American Journal of Pharmacological Sciences, 2021, Vol. 9, No. 2, 63-68
Available online at http://pubs.sciepub.com/ajps/9/2/3
Published by Science and Education Publishing
DOI:10.12691/ajps-9-2-3

Antioxidant Activity of Aqueous Extracts of Roasted Bean Seeds of *Coffea canephora* Rubiaceae

AKA Francis B. Angelo[1], Coulibaly Wacothon Karime[2], Titah T. James[3,*], Bea Gouanda Thibaut[4], Benié Anoubilé[5]

[1]Laboratoire de Biologie et Sante, UFR des Biosciences,
Université Félix HOUPHOUËT-BOIGNY, Abidjan, Côte d'Ivoire
[2]Département de Mathématiques, Physique, Chimie, UFR des Sciences Biologiques,
Université Peleforo GON COULIBALY, Korhogo, Côte d'Ivoire
[3]Department of Chemistry-Science & Mathematics, Tabor College, Hillsboro, Kansas, USA
[4]Laboratoire de Constitution et de Réaction de la Matière, UFR Sciences des Structures et Technologie,
Université Félix HOUPHOUËT-BOIGNY, Abidjan, Côte d'Ivoire
[5]Laboratoire de Chimie BioOrganique et de Substances Naturelles, UFR-SFA,
Université Nangui Abrogoua, Abidjan, Côte d'Ivoire
*Corresponding author: jamestitah@tabor.edu

Received November 22, 2021; Revised December 27, 2021; Accepted January 05, 2022

Abstract Phenolic compounds are highly solicited bioactive substances, present specifically in plants and playing a powerful antioxidant role. These secondary metabolites are endowed with several very interesting biological properties, which find applications in various fields, in particular in medicine, cosmetics and nutrition. Given the various virtues of these compounds, we were interested in determining by High Pressure Liquid Chromatography) HPLC their contents in the aqueous extract of roasted beans of *Coffea canephora* (EAGT*Cc*) and particularly the contents of chlorogenic acid, caffeic acid and catechol. In addition, the antioxidant activity was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH). Total polyphenols and total flavonoids were also assayed spectrophotometrically. HPLC showed levels of 3.7 % catechol, 5.4 % chlorogenic acid and 2.8 % caffeic acid in 100 g of dry matter of EAGT*Cc*. The concentration of EAGT*Cc* responsible for 50 % inhibition (IC_{50}) of the DPPH radical is 75.54 ± 2.08 µg / mL. The contents of total polyphenols and total flavonoids are 81.67 mg EAG / g and 293.33 mg EQ / g. This antioxidant activity present in the EAGT*Cc* is probably linked to the richness of the seeds of this plant in phenolic compounds. The results obtained are very interesting insofar as the use of natural compounds as antioxidants remains without side effects on the body and could replace the use of antioxidant molecules resulting from chemical synthesis.

Keywords: *extract, phenolic acid, coffea canephora, antioxidant activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH)*

Cite This Article: AKA Francis B. Angelo, Coulibaly Wacothon Karime, Titah T. James, Bea Gouanda Thibaut, and Benié Anoubilé, "Antioxidant Activity of Aqueous Extracts of Roasted Bean Seeds of *Coffea canephora* Rubiaceae." *American Journal of Pharmacological Sciences*, vol. 9, no. 2 (2021): 63-68. doi: 10.12691/ajps-9-2-3.

1. Introduction

Plants synthesize secondary metabolites such as polyphenols, which are a large group of natural phytochemicals. These polyphenols are made up of aromatic ring(s) to which at least one hydroxyl group is attached. The main group of polyphenols are the Flavonoids and Phenolic acids and have been used as bioactive compounds in controlling human diet. They can be found in fruits, vegetables, herbs, cocoa, coffee, tea, and some other alcoholic beverages such as beer and wine. In addition to controlling human diet, Flavonoids have consistently gained a lot of attention from researchers due to their biological functions such as antioxidant, hepatoprotective, antibacterial, anti-inflammatory and anti-cancer activities. Furthermore, phenolic acids and flavonoids are important organic compounds and their consumption provide in vivo protection against free radical damage and reduces the risk of degenerative diseases associated with oxidative stress [1-5]. Previous research on the bean seeds of coffee trees indicate that they contain phenolic compounds and flavonoids [6,7] and their consumption, especially in the Americans reveal some benefits, including overall mortality rates [8,9]. The antioxidant activity of most fruits, vegetables and foods have been determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) [3,10,11]. This DPPH method could also be extended to determine the antioxidant activity in many plant extracts including the bean seeds of coffee trees [11,12]. The coffee drink brewed in the coffee shops is a

complex mixture of more than a thousand different chemical compounds, many of which are said to be biologically active [13]. The coffee bean seeds contain many organic compounds such as flavonoids, chlorogenic acid and caffeic acid, etc, which have been used as antioxidant agents [6,14]. In this study, our aim is to measure the anti-free radical activity of an aqueous extract of roasted bean seed of *Coffea c.* using the DPPH method.

2. Materials and Methods

2.1. Plant Material

Roasted and finely ground green bean seeds of a coffee plant (*Coffea c.*) was use in this research. The green coffee bean seeds were obtained from Ivory Coast in Africa.

2.2. Experimentation

2.2.1. Preparation of the Aqueous Extract from the Roasted bean Seeds

Thirty grams (30 g) of roasted bean seeds of *Coffea c.* were ground into a powder and dissolved in 175.00 mL of hot distilled water. The resulting aqueouss solution was transferred into a *Philip*s brand filter coffee maker and allowed to brew for 10 minutes. The filtrate obtained was dried in an oven (*Binder*, Tuttlingen, Germany) at a temperature of 60°C to a solid powder. 3 g of the dried powder was recovered from the aqueous extract of roasted bean seeds of *Coffea c.*; percent recovery was 10 %.

2.2.1.1. Spectrophotometric Determination of Total Polyphenols

The determination of the total polyphenol content in our extract was done using the method proposed by method Vermerris and Nicholson [15]. 0.10 mL of the sample was stirred continuously with 2.0 mL of a 2 % sodium carbonate solution. After 5 minutes, 0.10 mL of Folin-Ciocalteu reagent (0.2 N) was added dropwise to the resulting mixture and allowed to react for 30 minutes. The reading was obtained using a spectrophotometer at 700 nm against a blank. A calibration curve was produced under the same condition as the extract with gallic acid at different concentrations ranging from 100 to 1000 µg / mL. The analyzes was repeated three more times and the results expressed in milligrams equivalent of gallic acid per gram of dry extract (mg EAG / g E).

2.2.1.2. Spectrophotometric Determination of Total Flavonoids

The colorimetric method reported by Ardestani and Yazdanparast [16] was used to determine the total flavonoids content in our sample. 500.00 µL of the extract was mixed with 2 mL of distilled water and 150 µL of 15 % sodium nitrite ($NaNO_2$) solution and incubated for six minutes. 150 µL of 10 % aluminum chloride ($AlCl_3$) was added dropwise to the resulting solution. On the other hand, 2.00 mL of 4 % sodium hydroxide (NaOH) was added to the different test tubes and diluted to 5 mL with distilled water. After 15 minutes of continuous stirring, the absorbance of the solution was measured using a spectrophotometer set at 510 nm against a blank. A calibration curve was produced under the same condition as the sample with quercetin at different concentrations (100 to 1000 µg / mL). The process was repeated three more times and the results expressed in milligram quercetin equivalent per gram of dry extract (mg EQ / g E).

2.2.2. Extraction and Determination of Phenolic Compounds

The extraction and determination of phenolic compound in our sample was done according the method proposed by Donovan et *al*. [17]. 25.00 mL of the aqueous extract of the bean seeds of *Coffea canephora* was added to 75.00 mL of 80 % dilute methanol solution (v/v) and saturated with sodium chloride solution. After continuous stirring for 30 minutes, the resulting solution was filtered using a Watchman No.4 paper. The filtrate obtained was concentrated at 35 °C using a *HEILDOLPH Laborata 4003 Control* rotary evaporator (*Schwabach*, Germany). The resulting mixture was diluted with 100 mL of distilled water and the total phenolic compounds in the sample determined using an HPLC instrument (*Shimadzu Corporation*, Japan) consisting of a pump (*Shimadzu* LC-20A Liquid Chromatograph), a UV detector (*Shimadzu* SPD-20A UV Spectrophotometry detector). The retention times of the resulting spectra were compared the retention times of the reference molecules (chlorogenic acid, caffeic acid and catechol) and an internal standards was prepared from powders of the reference substances. The procedure was replicated and the concentration of the different constituents determined using the equation below:

$$\mathbf{CE} = \frac{\mathbf{Aire\ E}}{\mathbf{Aire\ T}} \mathbf{x\ CT}$$

where
CE = sample concentration
CT = Witness concentration
Aire T = sample peak surface
Aire E = sample peak area

2.2.3. Measurement of the Anti-free Radical Activity of the Aqueous Extract of Roasted Bean Seeds of *Coffea c.* (EAGT*Cc*) Using DPPH Method

2.2-diphenyl-1-picrylhydrazyl (DPPH) is an unstable free radical, which becomes stable by accepting a free hydrogen atom. The antioxidants activity of DPPH is determined by its ability to accept it a hydrogen atom. The method described by Mansouri et *al*. [18] was used to measure the anti-free radical activity by DPPH. In this research, 50.00 µL of different concentrations of the EAGT*Cc* solution were added to 1,950.00 µL of a 0.025 mg/mL of DPPH solution. Similarly, a negative control was prepared in parallel, by adding 50.00 µL of methanol to 1,950.00 µL of a 0.025 mg/mL methanolic solution of DPPH. At the same time, a positive control was prepared

from ascorbic acid at different concentrations ranging from 0.02 to 0.2 mg / mL. The above solutions were incubated for 10 minutes, their absorbances determined using a spectrophotometer set at 515 nm. The equation below was used to express our calculations as a percentage.

$$\mathbf{DPPH}(\%) = \frac{\mathbf{OD_{control}} - \mathbf{OD_{sample}}}{\mathbf{OD_{control}}} \mathbf{x\ 100}$$

Where:
DPPH (%) = Percent reduction in DPPH
$\mathbf{OD_{control}}$ = Optical density of the negative control
$\mathbf{OD_{sample}}$ = Optical density of the sample.

The 50 % inhibition concentration (IC_{50}) is the concentration of the test sample necessary to reduce DPPH radical solution to 50 %. The IC_{50} values were determined graphically by linear or logarithmic regressions of the plots of the percentage inhibition as a function of the various final concentrations of the extracts tested [19]. This procedure was repeated three times.

2.2.4. Determination of Ions in the Aqueous Extracts of Roasted Bean Seeds of *Coffea c.* (EAGTC*c*)

Different methods were used to determine the number of ions present in our aqueous extracts (EAGTC*c*). The potassium ions (K^+) were determined by flame spectrophotometry or flame test. The titanium yellow spectrophotometric method was used to determine the presence of magnesium ions (Mg^{2+}). In addition, the complexometric method was to determine the presence of calcium ions (Ca^{2+}). Finally, the phosphorus, zinc and copper ions were determined using the absorption spectrophotometry method [20].

3. Results and Discussion

The total polyphenols and total flavonoids contents in the aqueous extracts our sample (EAGTC*c*) were determined to be 81.67 mg EAG / g and 293.33 mg EQ / g respectively (Table 1).

Table 1. Total polyphenols and total flavonoids content in the aqueous extracts of roasted bean seeds of *Coffea c.* (EAGTC*c*)

	Total Polyphenols (mg EAG/g)	Total Flavonoids (mg EQ/g)
Aqueous extract of roasted bean seeds of *Coffea c.* (EAGTC*c*)	81.67	293.33

EAG = Gallic Acid Equivalent; EQ = Quercetin Equivalent.

The total polyphenol content (81.67) determined in the aqueous extracts of roasted bean seeds of *Coffea c.* was seen to be higher than that reported from previous research done on aqueous extracts of roasted beans of *coffee robusta* (47.85 ± 0.077 mg EAG / g and 7 mg EAG / g to 42.37 mg EAG / g [21,22]. The only research that has reported higher values of total polyphenol content (561.91) compared to our result was in 2017 by Khadra obtained from aqueous extracts of green *robusta coffee* beans [23].

HPLC revealed levels of 3.7 % catechol, 5.4 % chlorogenic acid and 2.8 % caffeic acid in 100 g of dry matter of our sample (Figure 1 and Figure 2).

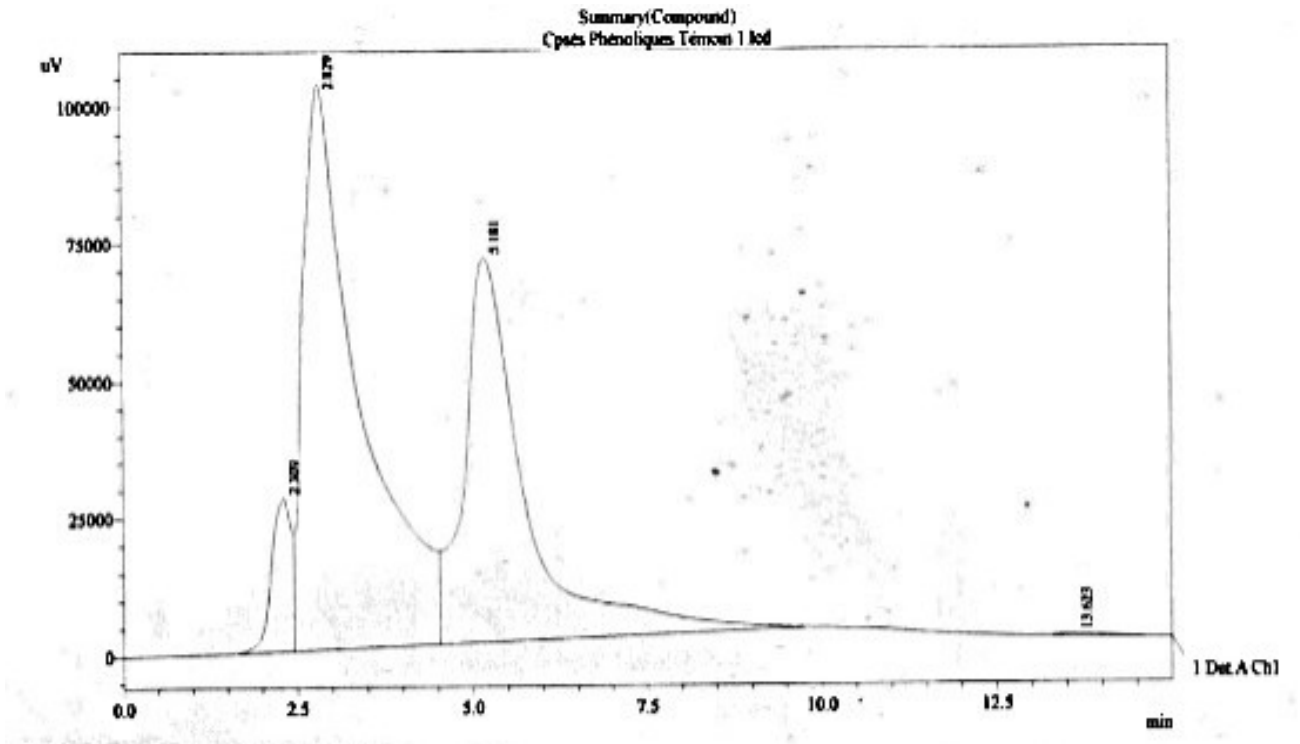

Figure 1. Chromatograms of control phenolic compounds: catechol (RT2.309), chlorogenic acid (RT2.829) and caffeic acid (RT5.181)

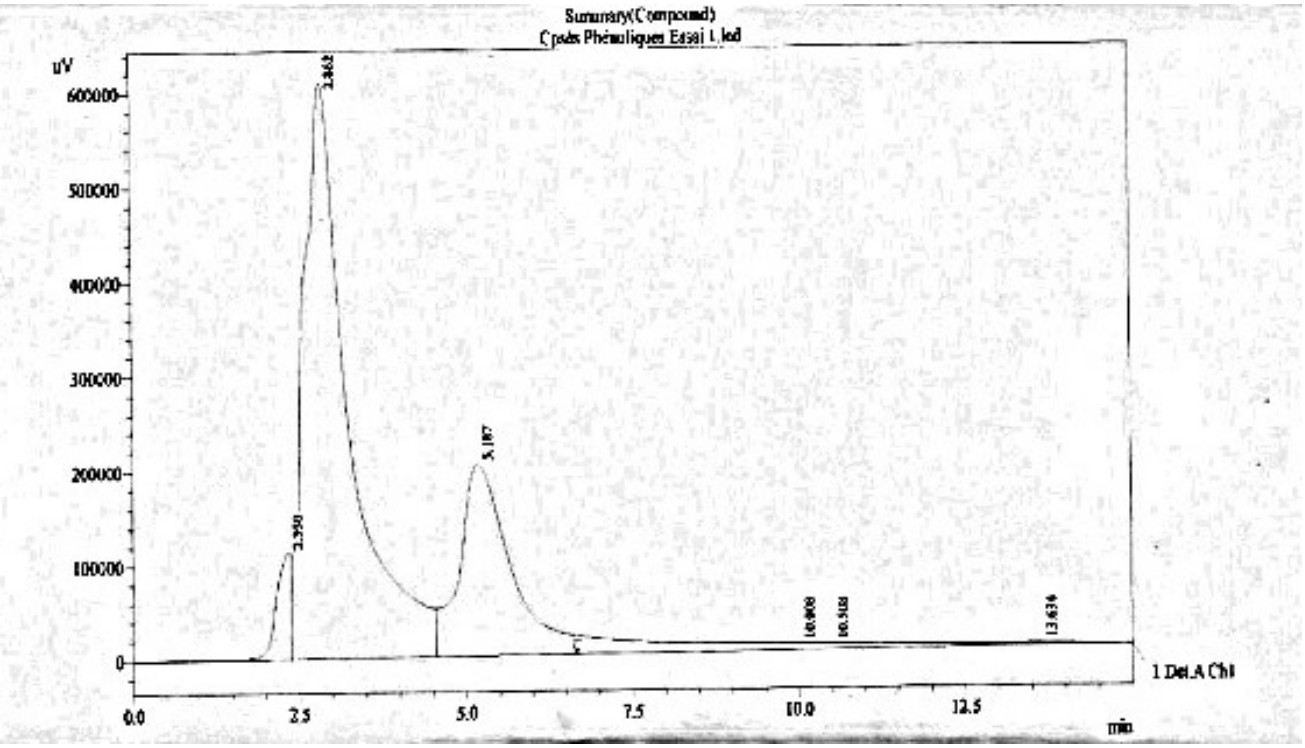

Figure 2. Chromatograms of the phenolic compounds contained in the EAGT*Cc*: catechol (RT2.330), chlorogenic acid (RT2.862) and caffeic acid (RT5.187)

From Figure 1 above, the chlorogenic acid content decrease with increase in temperature. This might be due to roasting of the seed. During roasting, chlorogen acid is decomposed into different phenol derivatives, including caffeic acid, quinic acid, catechol, pyrogallol and hydroquinone [24]. Previous research showed that during roasting, about 95 % of n-chlorogenic acid was observed at 200°C [25,26,27]. This gives the content of n-chlorogenic acid in roasted coffee beans to be between 0.5 % and 7 %. Therefore, our chlorogenic acid content after roasting of 5.4 % agrees with that obtained from previous reports [24]. However, some reports show a higher chlorogenic acid content (13.00 % - 14.73 %) after roasting of green bean seeds than that obtained in our study [28].

The concentration of EAGT*Cc* responsible for 50 % inhibition (IC_{50}) of the DPPH radical solution in our sample was higher than that of quercetin (75.54 ± 2.08 µg / mL versus 4.45 ± 1.02 µg / mL) (Table 2).

The value of IC_{50} correlated with its antioxidant power. A low IC_{50} value would reflect a higher antioxidant effect [29]. The IC_{50} value ofour extract (EAGT*Cc*) was observed to be 75.54 ± 2.08 µg / mL. This IC_{50} value is higher than that obtained with an aqueous extract of green *robusta coffee* bean seeds (51 µg / mL) [23] indicating that our extract has a higher antioxidant effect compared an aqueous extract of green *robusta coffee* bean seeds. Therefore, the antioxidant activity contained in the coffee drink is linked, among other things, to the chlorogenic, ferulic, caffeic and n-coumaric acid contentit contains [30,31]. Numerous studies have shown that polyphenols (mainly in the form of n-chlorogenic acid) and melanoidins have antioxidant activity and limit DNA degradation [32,33]. This activity could be due to the scavenging effect of the radical and/or to the chelating effects of the metal [34,35,36]. Thus, the antioxidant activity of coffee is linked to its richness in phenolic compounds [37,38]

Table 2. Evaluation of the anti-free radical activity of the aqueous extract of roasted bean seeds of *Coffea c.* (EAGT*Cc*) by 2,2-dipheny-l-picrylhydrazyl (DPPH)

	Anti-free radical activity (%)	
Concentration (µg/mL)	Quercetin	EAGT*Cc*
0	0	0
10	80.01	17.14
25	85.08	23.62
50	88.21	40.38
100	92.64	55.49
150	96.34	62.21
200	97.05	68.19

Therefore, the aqueous extract of roasted bean seeds of *Coffea c.* (EAGT*Cc*) contains 1,159.5 mg of potassium, 213.0 mg of magnesium, 106.2 mg of calcium, 100.5 mg of phosphorus, 31.0 mg of sodium, 3.3 mg of copper and 2.2 mg of zinc in 100 g of dry matter. These values correspond respectively to 1.16 % of potassium, 0.21 % of magnesium, 0.11 % of calcium, 0.10 % of phosphorus, 0.03 % of sodium, 0.003 % of copper and 0.002 % of zinc (Table 3).

Table 3. Electrolyte content in aqueous extract of roasted bean seeds of *Coffea c.* (EAGT*Cc*)

Ions	Potassium	Magnesium	Calcium	Phosphorus	Sodium	Copper	Zinc
Quantity (mg / 100g) of dry matter	1159.9	213	106.2	100.5	31	3.3	2.2

The different electrolyte contents in our extract could be due to the method of preparation of the coffee drink and the composition of the coffee bean seed used. In addition, the different electrolyte contents in our extracts could be caused environmental conditions such as the soil texture, the climate, the agricultural practices and the storage of the green coffee bean seeds. This would affect the physiology of the seeds and the chemical composition of the coffees [26,39]. The magnesium (0.212 % to 0.415 %) and calcium (0.106 % to 0.189 %) contents of our extract agrees to those in instant coffee made from a blend of robusta and arabica [40,41]. However, the contents of potassium (3.35 % to 4.76 %), phosphorus (0.223 % to 0.410 %), copper (0.005 % to 0.026 %) and zinc (0.003 % to 0.015 %) contents in our extract (EAGT*Cc*) are lower than those in coffee made from a blend of robusta and arabica.

4. Conclusion

In this study, we have successfully determine the organic content of an aqueous extract of roasted bean seeds of *Coffea c*. (EAGT*Cc*). This extract was shown to contains a good content of total polyphenols and can be used as a good antioxidant. The IC_{50} value (75.54 ± 2.08 µg / mL) was higher than that obtained with an aqueous extract of green *robusta coffee* bean seeds (51 µg / mL). In addition, the antioxidant activity of coffee could be attributed to its rich content in phenolic compounds different elements. Furthermore, these contents may vary due to environmental conditions. The antioxidant activity of coffee could be used to justify its use in preventing different diseases.

References

[1] Scalbert A, Johnson IT, Saltmarsh M. Polyphenols: antioxidants and beyond. American Journal Clinical Nutrition. 2005; 81: 215-217.

[2] Mokbel MS, Hashinaga F. Antibacterial and antioxidant activities of banana (Musa, AAA cv. Cavendish) fruits peel. Am J Biochem Biotechnol. 2005; 1(3): 125-31.

[3] Pellegrini N, Serafini M, Colombi, et al. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. J Nutr. 2003; 133: 2812-9.

[4] Fidrianny I, Windyaswari AS, Wirasutisna KR. Antioxidant capacities of various leaves extract from five colors varieties of sweet potatoes tubers using ABTS, DPPH assays and correlation with total flavonoid, phenolic, carotenoid content. Res J Med Plant. 2013; 7(3): 130-40.

[5] Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid content of some selected Iranian medicinal plants. Afr J Biotechnol. 2006; 5(11): 1142-5.

[6] Ramalakshmi K, Kubra IR, Rao LJ. Antioxidant potential of low-grade coffee beans. Food Res Int. 2008; 41: 96-103.

[7] Win M, Hamid A, Baharin B, Anwar FS, Pak-Dek MS. Phenolic compounds and antioxidant activity of peanut's skin, hull, raw kernel and roasted kernel flour. Pak J Bot. 2011; 43(3): 1635-42.

[8] Lopez-Garcia E, van Dam RM, Li Ty, et al. The relationship of coffee consumption with mortality. Ann Intern Med. 2008; 148: 904-14.

[9] Freedman ND, Park Y, Abnet CC, et al. Association of coffee drinking with total and cause-specific mortality. N Engl J Med. 2012; 366: 1891-904.

[10] Mashkor IM. Phenolic content and antioxidant activity of fenugreek seeds extract. Int J Pharmacogn Phytochem Res. 2014; 6(4): 841-4.

[11] Thaipong K, Boonprakob U, Crosby K, Zevallos LC, Byrne DH. Comparison of ABTS, DPPH, FRAP,and ORAC assays for estimating antioxidant activity from guava fruit xtracts. J Food Comp. Anal. 2006; 19: 669-75.

[12] Fidrianny I, Permatasari L, Wirasutisna KR. Antioxidant activities from various bulbs extracts, of three kinds allium using DPPH, ABTS assays and correlation with total phenolic, flavonoid, carotenoid content. Int J Res Pharm Sci. 2013; 4(3): 438-44.

[13] Butt MS, Smith MT. Coffee and its Consumption: Benefits and Risks. Critical Reviews in Food Science and Nutrition. 2011; 51: 363-373.

[14] Brezova V, Šlebodova A, Staško A. Coffee as a source of antioxidants: An EPR study. Food Chem. 2009; 114: 859-68.

[15] Vermerris W, Nicholson R. Phenolic compound Biochemistry. Springe. Dortrecht, The Netherland. 2006; 11-234.

[16] Ardestani A, Yazdanparast R. Inhibitory effects of ethyl acetate extract of *Teucrium polium* on in vitro protein glycoxidation. *Food and Chemical Toxicology*. 2007; 45: 2402-2411.

[17] Donovan JL, Manach C, Faulks RM, Kroon PA. Absorption and metabolism of dietary secondary metabolites. In: Plant Secondary Metabolites. Occurrence, Structure and Role in the Human Diet. Blackwell Publishing. Oxford (USA); 2006: 303-351.

[18] Mansouri A, Embarek G, Kokkalou E, Kefalas P. Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (*Phoenix dactylifera*). Food Chemistry. 2005; 89: 411- 420.

[19] Samarth RM, Panwar M, Kumar M, Soni A, Kumar, M, Kumar A. Evaluation of antioxidant and radical: scavenging activities of certain radioprotective plant extracts. Food chemistry. 2008; 106: 868-873.

[20] Kouassi JB, Cissé-camara M, Sess DE, Tiahou GG. Monde AA, Djohan FY. Détermination des teneurs en fer, en calcium, en cuivre et en zinc de deux variétés de gombo. Bulletin de la Société Royale des Sciences de Liège. 2013; 82: 22-32.

[21] Ivana H, Ana BC, Dunja H, Drazenka K. Comparative study of polyphenols and caffeine in different coffee varieties affected by the degree of roasting. Food Chemistry. 2011; 129: 991-1000.

[22] Anca CF, Sonia AS, Iulia B, al. Evaluation of Biofunctional Compounds Content from Brewed Cofee. Bulletin of Food Science and Technology 2014; 71: 2344-2347.

[23] Khadra B. Contribution à l'étude phytochimique et activité biologique des extraits des graines de *Coffea canephora*. Mémoire de Master en Biologie option Biochimie de l'Université Abou Bekr Belkaïd-Tlemcen, Tlemcen (Algérie). 2017: 57.

[24] Haler PNG, Le café: les effets bénéfiques et néfastes sur la santé. Thèse de doctorat d'Etat en pharmacie de l'Université de Lorraine, Lorraine (France). 2013: 175.

[25] Franca AS, Mendonça JCF, Oliveira MBPP. Composition of green and roasted coffees of different cup qualities. Elsevier. 2005; 38: 709-715.

[26] Franca AS, Oliveira MBPP, Mendonça JCF, Silva XA. Physical and chemical attributes of defective crude and roasted coffee beans. Food Chemistry. 2005; 90: 89-94.

[27] Farah A, Monteiro M, Donangelo CM, Lafay S. Chlorogenic acids from green coffee extract is highly bioavailable in humans. Journal Nutrition. 2008; 138: 2309-2315.

[28] Nemlin GJ, Irie ZB, Ban-koffi L, et al. Physico-chemical and organoleptic characteristics of robusta coffee (*coffea canephora l.*) according to terroirs and farming technics in Côte D'Ivoire. Agronomie Africaine. 2009; 21: 185-195.

[29] Mourad B, Etude de l'activité antioxydante et antimicrobienne d'extraits *d'Artemisia campestris L*. Mémoire de Magister en Biochimie de l'Université Ferhat Abbes, Sétif (Algérie). 2011: 99.

[30] Cuvelier C, Dotreppe O, Istasse L. Chimie, sources alimentaires et dosage de la vitamine E. Annales de Médecine Vétérinaire. 2003; 147: 315-324.

[31] Bee S, Brando CHJ, Brumen G, et al. The Raw Seed. In: Espresso Coffee, the Science of Quality. Proceeding of the 8th International Symposium on coffee, 2004: 25-28 November 2006. Salvador. Brazil. 87-178.

[32] Del Castillo MD, Ames JM. Effect of roasting on the antioxidant activity of coffee brews. Journal of Agricultural and Food Chemistry. 2002; 50: 3698-3703.

[33] Morales FJ. 2005. Assessing the non-specific hydroxyl radical scavenging properties of melanoidins in a Fenton-type reaction system. Analytica Chimica Acta. 2005; 534: 171-176.

[34] Rufian-Henares JA, Morales FJ. Effect of in vitro enzymatic digestion on antioxydant activity of coffee melanoidins and antihypertensive activities. Food Research International. 2007; 40: 995-1002.

[35] Gniechwing D, Reichard N, Ralph J, Blaud M, Steinhart H, Bunzed M. Isolation and caracterisation of a coffee melanoidin fraction. The Journal of the Science of Food and Agriculture. 2008; 88: 2153-2160.

[36] Vitaglione P, Morisco F, Mazzone G, et al. Coffee reduces liver damage in a rat model of steatohepatitis: the underlying mechanisms and the role of polyphenols and melanoidins. Hepatology. 2010; 52: 1652-1661.

[37] Higdon JV, Frei B. Coffee and health. A review of recent human research. Critical Review of Food Sciences and Nutrition, 2006; 46: 101-123.

[38] Kamel A. Les stilbénoïdeschez les Cypéracées : Isolation, identification et étude de leurs activités biologiques.Identification et dosage des stilbènes dans des vins Tunisiens. Thèse de doctorat en Nutrition de l'Université de bordeaux, bordeaux (France). 2014: 177.

[39] Perrone D, Neves YP, Brand~ao JM, Martinez HEP, Farah A. Influence de la fertilisation de zinc sur les acides chlorogénique et l'activité antioxydant des graines de café. 22 ème Conférence international sur le café, les 14-19 Septembre, Campinas (Brazil). 2008: 220-223.

[40] Dos Santos EJ, De Oliveira E. Determination of Mineral Nutrients and Toxic Elements in Brazilian Soluble Coffee by ICP-AES. Journal of Food Composition and Analysis. 2001; 14: 523-531.

[41] Krejová A, Cernohorský T. The determination of boron in tea and coffee by ICP–AES method. Food Chemistry. 2003; 82: 303-308.