Chromatographic analysis provided detection of nine fatty acids. The major fatty acids found in the species were oleic (40.0%), stearic (32.7%), arachidic (10.4%) and palmitic (8.0%). The acetylcholinesterase inhibition by fats from seeds was over 40.48%.

Keywords: brazilian amazon; *cupuaçu*; oleic acid; stearic acid; palmitic acid; Alzheimer disease

1. INTRODUCTION

Cupuaçu (*Theobroma grandiflorum*) from the Sterculiaceae family is an arboreal fruit species, that reaches a height of 15-20 m in the forest, but remains below 8 m when cultivated [1]. This species is still found wild in the eastern subregion of Brazilian Amazonia, especially in the states of Amazonas, Pará, Maranhão, Rondônia and Acre, while commercial crops can be found in other states, like in Bahia. Other tropical countries such as Costa Rica, Colombia, Ecuador, French Guyana, Guyana, and Surinam also cultivate this species [2].

The potential use of the agro-industrial byproducts of *cupuaçu* has only slightly been addressed by the scientific community [3]. Fat extracted from *T. grandiflorum* seeds is rich in saturated fatty acids [4] and may be used in food products, being extensively as cocoa butter substitute in chocolate-like derivatives [5] and as important ingredient in a variety of cosmetics [6].

The aim of this study was to verify the fatty acid profile present in *cupuaçu* seeds collected in Boa Vista, Roraima state, Brazil, as well as analyzing their acetylcholinesterase enzyme inhibition.

2. MATERIAL AND METHODS

Fruit obtaining and extract preparation

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Cupuaçu fruit was obtained in Boa Vista city (Roraima state, Brazil). The sample was taken to the Laboratory of Environmental Chemistry in the Center for Research and Post-Graduate in Science and Technology (NPPGCT) of the Federal University of Roraima, Boa Vista city, Roraima.

Pulp and seeds were manually separated. Seeds were dried, ground to a fine powder and extracted with hexane solvent in a Soxhlet apparatus. The solvent was then removed in a rotoevaporator and the residue was dried slowly under nitrogen stream to furnish an extract containing the fatty material [7].

Fatty acid composition

An aliquot of the extract (12 mg) was placed in a 2.0 mL cryogenic tube and 100 µL of a solution of ethanol (95%) / potassium hydroxide mol L^{-1} (5%) were added. After vortexing for 10 s, fat was hydrolyzed using a microwave oven (Panasonic Piccolo), at a power of 80W for 5 minutes. After cooling, 400 µL of hydrochloric acid 20% were added together with 20 mg of NaCl and 600 µL of ethyl acetate. After vortexing for 10 s, sample was left to rest for 5 minutes. An aliquot of 300 µL of the organic layer was removed, placed into micro centrifuge tubes, dried by evaporation, thus obtaining free fatty acids [8]. Subsequently, the free fatty acids were methylated with 100 µL of BF_3 / methanol (14%), by heating for 10 minutes in water bath at 60 °C. These samples were diluted in 400 µL of methanol and analyzed by gas chromatography.

The analyses were performed on a HP-7820A chromatograph (Agilent) equipped with a gas flame ionization detector (GC-FID). As data acquisition program EZChrom Elite Compact (Agilent) was used. BP-20 column 15 m x 0.25 mm x 0.20 µm with temperature gradient was used at 80 °C, 0 min, 7 ºC min^{-1} to 240 °C; injector (split 1/50) detector at 250 °C and 260 °C. Hydrogen was used as carrier gas (3.0 mL min^{-1}), Injection volume was 1 µL. Identification of the peaks was performed by comparison with standards of methylated fatty acids C14-C22 FAME (Supelco 18917) [8].

Acetylcholinesterase inhibition assay (*in vitro*)

Acetylcholinesterase inhibition assay was carried out as per the method of Ellman [9, 10] modified [11]. Aliquots of a working solution (25 µL) (sample in DMSO 10 mg mL^{-1}) were added to microplate wells and positive and negative controls were also prepared. To the first five wells of a column (positive control) 25 µL of an eserine solution prepared in Tris/HCl at pH 8.0 was added. Then, 25 µL of acetylthiocholine iodide (*Electrophorus electricus*, Sigma Aldrich) 1000 U mL^{-1}; the reaction mixture, 125 µL of 5'.5-dithio-bis (2- nitrobenzoate) (DTNB, Sigma D8130) (3 mM) and 50 µL of Tris/HCl (0.1% m/v, pH=8) containing bovine serum albumin was added to each well. Absorbance was measured at 405 nm every 1 min for 10 times.

3. RESULTS AND DISCUSSION

Fatty acid composition of the seed fat of *Cupuaçu*

The GC-FID analysis provided nine fatty acids (96.7%), as shown below in the chromatogram of Figure 1 and in Table 1.

Note that among the saturated fatty acids predominating in *cupuaçu* seeds fat are stearic, arachidic e palmitic acids (Table 1). Oleic acid was found in greater proportion in the studied sample (40.0%). The same applies to the unsaturated rate (43.4%). There is a slight difference in the the ω-9, ω-6 and ω-3 contents relating to the literature.

Comparison among chemical composition of fatty acids of fat from *cupuaçu* seeds and butter cocoa were qualitatively similar. However cocoa butter presented higher palmitic acid concentration (23.3%) which seems a disadvantage compared to *cupuaçu* fat since this saturated fatty acid is known to induce atherogenesis [12], weight gain, insulin resistance, inflammation, and is also capable of increasing production of reactive oxygen species [3]. On the other hand, the major fatty acid found in *cupuaçu* fat was oleic acid (40%), a monounsaturated fatty acid (MUFA) also detected in cocoa (36.8%) [13] and inversely correlated with insulin resistance in individuals dyslipidaemia [14].

Inhibition of Acetylcholinesterase

The results for acetylcholinesterase enzyme inhibition by *cupuaçu* seed fat was moderate, as shown in Table 2. This classification is given in the literature [15] in which values higher than 50% of inhibition were considered potent, inhibition values between 30-50% were rated as moderate and below 30% should be considered weak inhibitors.

This enzyme has biochemical importance in

humans, but its sudden increase may develop neurodegenerative conditions, one of them is Alzheimer's disease [16-17]. Use of extracts from *cupuaçu* seeds as additive in food could be beneficial for society to help in minimizing the effects of Alzheimer disease since according to the World Health Organization about 107 million people will develop Alzheimer's disease by 2050 [16].

Table 1. Fatty acids in *cupuaçu* seeds fat.

Fatty acid	Retention Time (min)	Amount (%)	*Cupuaçu* 1 [11]	*Cupuaçu* 2 [2]	*Cupuaçu* 3 [12]	cocoa butter [11]
Lauric acid (C12:0)	5.1	0.2	-	-	-	-
Myristic acid (C14:0)	7.8	0.6	-	-	-	0.1
Palmitic acid (C16:0)	10.4	8.0	7.0	7.54	7.68	23.3
Stearic acid (C18:0)	12.8	32.7	34.2	32.6	31.52	34.0
Oleic acid (ω-9) (C18:1)	13.0	40.0	41.9	41.31	42.55	36.8
Linoleic acid (ω-6) (C18:2)	13.5	3.1	3.2	4.9	3.76	2.7
Linolenic acid (ω-3(C18:3)	14.2	0.3	-	0.2	-	-
Arachidic acid (C20:0)	15.1	10.4	11.2	10.51	9.49	0.7
Behenic acid (22:0)	17.2	1.4	1.7	1.72	1.4	-
Saturated	-	53.3	54.3	52.81	50.09	58.1
Monounsaturated	-	40.0	42.3	42.12	42.55	36.8
Polyunsaturated	-	3.4	3.4	5.07	3.76	2.7

Cupuaçu 1, 2, 3 and cocoa butter to data from the literature.

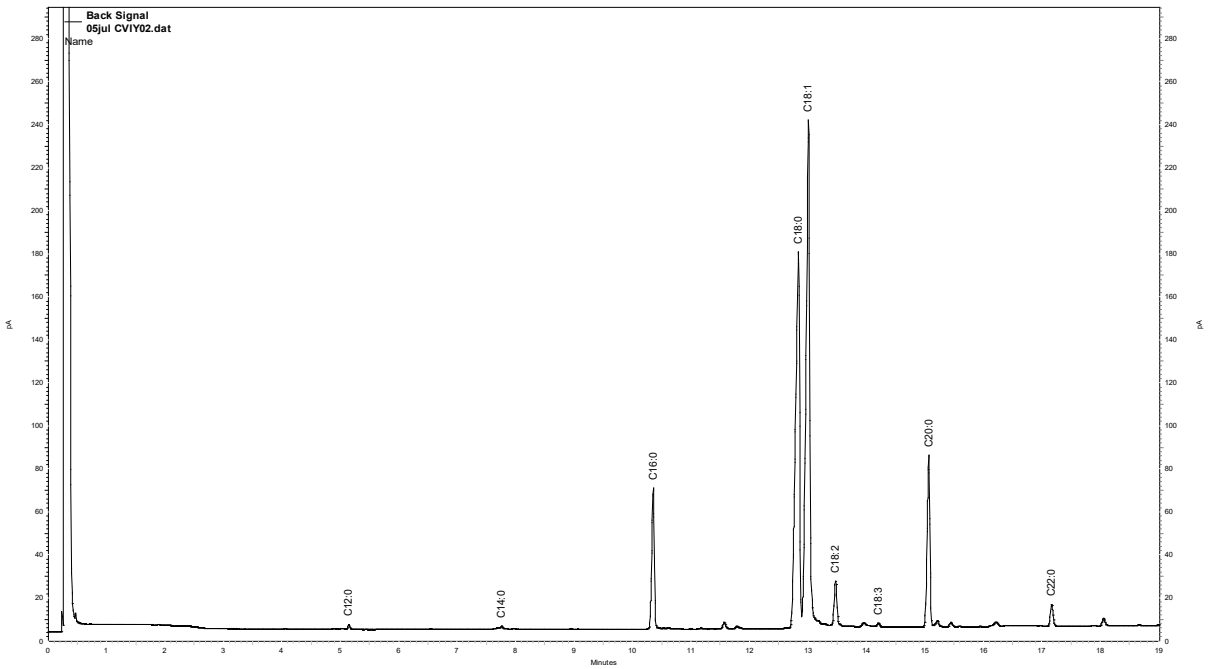

Figure 1. *Cupuaçu* seeds fat gas chromatogram.

Table 2. Percentage inhibition of acetylcholinesterase in *cupuaçu* seed fat.

Cupuaçu seed	% Inhibition	Classification
	40.48	moderate

4. CONCLUSION

The fatty acids from *cupuaçu* seeds have profile similar to other fats of importance such as cocoa butter. Its lipid content can bring benefits to human health and may find applications if pharmaceutical, cosmetics and foods industry. The inhibition of acetylcholinesterase reached almost 40%, being of interest since the seeds are an agroindustrial residue. The good results obtained of the fatty acid profile from *cupuaçu* seeds suggests future chemical and biotechnological applications of this fruit species with the intention of developing bioproducts for human health and cosmetic industry.

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