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Botanical Aspects, Caffeine Content and Antioxidant Activity of *Coffea arabica*

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Abstract

Brazil is the largest coffee exporter in the world. In addition, it occupies the second position, among the consuming countries of the drink. To investigate the chemical composition and quality of the coffee drink from *Coffea arabica* species, samples grown in the city of Ourinhos, the third most productive region in the state of São Paulo, a study of its properties and characteristics was conducted. The pharmacobotanical characteristics were investigated performed according to usual techniques in these researches through macroscopic and microscopic studies through cross-sections. The oil obtained for analysis was extracted by soxhlet, and the caffeine content was measured for green grains using High Performance Liquid Chromatography (HPLC) using as stationary phase, column C_{18} and gradient mobile phase formed by 80% water and 20% methanol. The concentration obtained was 44.983 ± 0.86 μg/mL. The antioxidant activity was measured in triplicate through the DPPH test of the *in natura* coffee oil, and presented antioxidant action of EC_{50} 25.89 ± 1.16 μg/mL.

Keywords

Coffea arabica, Botanical Characterization, Caffeine

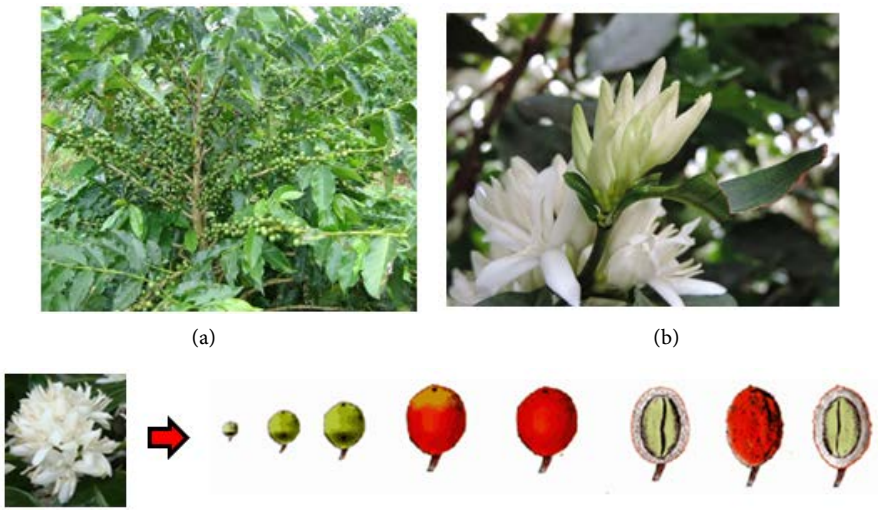
1. Introduction

The coffee is a plant belonging to the *Plantae* kingdom; class *Magnoliopsida*, family *Rubiaceae*, of the genus *Coffea*. The used parts of the plant were: fruit and grain [1]. The plant species *Coffea arabica*, which is cultivated on a large scale in the American continent, is a shrub with 2 to 4 meters high. The lateral branches, also called primary branches, which are long and flexible, containing secondary

and tertiary branches [2] (**Figure 1(a)** and **Figure 1(b)**). The leaves presents a short petiole, has a tan color when youth, and dark green when adult; shiny on the top and dull on the underside. The flowers are white or slightly pink. The green coffee grain (**Figure 1(c)**) is composed of glycines, mainly polysaccharides, lipids and proteins.

Also present are unsaponifiable lipids, sterols, hydrocarbons, tocopherols, furans, diterpene alcohols and phenolic acids such as caffeine and chlorogenic [1] [5]-[11]. The coffee oil is mainly composed of triacylglycerols with fatty acids in similar proportions as a common edible oil. The unsaponifiable fraction is relatively rich in diterpenes from the kauranes class, and derivatives of cafestol, kahweol and 16-*O*-methylcafestol, which have been receiving more attention in recent years due to their different physiological effects [12]. According to Savian [13], the green coffee oil helps in the regeneration of the lipids of the stratum corneum, re-structuring the skin barrier and avoiding dehydration. It has emollient properties from fatty acids and the ability to block the sun's rays to human skin, providing protection against UVB solar radiation. It has also been reported antibacterial [14] and antioxidant activity [5] [6] [9] [10] [15] [16]. The caffeine is the main methylxanthine in the coffee. It presents a wide spectrum of pharmacological activities, acting on the central nervous, cardiovascular, renal and digestive systems [17]. According to Koo *et al.* [18] in a study of mice exposed to UVB radiation, caffeine was used topically to treat the effects of photoaging, reducing the damage to the skin of the animal.

The coffee cultivation is the economic base of Brazil. It was developed only with national resources, the first being exclusively Brazilian, with the purpose of producing wealth. Even today it is one of the most important products of Brazil. The production of arabic coffee (*Coffea arabica*) is concentrated in São Paulo, Minas Gerais, Paraná, Bahia and part of Espirito Santo, while the robust coffee is



(a) (b)

(c)

Figure 1. *Coffea* (*Coffea arabica*) in Ourinhos, SP, Brazil (a); Coffee flowers (b) [3]; Development of grain coffee (c) [4].

planted mainly in Espirito Santo and Rondônia [19]. Considering this information, this work aims the botanical confirmation of the evaluated *Coffea arabica* plant, as well as extracting the oil from the green coffee grain, quantifying the caffeine content and evaluating its antioxidant activity.

2. Materials and Methods

2.1. Plant Material

The coffee used was *Coffea arabica* L., Mundo Novo variety. The coffee was harvested from a plantation in the Ourinhos city, on March 2017. The parts harvested were leaves and green grain.

2.2. Collection of Material

For the characterization of the *Coffea*, a sample was collected directly at the planting. In the botanical identification an exsiccate was prepared and the selection of the collected material was done avoiding the collect parts of the vegetable affected by diseases, parasites and also foreign materials. The place, time and date of collection were recorded, as the environment, time of day and time of year exerted a great influence on the production and accumulation of plant metabolites. An organoleptic and macroscopic evaluation of the plant was performed, with annotation of all relevant data (color, taste, smell, texture, shape, base, edge, apex and consistency) comparing such data with the literature.

2.3. Pharmacobotanical Characterization of Coffee Leaves and Fruits

Cross sections of the collected botanical material were made. The medial part of the leaf was cut with a thin blade, placed on watch glass with water; choosing the best ones that were then clarified in sodium hypochlorite. The clarified cuts were washed in water at least twice, for complete elimination of the sodium hypochlorite residues. The sections were stained with safranin for 30 seconds, washed in water, stained with Astra blue for 2 - 3 minutes, and washing at the end. They were mounted on slide with cover slip and observed under optical microscope in various increases up to 40× at the maximum. The same procedure was adopted for green coffee grain.

2.4. Obtaining Coffee Oil

The coffee oil was obtained by extraction with organic solvent. A soxhlet extractor was used, and ethyl ether was employed as extractive solvent. Samples of green coffee grain were cut and left under solvent for two hours under heating on a blanket. Subsequently, the obtained extract was filtered and dried in an air stream.

2.5. Determination of Caffeine

High Performance Liquid Chromatography (HPLC) equipment, model D-7000,

Merck-Hitachi, Germany was used. The stationary phase was a C_{18} (125 mm × 5 μm) column (Merck, Germany), the flow of 1.0 mL/min, the wavelength was 254 nm in the diode array detector (DAD) the injection volume was 5 μL. The mobile phase used was by gradient method, as described below: 80% water (solvent A) and 20% methanol (Merck, Germany) (solvent B) in a linear gradient system up to 40% A and 60% of B in 20 minutes. The running time was 30 minutes. For determination of caffeine, a standard curve with caffeine concentrations (Sigma, USA) was constructed between 0.1 and 1.0 mg/mL in methanol. From the standard curve, the concentration of caffeine in coffee oil was calculated. The quantitatively extracted coffee oil was diluted in methanol (1.0 mg/mL), filtered and injected into the chromatography apparatus.

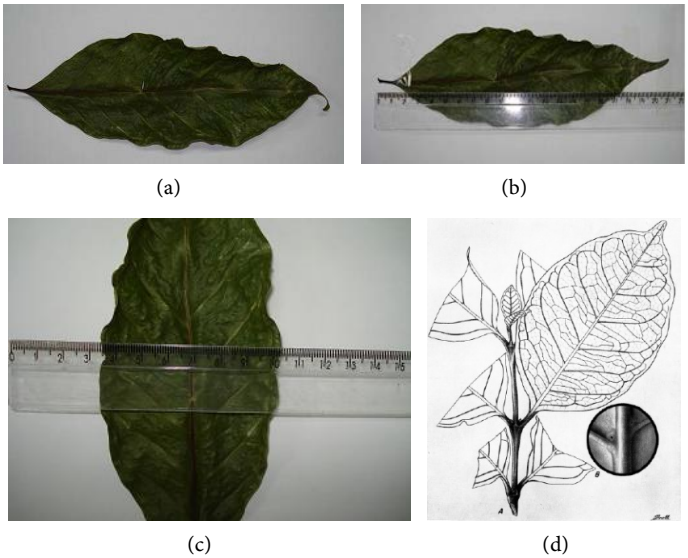
2.6. Antioxidant Activity

The antioxidant activity of coffee oil was determined using the diphenylpicrylhydrazyl radical (DPPH) (Sigma, USA) according to literature [20]. The experiment was performed in triplicate.

3. Results and Discussion

Pharmacobotanical Characterization of Coffee

The vegetal drug was evaluated for its pharmacobotanical characteristics. The evaluated samples presented dark green leaves with a characteristic odor (**Figure 2(a)**). They presents from 6 to 20 cm long (**Figure 2(b)**) and from 2 to 6 cm wide (**Figure 2(c)**). The leaf contour is lanceolate, leaf base attenuated and leaf apex acuminate; the margin is sinuous, reticulate venation and straight petiole; the leaves also present domatia in the axils of the main veins (**Figure 2(d)**). All these requirements are in accordance with the literature [21] [22] [23].



(a) (b) (c) (d)

Figure 2. General aspect of the coffee leaf (a); Length leaf (b); Width leaf (c); Botanical drawing of leaf with domatia in highlight (d) [21].

Through the microscopic analysis, the cross section evidenced, superior and inferior epidermis unstratified; palisadic parenchyma consisting of a layer of juxtaposed cells; lacunate parenchyma with large gaps in the layers just after the palisade, which condense into a dense shape in the last two layers (**Figure 3**). Absence of trichomes, starch grains, crystals or any other inclusion.

The coffee grains were already ripening and almost black. However, it was possible to analyze still the green grains. For the microscopic analysis of the green coffee grains, the bark was removed and made cross-sectional and longitudinal section (**Figure 4(a)**). The coffee grains were oblong and green. In microscopic analysis, the cross section of the endosperm green coffee grain presented lipid cells (**Figure 4(b)**). All these requirements are in accordance with the literature [24].

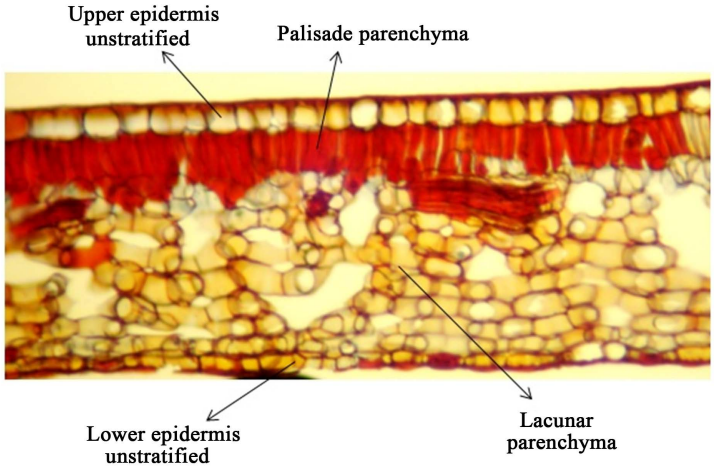
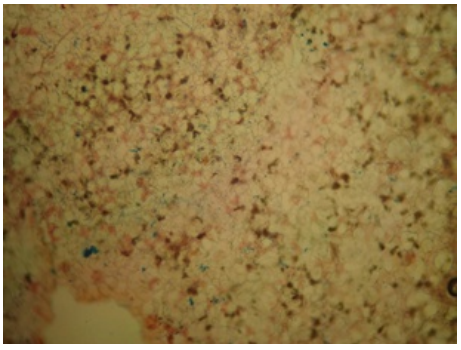


Figure 3. Cross section of the leaf.

(a)



(b)

Figure 4. Cross-sectional and longitudinal section of green coffee grain (a); Cross section of the endosperm coffee grain (b).

The results from of the coffee oil, as described in the experimental part, are described in Table 1 part 1. It was not possible to compare the results obtained in this process with the literature, since the method commonly used, occurs through presses and with large volume of green coffee grains. The concentration of caffeine calculated as a function of the area of the caffeine standard (Figure 5).

Table 1 shows the percentage of oil (part 1) and caffeine concentration values in the green coffee oil sample (part 2). Pérez-Hernandes *et al.* [9] found in different varieties of coffee grain, caffeine levels ranging from 1.29% to 1.74%. A similar range of caffeine content was also reported in other coffee grain varieties, ranging from 1.82% to 2.12% [16]. Other authors [11] described the range between 2.84% and 5.82% for roasted coffee grains from the Paraná State in Brazil. In all of these studies, results were reported from analysis of roasted coffee grains. In the present work, the caffeine content in green coffee grain oil was evaluated, and a lower content was obtained.

The antioxidant test was performed by DPPH with the coffee oil *in natura* and presented antioxidant activity of ED_{50}= 25.89 ± 1.16 μg/mL. The antioxidant activity of soluble coffees was evaluated by the DPPH method and the ED_{50} (μg/mL) found was in the range of 19.87 to 24.92 [11]. Cheong *et al.* [6] found

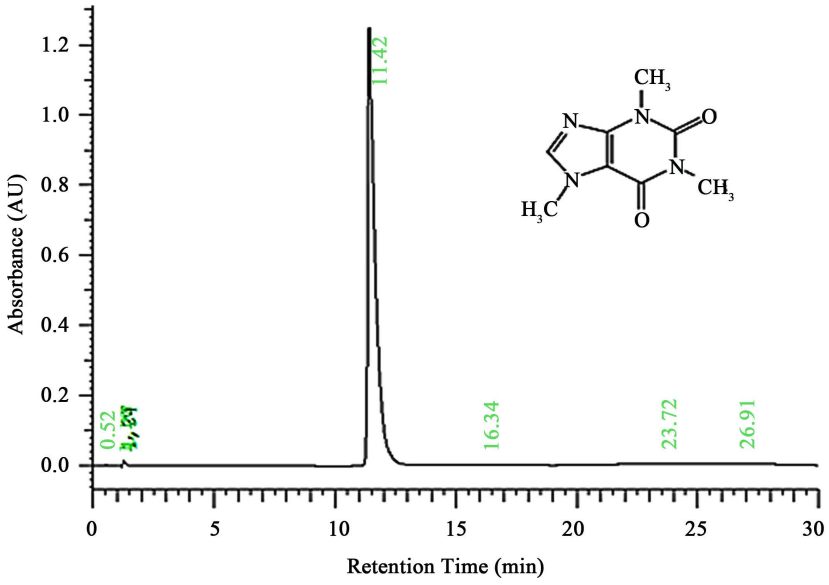


Figure 5. HPLC Chromatogram of the caffeine standard (Rt = 11.42 min).

Table 1. Coffee oil content (%) after extraction (part 1) and concentration of caffeine present in green coffee oil (%) (part 2).

Coffee grains (g)	Extracted oil (g)	Oil (%)
657,701	0.09365	0,14
Product	**Concentration of caffeine (mg/mL)**	**% (w/V)**
Coffee oil	449.83 ± 2.47	44.983 ± 0.86

values for antioxidant activity using the DPPH radical in different types of coffee from Thailand, Indonesia and China. The ranging was between 9.53 to 11.17 μg/mL (green coffee grains) and 8.23 to 9.96 μg/mL (roasted coffee grain). There are some reports in the literature about the antioxidant activity of coffee and green coffee oil, however the authors used other methods of evaluation, and it is not possible to establish a comparison.

4. Conclusion

The results obtained provide an overview of the pharmacobotanic characteristics, antioxidant activity and caffeine content of samples collected in the third largest coffee producing city in São Paulo State. From the morphological point of view, the leaves of *Coffea arabica*, present peculiar characteristics, and are in agreement with the literature. In relation to the chemical tests, the results show that the concentration of caffeine determined from the oil of the green coffee grains is lower to the concentrations reported in the literature; however, the literature analyzes other types of coffee grains. The antioxidant activity of the oil extracted from the green coffee grains presents a considerably high EC_{50} value, which is expected, since the antioxidant activity of green coffee grains is known to be lower comparing to grains that are submitted to processing methods.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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