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Phytochemical Composition and Physical-Chemical Properties of Fatty Acids from *Cinnamomun zeylanicum* Nees Seed Oil

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Cinnamomum zeylanicum Nees (Lauraceae), popularly known as cinnamon, has been widely investigated for presenting functional properties, nutraceuticals and other diverse bioactive activities, mainly for the beneficial effects to human health. That is why natural products made from different extraction methods and different cinnamon botanical structures have aroused interest in the biotechnology industry in different sectors of the world economy. The objective of this work was to determine the phytochemical composition and to characterize the physical-chemical properties of the fixed oil of the cinnamon seeds submitted to the conditions of Savana Tropical in the Brazilian Amazon by ^{1}H NRM and GC-FID. The following results were obtained for saturated fatty acids - FAS (97.21 %): lauric (88.60 %), the major constituent being; caprylic acid (2.34 %); myristic (2.70 %); pentadecyl ester (0.56 %); palmitic acid (1.89 %); stearic acid (0.96 %); and, arachidic acid (0.16 %). The percentage concentrations of linolenic (ω3), linoleic (ω6) and oleic (ω9) calculated by ^{1}H NRM and GC-FID, respectively, were: 0.20; 0.73; 1.86 %; and 0.19; 0.72; 1.88 %. The physico-chemical properties showed: Iodine content of 2.52 mg I_2/g; Saponification Index of 255.97 mg KOH / g; Acid value of 3.32 mg KOH / g; and, Molecular Weight of 604.09 g / mol. These exploratory results indicate the potential of the fixed oil of the cinnamon seeds as a possible therapeutic agent, as well as its use in the pharmaceutical and food industry.
Keywords: Essential Fatty Acids, Lauric acid, ^{1}H NMR, GC-FID.

1. Introduction

Cinnamomum zeylanicum, popularly known as cinnamon, belongs to the Lauraceae family, has known nutraceutical and functional properties (Tulini et al., 2017; Ostroschi et al., 2018), and has been widely investigated for its beneficial effects on the immune system (Perini et al., 2010), hormonal regulation associated with diabetes levels (Chatterji, Fogel, 2018) and cholesterol, as well as reducing the risk of cardiovascular disease. This botanical species also has significant ascorbic, palmitic, lauric, oleic, linoleic and linolenic acid levels, which, together with a balanced diet, can contribute in a prominent way to health and prevention of pathologies. Its oil, obtained from the different vegetal structures, especially of the leaves, barks and seeds, has aroused interest of the biotechnological industry in several sectors, mainly in the alimentary and pharmacological scope, to present characteristics of interest to human health.

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In this sense, the use and application of *C. zeylanicum* as well as species of the genus *Cinnamomum* has been widely reported in different areas of knowledge, especially in studies related to the bioactive, synergic (Kiani et al., 2018) or isolated potential of the constituents chemical properties of oils and extracts from their different plant structures. Investigations from different extraction methods of the secondary constituents of various cinnamic botanical structures, mainly demonstrate activity: antibacterial (Firmino et al., 2018; Teles et al., 2019); Antiviral (El-Hamid et al., 2019); In the control of noxious microorganisms in minimally processed foods (Park et al., 2018) and pre-and post-harvesting of olive groves (Ma et al., 2016); Antiamyloidogenic (Madhavadas et al., 2017); In inhibition of the enzyme acetylcholinesterase - AChE (Aumeeruddy-Elalfi et al., 2018) and butyrylcholinesterase - BchE (Arachchige et al., 2017); Anti-hepatotoxicity and antinephrotoxicity (Hussain et al., 2019); On agricultural pest arthropods (Jumbo et al., 2018); In the control of bacterial phytopathogens (Cadena et al., 2018); and Antinephrotoxic (Abdeen et al., 2019).

Chemiosystematic studies of lauráceas have been reported since the 70 (Gottlieb, 1972), and oils and extracts of *C. zeylanicum* generally reveal the presence of alkaloids, flavonoids, proanthocyanidins, tannins, triterpenoids and saponins, and the absence of steroids (Wickramasinghe et al., 2018), despite possible differences in chemical composition characteristics, depending on the structure of the analyzed plant (Legge, 1994), as well as the extraction method (Tullini et al., 2016). Very recent chemotaxonomic studies reported for the first time in the Lauraceae family glycoside compounds in *Cinnamomum cassia* (Guoruoluo et al., 2018).

The action of these compounds in reducing the risk of some diseases is directly related to the bioactivity of secondary metabolites such as polyunsaturated and saturated fatty acids, triglycerides, steroids, flavonoids, isoflavones, carotenoids, vitamin C and other antioxidants and some recent studies seek to elucidate the effects and benefits of therapeutic food consumption in the prevention and treatment of toenails (Do Prado et al., 2018). Some of these compounds, such as lauric acid, are present predominantly in the oil of the cinnamon seeds.

The physicochemical characteristics of a vegetable oil, also, may vary according to the composition of fatty acids that make up the triglycerides. This can occur as much of similar or distinct botanical species or families as well as by the botanical structure generally evaluated.

Thus, knowing the phytochemical composition and physical-chemical properties of vegetable oils with nutraceutical potential is relevant, especially when it is considered that the essential fatty acids are essential to the organism and cannot be synthesized by the same. Among these, polyunsaturated fatty acids (ω-3, ω6 and ω-9), without which the human organism does not function properly, should therefore be offered through diet or food supplementation (Baeza-Jiménez et al., 2017).

However, there are few approaches to phytochemical composition and physicochemical properties specifically related to the fatty acids present in the fixed oil of *C. Zeylanicum* seeds. In the literature, no studies of this nature were found in *C. zeylanicum* chemotypes submitted to the edafoclimatic and ecophysiological conditions of the Tropical Savannah in the Brazilian Amazon. In this way, the objective of this work was to determine the phytochemical composition and to characterize the physical-chemical properties of the fixed oil of the *C. Zeylanicum* seeds submitted to the conditions of Savana, Brazilian Amazonia, through High Resolution Gas Chromatography (GC-FID) and Analysis Nuclear Magnetic Resonance (^{1}H NMR).

2. Materials and methods

2.1 Collection, Exchange and processing

Cinnamon seeds were collected in the municipality of Boa Vista (2°49'46"N, 60°43'11"W), urban perimeter, Brazil, Roraima, Boa Vista. The species was duly identified and an exsicta deposited in the indexed Herbarium of the National Institute of Amazonian Research (INPA), Manaus, Amazon, Brazil, under registry nº 268121. The climate of this region, Brazilian Amazon Savannah, is characterized as Tropical Rainy (Aw), according to the types of climatic classification of Köppen. The access was duly registered in the National System of Management of Genetic Heritage and Associated Traditional Knowledge - SisGen linked to the Ministry of the Environment - MMA, according to specific Brazilian legislation, under number A87FF8B.

For the processing of the vegetal material and obtaining of the fixed oil, the samples were destined to the Laboratory of Environmental Chemistry of the Federal University of Roraima - UFRR and the seeds were selected, washed, dried until reaching constant weight, and later submitted to the extraction of the oil.

2.2 Extraction of fixed oil from seeds and melting point

The extraction of the oil from the cinnamon seed was carried out in triplicate using the Soxhlet system and the solvent hexane. In order to achieve this the following procedures were performed: i) Initially, the cream solid material stored in the amber flask was weighed in triplicate and placed inside; ii) The cartridges were placed in the Soxhlet and together with 1000 mL vials containing 500 mL of hexane; iii) The systems were placed in blankets and heating plates, heating to the boiling point of the solvent (65 °C); iv) The system was turned off after six hours of extraction, cooled to room temperature and then filtered and immediately after the start of solvent and cinnamon oil separation system, via a rotating-evaporation system; v) Three amber vials were weighed and tared and then added to the three broken-evaporated samples; vi) The excess hexane was removed through gaseous nitrogen, finally the three weighings were made; vii) These samples (solid samples, CSB, light yellow in color at 27 °C) were stored in an amber bottle and nitrogen atmosphere until the analyzes were carried out under nitrogen atmosphere for further analysis and viii) The melt temperature started at 39.2 °C and fused to 43.2 °C of the CSB sample.

2.3 Profile phytochemical of fatty acids

The profile phytochemicals of fatty acids were provided by High Resolution Gas Chromatography (GC-FID) and Hydrogen Nuclear Magnetic Resonance Analysis (^{1}H NMR). These procedures were performed in of the Federal University of Minas Gerais - UFMG and at the State University of São Paulo – USP, Brazil.

The analyzes were performed on an HP7820A gas chromatograph (Agilent) equipped with a flame ionization detector. As a data acquisition program, the EZChrom Elite Compact (Agilent) was used. Column HPINNOWAX 15m x 0.25mm x 0.20µm (HP) was used with a temperature gradient: 120 °C, 7 °C min^{-1} up to 240 °C maximum; injector (Split 1/50) detector at 250 °C and 260 °C. Hydrogen was used as drag gas (3.0 mL min^{-1}) and injection volume of 1 mL. Peak identification was performed by comparison with FAME methylated C_{14}-C_{22} fatty acid standards (Supelco cat no 18917), as described by Melo Filho et al. (2018). Analysis of Nuclear Magnetic Resonance of Hydrogen (^{1}H NMR) were performed according Dos Santos et al. (2017). The ^{1}H NMR spectrum were processed by free software SpinWork 4.2.0.

2.4 Characterization of Physical-Chemical Properties

The following parameters were analyzed: i) iodine content; ii) saponification index; iii) acid value; iv) Molecular Mass; v) melting temperature; and, vi) seed moisture content. For that, the reference methodologies described by Carneiro et al. (2005) and REDA; CARNEIRO (2006) were used.

3. Results and discussion

The fixed oil extracted from the cinnamon seed was characterized as a butter, behaving as a solid at room temperature of 25 ºC (Brazil, 2005). In this sense, the final product obtained, given its physical characteristics, came to be denominated Cinnamon Seed Butter - CSB. Its appearance is similar to that of cocoa butter (*Theobroma cacao*). This can be explained, mainly, by the presence of saturated fatty acids.

CSB also showed a color ranging from yellow to brown, which darken and assume a more solidified consistency after its oxidative stability. The mean yield of CSB was 50.29 % from the crude extract (20.40 g) and the mean moisture content was 11.28%, and the melt temperature started at 39.2 °C and fused to 43.2 °C, close to the lauric acid melting temperature (Kant, Shukla, Sharma, 2016).

The iodine content found in CSB, 2.52 mg I2/g, is below those found in the oils of the seeds of Carapa guianensis and Citrus sp. investigated by Farias (2013) and Reda et al. (2005), respectively. However, the values related to the acid number and the saponification index of 3.32 (mg KOH g-1) and 255.97 (mg KOH g-1), in due order, were higher than those observed by the same researchers mentioned above.

According to the classification determined from the Resolution of the Collegiate Board - RDC nº 270 (Brazil, 2005) of the National Agency of Sanitary Surveillance - ANVISA, linked to the Ministry of Health - MS, Brazil, refined oils and fats must present the maximum capacity of absorption of 0.6 mg KOH g^{-1}. In this sense, the CSB has an acid value higher than stipulated and this can be explained by the presence of lauric compound in the majority. This result also indicates the need to establish criteria for conservation of this product, considering its possible economic commercial use.

The chemical composition of CSB for Saturated Fatty Acids - SFA (97.21%), presented the following profile: lauric (88.60%); capric (2.34%); myristic (2.70%); pentadecyl ether (0.56%); palmitic acid (1.89%); stearic acid (0.96%); arachidic (0.16%). For the unsaturated fatty acids - UFA (2.79%), the following constituents were verified: oleic, ω-9 (1.88%); linoleic, ω-6 (0.72%); linolenic acid, ω-3 (0.19%), and thus lauric acid is the major compound.

Studies similar to *C. Zeylanicum* have not been identified in the literature. However, Albarracin et al. (2017), when studying the chemical composition of *Endlicheria oreocola* (Lauraceae), a species of the same family of cinnamon, found the majority of saturated fatty acids, the most common being lauric acid and, to a lesser degree, palmitic acid. This points out the potential of lauraceae species with source of lauric acid, mainly.

Some investigations of phytochemical composition related to the presence of fatty acids from oils of the seeds of other botanical species also report the presence of lauric acid in the fixed oil of its seminiferous structures, however, in concentrations lower than those found in the CSB of the present study.

Investigating the oil of the seeds of tucumã (*Astrocaryum vulgare*), Arecaceae, native species of the Brazilian Amazonian forest, Santos et al. (2013) found only 4.6% of lauric acid in its composition. Jorge, Luzia (2012) and Belén-Camacho et al. (2005) studying the oils of the seeds of *Pachira aquatica* (Bombacaceae) and *Acrocomia aculeata* (Arecaceae), in due order, did not verify the presence of lauric acid in the chemical profile of their samples. Machado et al. (2006), investigating the oil of coco-babassu (*Attalea speciosa*) seeds, Palmaceae, found concentrations of lauric acid varying between 44.96 and 44.13% when submitted to extraction at different temperatures. Osorio et al. (2012) when analyzing the oil of the seeds of the palm tree *Bactris gasipaes* (Arecaceae), verified the presence of only 0.014 % of the lauric acid.

It is worth mentioning that CSB presented a lower concentration of unsaturated fatty acids (2.79%) in relation to the other oils mentioned above. When evaluating the fatty acid profile in the fixed oil of annatto (*Bixa orellana*) seeds, Bixaceae, Costa et al. (2013) also found values higher than the unsaturated acids present in CSB.

In this context, the importance of the levels of lauric acid present in the CSB to perform new chemosystematic studies, as well as possible biotechnological prospects from this product is evidenced. However, investigations with chemo-types from other regions of Brazil and the world where cinnamon is distributed are recommended. The realization of bioassays to prove the biological, nutraceutical, functional potential, among other functions of the lauric acid present in CBS and that can bring benefits to human health are also suggested.

In relation to the unsaturated fatty acids, the reason of ω6/ω3 (3.8:1) of CSB has values higher than those found in linseed (*Linum usitatissimum*) and canola (*Brassica napus*) (Martins et al., 2006), being these values, recommended by the major global health institutions as appropriate for human consumption. These same authors corroborate that ratios between 2:1 and 3:1 are considered acceptable, above all, because it enables a positive conversion of α-linolenic acid to docosahexaenoic acid (DHA) in the human organism, bringing health benefits.

4. Conclusions

These exploratory and preliminary results indicate the potential of *Cinnamomum zeylanicum* seed oil as a potential therapeutic agent. In addition, they point out possibilities of prospecting for medicines and demonstrate their possible use in the food industry with functional objectives and nutraceuticals. However, applied investigations related to the biological effects of this product should be performed to ensure the therapeutic safety and pharmaceutical efficacy of its bioactive compounds.

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