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**EXTRACTION AND EVALUATION OF BIOACTIVE COMPOUNDS  
WITH ANTIOXIDANT POTENTIAL FROM  
GREEN ARABICA COFFEE EXTRACT**

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During the last decade researches concerning the essential role of coffee in health and disease prevention showed an increased development. In the present study we obtained extracts from three green Arabica coffee varieties which demonstrated a significant antioxidant potential due to the presence in their composition of two bioactive compounds, caffeine and chlorogenic acids. The content and antioxidant activity of bioactive compounds were evaluated by qualitative and quantitative analyses using spectrophotometric and chromatography methods. The chlorogenic acid was found in high concentrations, being followed by gallic, p-coumaric and ferulic acids. The highest caffeine contents were found in the green coffee extracts of the Supremo–Columbia and Top Quality–Kenya products.

**Keywords:** green coffee, bioactive compounds, antioxidant activity

**Introduction**

Coffee is one of the most popular plant products used as beverage with essential stimulant properties. The most commercialized varieties of coffee are Coffee Arabica (Arabica) and *Coffea canephora* (Robusta) (Esquivel *et al.*, 2012). This common beverage contains multiple bioactive compounds (caffeine, chlorogenic acid, cafestol, trigonelline and kahweol) with significant potential as antioxidants and free radical scavengers. These substances have various therapeutic applications such as liberation of free fatty acids from human adipocytes to prevent body fat accumulation, inflammation control by reducing the inflammatory markers, and

sun protection factor due to the lipid fraction of green coffee extract (Onakpoya *et al.*, 2011, Wagemaker *et al.*, 2011, Frost-Meyer *et al.*, 2012, Flanagan *et al.*, 2013, Martinez-Saez *et al.*, 2014).

In literature, various methods are proposed to evaluate the bioactive compound content from coffee products and their antioxidant capacity. Therefore, the caffeine content of tea and instant coffee brands on the world market and its antioxidant activity were analyzed by high performance liquid chromatography (HPLC), UV/VIS spectrophotometric methods and colorimetric assays (Folin-Ciocalteu, ABTS and DPPH), respectively. (Wanyika *et al.*, 2010, Ludwinget *et al.*, 2012, van der Werf *et al.*, 2014). Chlorogenic acids from green coffee extracts were isolated using activated carbon (Suárez-Quiroz *et al.*, 2014) and quantified by liquid chromatography-mass spectrometry (LC-MS) and HPLC (Mills *et al.*, 2013). The most effective technique for preserving the functional properties of coffee extracts is block freeze concentration by increasing the concentration of bioactive compounds and the antioxidant activity of the coffee extract (Moreno *et al.*, 2014). Both caffeine and chlorogenic acids can be used as natural antioxidants in food or pharmaceutical products, as they are absorbed and metabolized in the human body (Farah *et al.*, 2008).

The aim of this paper is to extract and evaluate two essential bioactive compounds, caffeine and chlorogenic acid, from five green Arabica coffee varieties. Qualitative and quantitative analyses of caffeine and chlorogenic acid were performed in order to show their significant antioxidant potential.

## **Materials and methods**

All the reagents used for the extraction and evaluation of bioactive compounds with antioxidant activity from green coffee extracts were purchased from Merck.

### ***Plant material***

For the qualitative and quantitative analyses of xanthine alkaloids (caffeine) and the antioxidant activity evaluation of bioactive compounds (caffeine, polyphenols) from various commercial species of green coffee, there were used five varieties of green Arabica coffee purchased from Romanian hypermarkets: Estrellas special-Panama (P1), Supremo-Columbia (P2), Top Quality-Kenya AA (P3), Huehuetenango-Guatemala (P4), Kilimanjaro-Tanzania (P5).

### ***Caffeine extraction***

Caffeine extraction was performed by the modified method proposed by Komes *et al.* (2009) using the five commercial samples of green coffee. 20 g of each sample were added into a beaker with 90mL of distilled water and left to reflux for 30 minutes. The final solution was filtered twice through a Buchner flask, mixed with 12.5 mL of  $(\text{CH}_3\text{COO})_2\text{Pb}$  and heated for 5 minutes. Afterwards, the filtered solution was extracted with 40 mL of chloroform, which was removed from the extract by rotary evaporation. The chloroform phase was washed with KOH and distilled water. The residue obtained after evaporation was expressed as g caffeine/100g plant product.

### ***Caffeine identification***

The identification of caffeine from the five samples of green Arabica coffee was developed using some specific chemical reactions with  $\text{AgNO}_3$ ,  $\text{K}_2\text{Cr}_2\text{O}_7$  and thin layer chromatography (TLC). The chromatographic analysis involved the spotting of chloroform solutions of the pure caffeine and the green coffee extracts on silica gel sheets (60, F<sub>254</sub>). The solvents mixture used was butanol saturated with distilled water, and formic acid 10% (9:1). The detection of caffeine on the chromatogram was performed by exposing the chromatographic sheet to iodine vapor until the spots were stained in brown or by viewing the spots at the UV lamp on  $\lambda = 265$  nm.

### ***Caffeine dosage***

The caffeine content from green coffee extracts was measured by the UV-Vis spectrophotometric method using UV-VIS Double Beam PC 8 Scanning Autocell equipment, UVD-3200, Labomed Inc., U.S.A. The spectrophotometric method consisted of two steps: (1) plotting of calibration curve and (2) preparation of test solution. In order to plot the calibration curve, some standard solutions of caffeine (1 mg/mL) of different concentrations (0, 1, 2, 4, 6, 8 mL) were mixed with 1 mL of HCl 0.01 M and distilled water in 6 volumetric flasks of 25 mL. Finally, the absorbance at 247 nm was recorded and the extinction versus the concentrations of standard solutions of caffeine ( $E = f(c)$ ) was plotted. The test solution was obtained by mixing 0.5 g of green coffee with 20 mL of hot water, 10 mL HCl 0.01 M, 2 mL  $(\text{CH}_3\text{COOH})_2\text{Pb}$  and distilled water in a 250 mL volumetric flask. To measure the absorbance of the test solution at 247 nm, 50 mL of this sample were mixed with 0.2 mL  $\text{H}_2\text{SO}_4$  and distilled water in a 100 mL volumetric flask. The concentration of the test solution ( $c_x$ ) was established from the calibration curve and expressed as  $\mu\text{g/mL}$ .

### ***Antioxidant activity of green coffee extracts***

The antioxidant activity of the green coffee extract was evaluated by measuring the radical scavenging capacity (RSC) using the DPPH method and by identifying chlorogenic acid from the extract using the HPLC method, respectively. The DPPH method was carried out by mixing 0.1 mL of green coffee extract with 1 mL of 2,2'-diphenyl-1-picrylhydrazyl (DPPH) and 3 mL of methanol 95%. Simultaneously, a blank sample from 1 mL of DPPH and 3 mL of methanol 95% was obtained. After 1 hour, the absorbance at 515 nm for each sample was recorded and the radical scavenging capacity was estimated. The identification of chlorogenic acid by the HPLC method involved two main steps: (1) extraction of chlorogenic acid and (2) HPLC analysis. To extract the chlorogenic acid, 1 g of green coffee powder was mixed with 100 mL of methanol/distilled water (70/30) and 0.5%  $\text{Na}_2\text{SO}_3$ . This mixture was continuously stirred for 24 hours at room temperature. Afterwards, the methanolic phase was filtered and a homogenous powder of green coffee extract was obtained. HPLC analysis was performed on the interdisciplinary research and training "BIOALIMENT" Platform from "Dunarea de Jos" University of Galati. The identification of chlorogenic acid was carried out using the Thermo Scientific Finnigan Surveyor Plus HPLC System. The analytical column used was BDS HYPERSIL C18 column (150 × 4.6 mm i.d. particle size 5

µm). The mobile phase involved 3% formic acid (A) and methanol (B). The chlorogenic acid was separated at 30°C using the following gradient: 0-43 min, 10-56% B, 43-45 min, 56-100% B. The mobile phase debit was 0.7 mL/min. 10 µL was injected from each sample. The detection of chlorogenic acid was performed at 325 nm using UV detection provided by the Thermo Scientific Finnigan Surveyor PDA Plus detector.

All the experiments were done in triplicate and the data presented here represents the mean of these replicates.

## Results and discussion

### *Qualitative and quantitative analysis of caffeine*

Green coffee is used as raw material for the extraction of caffeine and has stimulative action on the central nervous system (CNS). This plant product has the following chemical composition: 0.6-2.2% caffeine, 0.001% theophylline, 0.002% theobromine, trigonelline, over 50% carbohydrates (mainly polyholosides), 10-12% proteins, 10-18% lipids, over 10% insaponifiable substances (hydrocarbons, tocopherols, sterols, diterpene alcohols – cafestol, kahweol, kaurenoic derivatives), non-diterpene glycoside - esters (atractylosides), 3-5% polyphenolicacids (caffeic, quinic, ferulic and chlorogenic acid) (Esquivel *et al.*, 2012, Garrett *et al.*, 2015).

The presence of caffeine in the green Arabica coffee extracts was highlighted by chemical reactions with AgNO<sub>3</sub> 2% and K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> 0.5%, showing a red coloration.

In the case of the five green Arabica coffee samples, the next caffeine content has been identified: **P1** – 1.204 g caffeine/100 g plant product; **P2** – 1.322 g caffeine/100 g plant product; **P3** – 1.310 g caffeine/100 g plant product; **P4** – 1.282 g caffeine/100 g plant product; **P5** – 1.100 g caffeine/100 g plant product. These results are according to data found in literature: **P1** – 1.310 g caffeine/100 g plant product; **P2** – 1.370 g caffeine/100 g plant product; **P3** – 1.360 g caffeine/100 g plant product; **P4** – 1.320 g caffeine/100 g plant product; **P5** – 1.250 g caffeine/100 g plant product (Preedy, 2015).

The identification of caffeine in green coffee extracts was also achieved by thin layer chromatography (TLC) (Suárez-Quiroz *et al.*, 2014). First, the retention factor values of extract spots have been calculated with formula (1).

$$R_f = x_i/H \quad (1)$$

where, R<sub>f</sub> = retention factor; x<sub>i</sub> = the distance of the start line to the solvent front; H = solvent front.

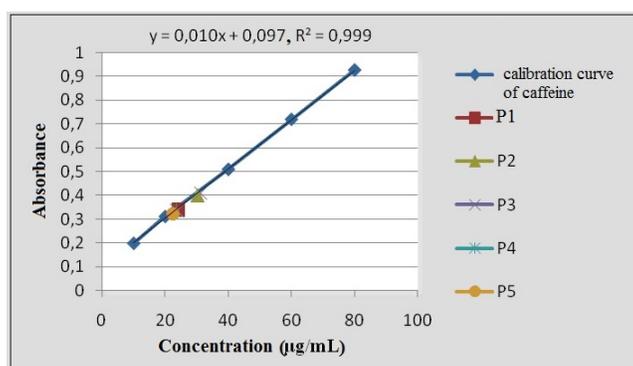
The results were compared to the retention factor value of standard caffeine (Table 1). The values of x<sub>i</sub> and H used in this analysis were: H<sub>blank</sub> = 8 cm; x<sub>blank</sub> = 3.6 cm; x<sub>1</sub> = 3.55 cm; x<sub>2</sub> = 3.6 cm; x<sub>3</sub> = 3.6 cm; x<sub>4</sub> = 3.58 cm; x<sub>5</sub> = 3.6 cm.

The TLC analysis showed that the extraction method was successful, obtaining a significant purity of the caffeine from the five green Arabica coffee extracts. The values of the retention factor of the caffeine extracted from green Arabica coffee varieties were close to the one of standard caffeine solution (R<sub>f blank</sub> = 0.4500).

**Table 1.** Retention factor ( $R_f$ ) values for identification of the caffeine by TLC

Sample	$R_{f\text{ sample}}$	$R_{f\text{ blank}}$
P1	0.4437	
P2	0.4500	
P3	0.4500	0.4500
P4	0.4475	
P5	0.4500	

The concentration of caffeine from green coffee extract was measured by plotting the calibration curve of caffeine and green Arabica coffee extracts (Figure 1).

**Figure 1.** Calibration curve of caffeine and green Arabica coffee extracts

The concentration of caffeine was in the 22.5÷31.3 µg/mL range. The highest caffeine content was recorded for sample P2 (Supremo-Columbia).

#### ***Evaluation of the antioxidant activity of green coffee extracts***

The antioxidant activity of green coffee extracts was demonstrated by their ability to eliminate free radicals using the DPPH method (Oliveira-Neto *et al.*, 2015; Xu *et al.*, 2015). The method consists in the spectrophotometric measurement of the color exchange from purple to colorless of methanol solution of DPPH and calculation of the radical scavenging capacity (RSC) using equation (2) below:

$$\text{RSC \%} = 100(A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \quad (2)$$

where,  $A_{\text{blank}}$  = absorbance of the blank sample;  $A_{\text{sample}}$  = absorbance of the green coffee extract.

From the evaluation profile of the antioxidant activity of green coffee by the DPPH method (Figure 2), it is observed that P2 and P3 have a strong antioxidant activity compared to the other three samples.

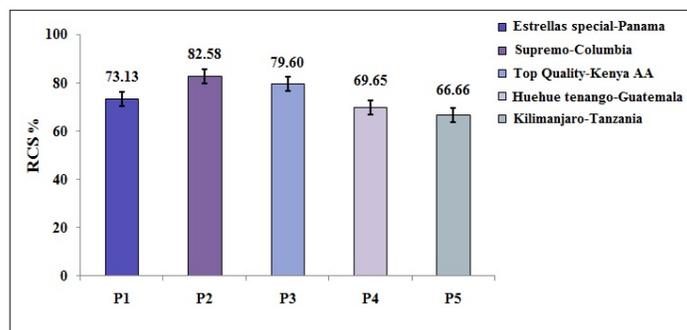


Figure 2. Antioxidant activity of green coffee extracts by DPPH method

The evaluation of the antioxidant activity of green coffee extracts was also achieved by the identification of chlorogenic acid by the HPLC method. Green coffee has a high content of polyphenolic compounds, the chlorogenic acid being present in the highest amount (Naidu *et al.*, 2008).

In order to highlight the presence of compounds with antioxidant properties in green coffee extracts, only P2 (Supremo-Columbia) was analyzed by the HPLC method. Thus, Figure 3 shows the chromatographic profile of polyphenols found in the alcoholic extract of green coffee (P2) and standard phenolic acids, respectively (Belguidoum *et al.*, 2014).

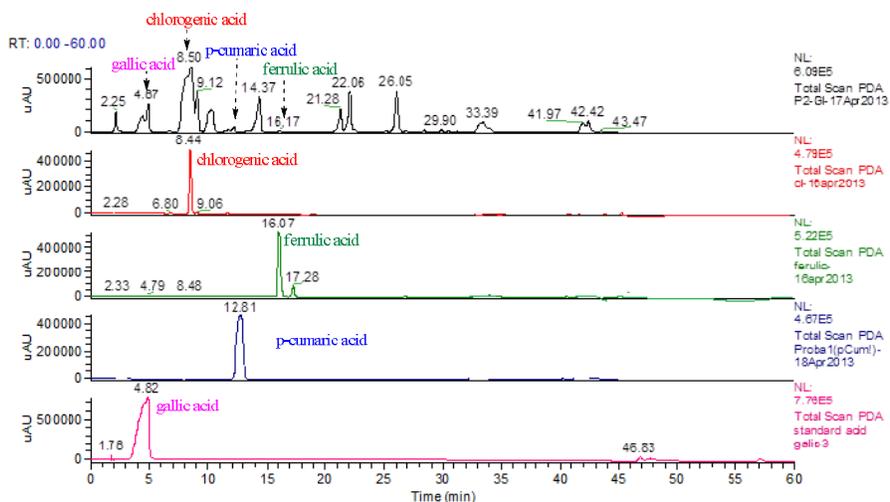


Figure 3. HPLC chromatogram of sample P2 and standard phenolic acids

The HPLC analysis of the green coffee extract detected the following phenolic acids: gallic, p-coumaric, ferulic, and chlorogenic acids. From Figure 3, it can be observed that the chlorogenic acid has the highest concentration in green coffee

extract followed by gallic, p-coumaric and ferulic acids. The presence of these compounds in the green Arabica coffee extracts demonstrates the antioxidant activity of green coffee.

### Conclusions

Due to the significant therapeutic applications of caffeine, the main goal of this study was to extract and evaluate the antioxidant potential of this xanthenic alkaloid from various green Arabica coffee varieties. The green coffee extracts are characterized by the presence of certain bioactive compounds with antioxidant properties such as caffeine and polyphenols. The assessment of these compounds was carried out by qualitative and quantitative analyses. The qualitative analysis involved the chemical identification reactions and development of the TLC and HPLC methods, which highlight in large amount the presence of polyphenolic compounds with antioxidant activity. The quantitative analysis consisted of the extraction and spectrophotometric determination of the caffeine using the DPPH method. The green coffee extracts with a significant amount of caffeine were Supremo–Columbia and Top Quality–Kenya. The validation of the antioxidant character was also performed through HPLC analysis by detecting the chlorogenic acid in green Supremo–Columbia coffee extract. The chlorogenic acid was found in appreciable concentration, being followed by gallic, p-coumaric and ferulic acids.

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### References

- Belguidoum, K., Amira-Guebailia, H., Boulmouk, Y., Houache, O. 2014. HPLC coupled to UV–vis detection for quantitative determination of phenolic compounds and caffeine in different brands of coffee in the Algerian market. *Journal of the Taiwan Institute of Chemical Engineers*, **45**, 1314-1320.
- Esquivel, P., Jiménez, V.M. 2012. Functional properties of coffee and coffee by-products. *Food Research International*, **46**, 488-495.
- Farah, A., Monteiro, M., Donangelo, C.M., Lafay, S. 2008. Chlorogenic acids from green coffee extract are highly bioavailable in humans. *The Journal of Nutrition*, **138**(12), 2309-2315.
- Flanagan, J., Bily, A., Rolland, Y., Roller, M. 2013. Lipolytic activity of svetol, a decaffeinated green coffee bean extract. *Phytotherapy Research*, **28**(6), 946-948.
- Frost-Meyer, N.J., Logomarsino, J.V. 2012. Impact of coffee components on inflammatory markers: A review. *Journal of Functional Foods*, **4**(4), 819-830.

- Garrett, R., Rezende, C.M., Ifa, D.R. 2015. Revealing the spatial distribution of chlorogenic acids and sucrose across coffee bean endosperm by desorption electrospray ionization-mass spectrometry imaging. *LWT - Food Science and Technology*, **65**, 711-717.
- Komes, D., Horzic, D., Belscak, A., Ganic, K., Baljak, A. 2009. Determination of caffeine content in tea and maté tea by using different methods. *Czech Journal of Food Sciences*, **27**, S213-S216.
- Ludwing, I.A., Sanchez, L., Caemmerer, B., Kroh, L.W., De Pena, M.P., Cid, C. 2012. Extraction of coffee antioxidants: Impact of brewing time and method. *Food Research International*, **48**, 57-64.
- Martinez-Saez, N., Ullate, M., Martin-Cabrejas, M.A., Martorell, P., Genovés, S., Ramon, D., del Castillo, M.D. 2014. A novel antioxidant beverage for body weight control based on coffee silverskin. *Food Chemistry*, **150**, 227-234.
- Mills, C.E., Oruno-Concha, M.J., Mottram, D.S., Gibson, G.R., Spencer, J.P.E. 2013. The effect of processing on chlorogenic acid content of commercially available coffee. *Food Chemistry*, **141**, 3335-3340.
- Moreno, F.L., Raventós, M., Hernández, E., Ruiz, Y. 2014. Block freeze-concentration of coffee extract: Effect of freezing and thawing stages on solute recovery and bioactive compounds. *Journal of Food Engineering*, **120**, 158-166.
- Naidu, M.M., Sulochanamma, G., Sampathu, S.R., Srinivas, P. 2008. Studies on extraction and antioxidant potential of green coffee. *Food Chemistry*, **107**, 377-384.
- Oliveira-Neto, J.R., Garcia Rezende, S., de Fátima Reis, C., Rathinaraj Benjamin, S., Lavorenti Rocha, M., de Souza Gil, E. 2015. Electrochemical behavior and determination of major phenolic antioxidants in selected coffee samples. *Food Chemistry*, **190**, 506-512.
- Onakpoya, I., Terry, R., & Ernst, E. 2011. The use of green coffee extract as a weight loss supplement: a systematic review and meta-analysis of randomised clinical trials. *Gastroenterology Research and Practice*, 1-7.
- Preedy, V.R. 2015. *Coffee in Health and Disease Prevention*. Elsevier Inc. Academic Press.
- Suárez-Quiroz, M., Campos, A.A., Alfaro, G.V., González-Rios, O., Villeneuve, P., Figueroa-Espinoza, M.C. 2014. Isolation of green coffee chlorogenic acids using activated carbon. *Journal of Food Composition and Analysis*, **33**, 55-58.
- Van der Werf, R., Marcic, C., Khalil, A., Sigrist, S., Marchioni, E. 2014. ABTS radical scavenging capacity in green and roasted coffee extracts. *LWT-Food Science and Technology*, **58**, 77-85.
- Wagemaker, T.A.L., Carvalho, C.R.L., Maia, N.B., Baggio, S.R., Filho, O.G. 2011. Sun protection factor, content and composition of lipid fraction of green coffee beans. *Industrial Crops and Products*, **33**, 469-473.
- Wanyika, H.N., Gatebe, E.G., Gitu, L.M., Ngumba, E.K., Maritim, C.W. 2010. Determination of caffeine content of tea and instant coffee brands found in the Kenyan market. *African Journal of Food Science*, **4**(6), 353-358.
- Xu, H., Wang, W., Liu, X., Yuan, F., Gao, Y. 2015. Antioxidative phenolics obtained from spent coffee grounds (*Coffea arabica* L.) by subcritical water extraction. *Industrial Crops and Products*, **76**, 946-954.