

PHYTOCHEMICAL STUDIES ON GREEN COFFEE BEAN (COFFEE ARABICA)

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ABSTRACT

The consumption of coffee (roasted) all over the world is second only to water. Green coffee bean generally referred to as unroasted coffee beans has been used as a weight loss supplement and for other medicinal purposes. The unroasted coffee beans were air dried and powdered for this study. The extract was subjected to chemical tests to determine the phytochemical constituents using standard procedures. Qualitatively, alkaloids, saponins, tannins, flavonoids, terpenoids, steroids, proteins, starch and phenols were identified while flavonoids, alkaloids, tannins and saponins were quantitatively determined and found to measure 6.0%, 4.4%, 4.2% and 1.0% respectively. These findings give credence to its use in therapies and in drug production.

KEYWORD: Coffee, phytochemicals, qualitative, quantitative.

INTRODUCTION

Coffee has been established as the most consumed drink all over the world after water. Consumption of coffee is around 255 kg per second or 8 million tonnes per year worldwide.^[1] The family of coffee is *Rubiacea* and belongs to the genus *Coffea*. In all over the world, there are more than 80 coffee species identified.^[2] Despite this enormous diversity, only two species are actually of economic and therapeutic importance in the world market, *Coffea arabica* L. and *Coffea canephora* Pierre.^[3]

Green coffee bean extracts are taken as weight loss supplement and often marketed under different brand names.^[4,5] Due to antioxidant activity property of the green coffee extract, it has been found to reduce the risk of cancer, diabetes and liver disease. Green coffee extract

can also be used against Parkinson's disease and reduces blood pressure.^[6,7] Chlorogenic acid and Caffeic acid a component of coffee bean extract have been reported to possess anti-hepatitis B virus activity and also a significant antiviral effects against several viruses, including HIV, Adenovirus, and Herpes Simplex virus.^[8,9,10,11] Chlorogenic acid has also been shown to exert antifungal property on *Aspergillus* genus as *Aspergillus flavidus*, *Aspergillus nominus*, *Aspergillus ochraceus*, *Aspergillus parasiticus* and *Aspergillus westerdijkiae*.^[12] This research work is therefore tailored to assess the bioactive phytochemicals in *coffea arabica*.

MATERIALS AND METHOD

This study was conducted in the Department of Pharmacology and Toxicology, College of Pharmacy, Nnamdi Azikiwe University Agulu Campus.

Plant collection

Unroasted green coffee beans (*Coffea arabica*) were purchase from a local market in Lagos, Nigeria. The beans was authenticated by a botanist and deposited in the herbarium of the department of Botany, Nnamdi Azikiwe University, Awka. The beans used for the quantitative and qualitative screening was finely powered with a mechanical grinder.

Qualitative phytochemical screening

1. Test for Alkaloids – 5g of the powdered coffee bean was placed in a test tube and 20ml methanol poured/ added into it. The mixture was allowed to boil for 2 minutes in water bath, cooled and filtered. Two drops of Dragendoff's reagent was added to 2ml of filtrate; two drops of Wagner's reagent was added to 5ml of the filtrate; two drops of Meyers reagent was added to another 2ml of filtrate and two drops of Hager's reagent was added to a 5ml of filtrate. Alkaloids precipitated from the above solutions while giving off different characteristic colours – reddish brown, reddish brown, cream and yellow respectively.
2. Test for Saponin – 2g of the powdered coffee bean sample was boiled in 20ml of distilled water in water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with three drops of olive oil and shaken vigorously, then observed for the formation of emulsion.
3. Test for Tannin – 0.5g of the powdered coffee bean sample was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black colouration.

4. Test for flavonoid – 10ml of ethyl acetate was added to about 0.2g of the powdered coffee bean and heated on a water bath for 3 minutes. The mixture was cooled, filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. The layers are allowed to separate and the yellow colour in the Ammonical layer indicates the presence of flavonoids.
5. Test for Terpenoids: 5ml of the powdered coffee bean were mixed in 2ml of chloroform and 3ml concentrated sulphuric acid which was carefully added to form a layer. A reddish brown colour interface was formed to show positive results for Terpenoids.
6. Test for steroids – A 9ml portion of ethanol was added to 1g of the powdered coffee bean. This was refluxed for a few minutes and filtered. The filtrate was concentrated to 2.5ml on a boiling water bath and 5ml of hot water added. The mixture was allowed to stand for an hour and the waxy matter filtered off. The filtrate was extracted with 2.5ml of chloroform using a separating funnel. To 0.5ml of the chloroform extract in a test tube, 1ml of concentrated sulphuric acid was added to form a lower layer. A reddish brown interface shows the presence of steroids.
7. Test for protein – few drops of picric acid was added to a little portion of the aqueous extract of the coffee bean. A yellow precipitate indicates the presence of proteins.
8. Test for starch: 0.1g of the powdered plant was mixed with a drop of iodine solution in a test tube. A blue-black coloration indicates the presence of starch.

Quantitative determination of phytochemical constituents

1. Alkaloid quantification was done using a method by Harborne (1973):^[13] 5g of the powdered coffee bean was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added, covered and then allowed to stand for 4hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected, washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid which was dried and weighed.
2. Saponin quantification following the method by (Obadoni and Ochuko, 2001):^[14] The 20g of powdered coffee bean sample was placed into a conical flask and 100cm³ of 20% aqueous ethanol was added. This was heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue re- extracted with another 200ml 20% ethanol. The combined extracts were reduced to 40ml over

water bath at about 90°C. The concentrate was transferred into a 250ml separating funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while discarding the ether layer. The purification process was repeated. 60ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride and the remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight.

3. Flavonoid quantification using Bohrn and Kocipal-abyazan (1994) method:^[15] 10g of the powdered coffee bean was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatmann filter paper No 42. The filtrate was later transformed into a crucible and evaporated into dryness over a water bath to a constant weight.
4. Tannin quantification by Van- burden and Robinson (1981) method:^[16] 500mg of the powdered coffee bean was weighed into a 50ml plastic bottle. 50ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the filtrate was pipetted out into a test tube and mixed with 2ml of 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 605 nm within 10mins.

The flavonoid, alkaloid and saponin content were calculated as percentage using the formula:

$$(\text{Final weight obtained (in grams)} \times 100) \div (\text{Initial dried sample used (in grams)})$$

The percentage tannin is calculated thus:

$((A_n \div A_s) \times C \times (100 \div 10) \times (V_f \div V_a))$ where

A_n = Absorbance of test sample; A_s = Absorbance of Standard Solution

C = Concentration of Standard Solution; V_f = Volume of Extract used; V_a = Volume of Extract Analysed

RESULTS

The result of this study revealed the presence of active phytochemicals in coffee bean (*Coffea arabica*) as summarized in tables 1.0 and 2.0. The qualitative estimation of the *Coffea arabica* saw the presence of alkaloid, saponin, tannin, flavonoid, terpenoid, steroids, protein, starch and phenols either in mildly present and abundantly present measure.

Quantitative analysis (in percentages) of four active constituent of *Coffea Arabica* yielded a high content of flavonoids (6.0%), alkaloids (4.4%) and tannins (4.2%) with a low content of saponins (1.0%) (Table 2.0).

Table 1: Qualitative analysis of Coffea arabica constituent.

Phytochemicals	Alkaloid	Saponin	Tannin	Flavonoid	Terpenoid	Steroid	Protein	Starch	Phenol
Quantity	++	++	++	++	++	++	++	+	++

Key: + = mildly present; ++ = abundantly present

Table 2: Quantitative analysis of Coffea bean constituent.

Phytochemical	Quantity (%)
Alkaloids	4.4
Saponins	1.0
Flavonoid	6.0
Tannins	4.2

A table showing the percentages of different bioactive phytochemicals in the coffee extract.

DISCUSSION

Medicinal plants are the main source for the preparation and extraction of various modern drugs and pharmaceuticals. Recently using of phytochemicals is considered to be safer and congenial to the biology of the human body while producing specific physiological action.^[17]

The phytochemical constituents of *Coffea arabica* grown in Nigeria are presented in Table 1.0 above. It is seen to contain alkaloids, flavonoids, saponins, steroids, starch, tannins, proteins, terpenoids and phenols as bioactive phytochemicals. Studies have reported that the isoquinoline alkaloid extract from *Fumaria capreolata* exerts antioxidant, analgesic, and intestinal anti-inflammatory activities in DNBS model of experimental colitis in mice.^[18,19,20]

It has been also shown to have strong anti-cough effect. In agreement to this research, literature shows that saponins exhibit a biological role and medicinal properties such as anti-inflammatory, antibacterial, insecticidal, anticancer and cytotoxic effect. In addition, saponins are reported to exhibit cholesterol-lowering action in animals and human.^[21,22,23,24,25,26,27]

Substantial pharmacological properties of flavonoids comprise radical scavenging, anti-inflammatory, anticancer and cardioprotective effects.^[28] Naringenin (a flavonone) may be used for treating dyslipidemia and improving endothelial dysfunctions.^[29] They have therapeutic effects in cardiovascular diseases, certain types of cancer, visual health, diabetes mellitus, obesity, and neurological disorders.^[30] Tannins possess some biological properties such as anti-inflammatory and antiallergic effects, anthelmintic, and antiviral effects against enteric virus, herpes simplex virus, poliovirus, etc.^[31,32,33]

Chlorogenic acid in green coffee bean has been noted to reduce body weight and BMI, lower the risk of neurodegenerative diseases and cancer, and effective against the development of Porcine Reproductive Respiratory Syndrome Virus (PRRSV).^[34,35,36,37] This therefore validates the presence of bioactive medicinal phytochemicals in *coffea arabica*.

CONCLUSION

Green coffee bean extract of coffee arabica has been scientifically and globally acknowledged and taken as a weight loss supplement and has been found to be effective against some other disease conditions. The phytochemicals found and noted in this study shows the plant's potency in use in the pharmaceutical as a source of bioactive components in drug production.

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REFERENCES

1. International Coffee Organization, (2018). Annual review; 2017/18, London.
2. Clarke RJ. Coffee: green coffee/roast and ground. In: Encyclopaedia of Food Science and Nutrition. 2nd edition, Caballero, B., Trugo, L. C., Finglas, P., eds. Academic Press: Oxford., 2003.
3. Anthony F, Combes MC, Astorga C, Bertrand B, Graziosi G, Lashermes P. The origin of cultivated *Coffea arabica* L. varieties revealed by AFLP and SSR markers. *Theor. Appl. Genet*, 2002; 104(5): 894-900.
4. Shimoda H, Seki E, Aitani M. Inhibitory effect of green coffee bean extract on fat accumulation and body weight gain in mice. *BMC Complement Altern. Med.*, 2006; 6: 9.
5. Cho AS, Jeon SM, Kim MJ, Yeo J, Seo KI, Choi MS, Lee MK. Chlorogenic acid exhibits anti-obesity property and improves lipid metabolism in high-fat diet-induced-obese mice. *Food Chem. Toxicol.*, 2010; 48: 937–943.
6. Bhupathiraju, SN, Pan A, Malik VS, Manson JE, Willett WC, van Dam RM, Hu FB. Caffeinated and caffeine-free beverages and risk of type 2 diabetes. *Am. J. Clin. Nutr.*, 2013; 97: 155–166.
7. Suzuki A, Kagawa D, Ochiai R, Tokimitsu I, Saito I. Green coffee bean extract and its metabolites have a hypotensive effect in spontaneously hypertensive rats. *Hyperten. Res.*, 2002; 25: 99–107.

8. Wang X, Fan X, Yuan S, Jiao W, Liu B, Cao J, Jiang W. Chlorogenic acid protects against aluminium-induced cytotoxicity through chelation and antioxidant actions in primary hippocampal neuronal cells. *Food and Funct*, 2017; 8(8): 2924–2934.
9. Tamura H, Akioka T, Ueno K, Chujyo T, Okazaki K, King OJ, Robinson Jr WE. Anti-human immunodeficiency virus activity of 3,4,5-tricaffeoylquinic acid in cultured cells of lettuce leaves. *Mol. Nutr. Food Res.*, 2006; 50: 396–400.
10. Chiang LC, Chiang W, Chang MY, Ng LT, Lin CC, Antiviral activity of *Plantago major* extracts and related compounds in vitro. *Antiviral research*, 2002; 55: 53–62.
11. Khan MT, Ather A, Thompson KD, Gambari R. Extracts and molecules from medicinal plants against herpes simplex viruses. *Antiviral Res.*, 2005; 67: 107–119.
12. Suarez-Quiroz ML, Taillefer W, Lopez-Mendez EM, Gonzalez-Rios O, Villeneuve P, Figuero-Espinoza MC, (2013). Antibacterial activity and antifungal and anti-mycotoxigenic activities against *Aspergillus flavus* and *Aspergillus ochraceus* of green coffee chlorogenic acids and dodecyl chlorogenates. *J. Food Saf.*, 2013; 33: 360–368.
13. Harbone JB. *Phytochemical methods*, London Chapman and Hall ltd., 1973; 49-188.
14. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta states of Nigeria. *Global J Pure Appl. Sci.*, 2001; b: 203-208.
15. Boham BA, Kocipai-Abyazan R. Flavonoids and Condensed Tannins from Leaves Of Hawaiian *Vaccinium vaticulatum* And *Calycinium*. *Pacific Sci.*, 1974; 48: 458-463.
16. Van-Burden TP, Robinson WC. Formation of complexes between protein and tannic acid. *J. Agric Food Chem.*, 1931; 1: 77.
17. El Aziz MMA, Ashour AS, Melad ASG. A review on saponins from medicinal plants: chemistry, isolation, and determination. *J Nanomed Res.*, 2019; 7(4): 282–288.
18. Bribi N, Bouguezza Y, Maiza F. Evaluation of erythrocytes toxicity and antioxidant activity of alkaloids of *fumaria capreolata*. *Inter J of Pharma and Bio Sci.*, 2013; 4: 770-776.
19. Bribi N, Algieri F, Rodriguez-Nogales A, et al. Anti-nociceptive and anti-inflammatory effects of total alkaloid extract from *fumaria capreolata*. *eCAM*, 2015; 736895(7): 1-8.
20. Bribi N, Belmouhoub M, Maiza F. Analgesic and anti-inflammatory activities of ethanolic extract of *Fumaria capreolata*. *Phytothérapie*, 2017; 15(4): 211-216.
21. Just MJ, Recio MC, Giner RM, et al. Anti-inflammatory activity of unusual lupane saponins from *Bupleurum fruticosens*. *Planta Medica*, 1998; 64(5): 404–407.

22. Sparg SG, Light ME, van Staden J. Biological activities and distribution of plant saponins. *J. Ethnopharmacol.*, 2004; 94(2–3): 219–243.
23. De Geyter E, Lambert E, Geelen D, et al. Novel advances with plant saponins as natural insecticides to control pest insects. *Pest Technol*, 2007; 1(2): 96–105.
24. Cheng TC, Lu JF, Wang JS, et al. Antiproliferation effect and apoptosis mechanism of prostate cancer cell PC-3 by flavonoids and saponins prepared from *Gynostemma pentaphyllum*. *J Agric Food Chem.*, 2011; 59(20): 11319–11329.
25. Mbaveng AT, Ndontsa BL, Kuete V, et al. A naturally occurring triterpene saponin ardisiacrispin B displayed cytotoxic effects in multi-factorial drug resistant cancer cells via ferroptotic and apoptotic cell death. *Phytomedicine*, 2018; 43(1): 78–85.
26. Oboh HA, Omofoma CO. The effects of heat treated lima beans (*Phaseolus lunatus*) on plasma lipids in hypercholesterolemic rats. *Pak J Nutr.*, 2008; 7(5): 636–639.
27. Moghimipour E, Handali S. Saponin: properties, methods of evaluation and applications. *Annu. Res. Rev. Biol.*, 2015; 5(3): 207–220.
28. Khan MK, Dangles O. A comprehensive review on flavanones, the major citrus polyphenols. *J Food Compos Anal*, 2014; 33: 85–104.
29. Erlund I. Review of the flavonoids quercetin, hesperetin, and naringenin. Dietary sources, bioactivities, bioavailability, and epidemiology. *Nutr Res.*, 2004; 24: 851–874.
30. Khoo HE, Azlan A, Tang ST, Lim SM. Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food Nutr Res.*, 2017; 61: 1361779.
31. Ghosh D. Tannins from foods to combat diseases. *Int. J. Pharm Sci. Rev. Res.*, 2015; 4(5): 40–44.
32. Ketzis JK et al. Evaluation of efficacy expectations for novel and non-chemical helminth control strategies in ruminants. *Vet. Parasitol.*, 2006; 139(4): 321–335.
33. Ashok PK and Upadhyaya K. Tannins are astringent. *J. Pharmacogn. Phytochem.*, 2012; 1(3): 45–50.
34. Roshan H, Nikpayam O, Sedaghat M, Sohrab G. Effects of green coffee extract supplementation on anthropometric indices, glycaemic control, blood pressure, lipid profile, insulin resistance and appetite in patients with the metabolic syndrome: A randomised clinical trial. *Brit. J. Nutr.*, 2018; 119: 250–258.
35. Miao M, Cao L, Li R, Fang X, Miao Y. Protective effect of chlorogenic acid on the focal cerebral ischemia reperfusion rat models. *Saudi Pharm J.*, 2017; 25: 556–563.

36. Suzuki A, Kagawa D, Fujii A, Ochiai R, Tokimitsu I, Saito I. Short- and long-term effects of ferulic acid on blood pressure in spontaneously hypertensive rats. *Am. J. Hyperten*, 2002; 15: 351-357.
37. Bello-Onaghise G, Wang G, Han X. Antiviral strategies of Chinese herbal medicine against PRRSV infection. *Front. Microbiol*, 2020; 11: 1756.