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The determination of antioxidant activity of green *Coffea arabica L.* coffee beans

Research article

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Abstract
An infusion of green coffee is a commonly consumed beverage, famous for its health-promoting properties. Green coffee owes its properties to the richness of active phytochemicals and is thought to have impacts on body mass, blood glucose and cholesterol levels, blood pressure, and cardiovascular disease prevention due to the antioxidant action of chlorogenic acid.
The aim of this work was to assess the antioxidant potential and chlorogenic acid content of the green coffee. Chlorogenic acid (CGA) is one of the compounds found in coffee beans and other parts of coffee plant. The assessment of antioxidant activity was done by DPPH (2,2-diphenyl-1-picrylhydrazyl) method and to determine the content of CGA was used an UV-Vis spectrophotometric method. Results indicated a percentage of 5.15% in CGA in green coffee beans and a percentage of 79.2% for antioxidant activity.

Keywords: green coffee, antioxidant activity, chlorogenic acid

1. INTRODUCTION

In the international trade market, coffee is an economically important commodity traded as green coffee [1].

There are about 100 varieties of the coffee genus available, including *Coffea canephora L.* and *Coffea arabica L. Coffea arabica L.* is the essential variety for commercial purposes. Green coffee consists of various primary components. These primary components include polysaccharides, lipids, proteins, polyphenols, caffeine, simple sugars and free amino acids. Polysaccharides account for nearly half of the dry weight of coffee beans [2]. Tannins, anthocyanins, and lignans are presented in seeds in low amounts.

Amino acids conjugated with hydroxycinnamic acids called as cinnamoyl amides or glycosides known as cinnamoyl glycosides, which are seen in green coffee and possess good antioxidant property [3]. Polyphenols from coffee are excellent antioxidants and possess free radical scavenging properties [4]. Major polyphenols from green coffee beans are hydroxycinnamic acids and quinic acid, together known as chlorogenic acids [5]. Antioxidant activity of coffee beans depends on the characteristics of phenolic compounds, especially chlorogenic acids [6]. In Figure 1 are presented chlorogenic acids found in coffee.

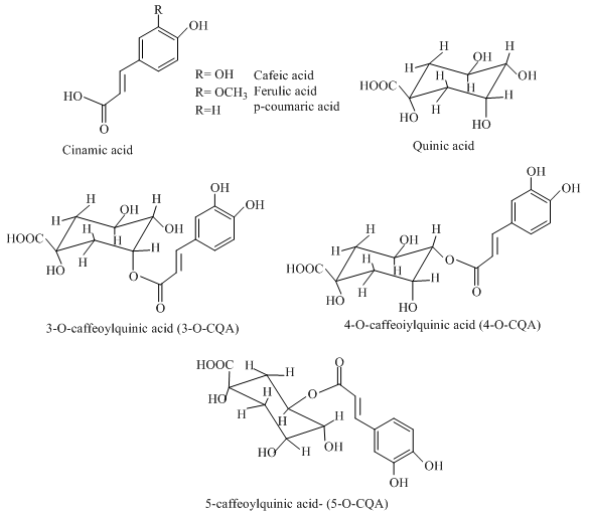

Figure 1. Chemical structures of chlorogenic acids from coffee

Measuring the antioxidant activity

To determine the antioxidant activity of one component, are used methods based on single electron transfer reactions. These procedures are widely used, especially for determinations made on extracts obtained from natural products. In Table 1 are presented different spectrometric techniques of antioxidant activity assay [7].

Table 1. Different spectrometric techniques of antioxidant activity assay

Antioxidant capacity assay	Principle of the method	End-product determination
	Spectrometry	
DPPH	Antioxidant reaction with the organic radical 2,2-diphenyl-1-picrylhydrazyl (DPPH)	Colorimetry
ABTS	Antioxidant reaction with the organic cation radical 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)	Colorimetry
FRAP	Antioxidant reaction with Fe^{3+}—TPTZ (iron[III]-2,4,6-tripyridyl-S-triazine) complex	Colorimetry
CUPRAC	Cu (II) reduction to Cu (I) by antioxidants	Colorimetry
ORAC	Antioxidant reaction with peroxyl radicals, induced by AAPH (2,2'-azobis-2-amidino-propane)	Loss of fluorescence of fluorescein
TRAP	Antioxidant capacity to scavenge luminol-derived radicals, generated from AAPH decomposition	Chemiluminescence quenching
Fluorimetry	Emission of light by a substance that has absorbed light or other electromagnetic radiation of a different wavelength	Recording of fluorescence excitation/emission spectra

In Table 2 are presented most popular assays of total antioxidant activity [8].

Table 2. Most popular assays of total antioxidant activity

Method	Principle of Measurement
ABTS* reduction	Decrease in absorbance of solution of pre-formed ABTS* radical (usually at 734 or 414 nm)
DPPH* reduction	Decrease in absorbance of solution of the stable DPPH* radical (around 517 nm)
FRAP	Increase in absorbance of Fe^{2+} -TPTZ complex upon reduction of Fe^{3+} to Fe^{2+} (at 593 nm)
CUPRAC	Increase in absorbance of bis(neocuproine)copper$^{(+)}$ upon reduction of Cu^{2+} to Cu^{+} (at 540 nm)
ORAC	Inhibition of fluorescence decrease of R-phycoerythrin or fluorescein induced by a source of peroxyl radicals

2. MATERIALS AND METHODS

2.1. Materials

Green coffee was commercially purchased. The samples were thoroughly washed with tap water, wet sorted, dried, and grinded into powder in a coffee grinder.
2,2-diphenyl-1-picrylhydrazyl (DPPH) Sigma Aldrich; Clorogenic acid

2.2. Obtaining the coffee extract

25 g of ground green coffee was subjected to the extraction process with 70% water and 30% methyl alcohol as a solvent. The extraction took place in an Erlenmeyer flask at a temperature of 80°C for 2 hours with magnetic stirring. After filtration, the mixture was stored at 5-6°C until use.

2.3. Analysis methods

2.3.1. Antioxidant activity assay using DPPH method

The DPPH radical neutralization activity was used to measure the antioxidant activity according to the method of Latief et al., 2022 [9] with minor modifications.

0.2 mL of the sample solution was pipetted with a micro pipette into the a vial, then 3.8 mL of 50 μM DPPH solution was added. The solution mixture was homogenized and left for 30 minutes in the dark at room temperature (25°C). The absorbance was measured by a UV-Vis spectrophotometer at a wavelength of 519 nm. Antioxidant activity was expressed as the inhibition percentage and was calculated by the following equation:

% inhibition = [($Abs_{control}$ – Abs_{sample}) / $Abs_{control}$] × 100 where $Abs_{control}$ is the absorbance of DPPH radical + methanol; Abs_{sample} is the absorbance of DPPH radical + sample extract.

In Figure 2 is presented the mechanism of 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition with the antioxidant.

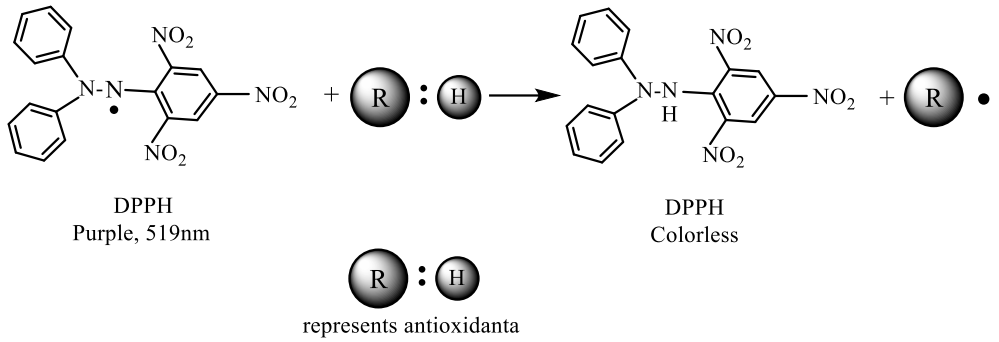

Figure 2. Reaction mechanism of 2,2-diphenyl-1-picrylhydrazyl (DPPH) with antioxidant; R:H = antioxidant radical scavenger, R· = antioxidant radical

2.3.2. The determination of chlorogenic acid (calibration curve)

100 mg of standard chlorogenic acid were dissolved in methanol in a 100 mL Erlenmeyer flask.

The calibration curve was prepared using solutions of different concentrations ranging from 100 μg/ mL to 400 μg/ mL and determining their absorbance at 290 nm on a UV-Vis spectrophotometer Cary On-50 (Figure 3).

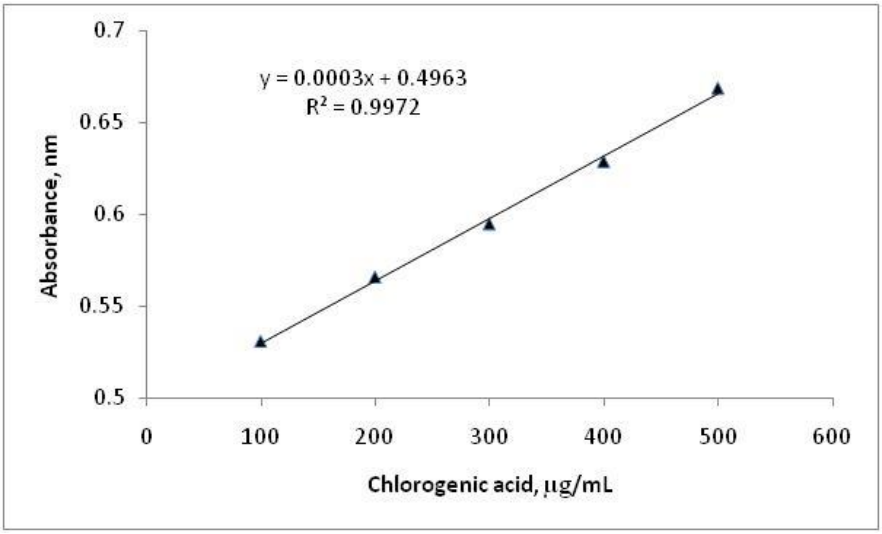

Figure 3. The calibration curve of chlorogenic acid

2.3.3. Sample preparation for measurement of the chlorogenic acid content

Green coffee extract was obtained using the method described by Misto et al., 2022 [10] with small modifications. 1 g of ground green coffee was dissolved in 100 mL of boiling water. After 10 minutes, the coffee solution was filtered on filter paper. 7.5 g of calcium carbonate were added to the filtrate solution to remove caffeine. Furthermore, the chlorogenic acid was extracted by adding 25 mL of chloroform into a separating funnel. After shaking, the upper layer was separated. The extracted liquid was filtered again and then diluted in a 50 mL volumetric flask using methanol. The absorbance of the obtained extract was determined at a wavelength of 290 nm. The chlorogenic acid content were calculated according to following equation that was obtained from the calibration curve: $y = 0.003x + 0.4963$, $R^2 = 0.9973$ (Figure 3). The content of chlorogenic acids was expressed in percentage.

3. RESULTS AND DISCUSSION

Chlorogenic acids (CGAs) are important biologically active dietary polyphenols produced by certain plant species. Chlorogenic

acids are widely distributed in plants, vegetable and fruits. They are some of the most used polyphenols in the human diet [11]. The quantity of chlorogenic acid in most plants is very small. However, a few types of plants accumulate chlorogenic acid: **vegetables** (artichoke 1.1-1.8 $g \cdot Kg^{-1}$ [12]; carrot 0.3-18.8 $g \cdot Kg^{-1}$ [13]; eggplant 14.1-28.0 $g \cdot Kg^{-1}$ [14]; potato 0.01-4.6 $g \cdot Kg^{-1}$ [15]; tomato 0.2-0.4 $g \cdot Kg^{-1}$ [16]; **fruits** (apple 0.4-1.2 $g \cdot Kg^{-1}$ [17]; peach; 0.1-1.6 $g \cdot Kg^{-1}$ [18]; apricot 0.02-0.51 $g \cdot Kg^{-1}$ [19]; cherry 0.02-0.09 $g \cdot Kg^{-1}$ [20]; plum 0.4 $g \cdot Kg^{-1}$ [21].

Unroasted coffee beans represent the richest dietary source of chlorogenic and caffeic acids [22]. Polyphenolic materials found in green coffee especially chlorogenic acid have an important role due to its high antioxidant activity [23].

Several studies have associated CGAs with beneficial health properties, such as antioxidant, antiviral, antibacterial, anticancer, and anti-inflammatory activity, reduce the relative risk of type 2 diabetes and Alzheimer's disease [24], reduced carbohydrate absorption in the digestive tract, decreasing insulin resistance, stimulation of insulin secretion, hypotensive effect, antidyslipidemic and antiatherosclerotic effect (cardio-protective), and neuroprotective effects [25]. Chlorogenic acid may provide a non-pharmacological and non-invasive approach for treatment or prevention of some chronic diseases [26].

The determination of the chlorogenic acid content carried out on the green coffee samples (*Coffea Arabica L.)* was 5.15%, a value that is within the wide range of the values presented by other authors for different food products and in accordance with the values obtained by Nogaim. et al., 2013 [27] by analysing 70 samples of coffee Arabica, values that varied between 4.24-11.62%.

The antioxidant activity value determined by the DPPH method of Arabica coffee samples was 79.2%, a value that is in good agreement with results obtained by Bijla and co-workers [28]. The obtained values highlight the role played by green coffee in supplying active components with antioxidant activity beneficial to the human body.

4. CONCLUSION

Chlorogenic acids, along with other active components, play an important role in the expression of the antioxidant properties of green

Arabica coffee. The chlorogenic acid content and the antioxidant activity that we have determined on the green coffee samples (*Coffea Arabica L.)* are high enough to recommend this product for the maintenance of human health, constituting an important source in the production of pharmaceutical preparations.

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