

Full Length Research Paper

Quantification of some phenolics acids and flavonoids in *Cola nitida*, *Garcinia kola* and *Buchholzia coriacea* using high performance liquid chromatography- diode array detection (HPLC-DAD)

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Received 25, September, 2019; Accepted 9 December, 2019

Kola seeds are highly valued in most African communities due to their medicinal benefits and socio-cultural application during ceremonies. Apart from their neuro-stimulatory effect, the other health benefits such as antioxidant activities which are closely associated with the level of phenolic constituents have also been widely reported. The main purpose of this study is to determine the quantity of quercetin, caffeic acid, gallic acid, ferulic acid, rutin and chlorogenic acid in *Cola nitida*, *Garcinia kola* and *Buchholzia coriacea* using HPLC-DAD. Ethanolic extracts of the selected kola plants was obtained by cold maceration and analysed by HPLC-DAD in accordance with standard methods. The regression coefficient (r^2) from the calibration curve for caffeic acid = 0.999, chlorogenic acid = 0.998, gallic acid = 0.999, ferulic acid = 0.998, quercetin = 0.996 and rutin = 0.997. The repeatability gave % RSD of 0.6, 1.63, 0.44, 1.55, 3.65 and 4.67 for caffeic acid, chlorogenic acid, gallic acid, ferulic acid, quercetin and rutin respectively. The quantity of these compounds in *C. nitida* was caffeic acid (101.24 mg/g), chlorogenic acid (36.35 mg/g), gallic acid (16.99 mg/g) ferulic acid (1.47 mg/g) while quercetin and rutin were not detected. In *Garcinia kola*, caffeic acid was (0.84 mg/g), gallic acid (1.02 mg/g), ferulic acid (21.83 mg/g), quercetin (53.24 mg/g), rutin (0.49 mg/g) and chlorogenic acid was not detected. *B. coriacea* had caffeic acid (1.03 mg/g), chlorogenic acid (0.33 mg/g), gallic acid (1.07 mg/g) while ferulic acid, quercetin and rutin were not detected. Using this analytical method, the quantities of some phenolics and flavonoids compounds were determined, and the most abundant compound in the three species of kola was caffeic acid in *C. nitida* and quercetin in *Garcinia kola*. This study also showed that *C. nitida* contains high amounts of phenolics compounds as compare to the other species of kola seeds investigated in the study.

Key word: Phenolic acids, flavonoids, high-performance liquid chromatography- diode-array detector (HPLC-DAD), *Cola nitida*, *Garcinia kola*, *Buchholzia coriacea*.

INTRODUCTION

Cola nitida (kola nut) belongs to the plant family sterculiaceae, it grows as a tree. It is believed that kola nut originates from Ghana and Ivory Coast. There are

over fifty species of Kola plant but only seven have edible nuts (Okoli et al., 2012). Kola nut contain large amount of caffeine and threobromine hence, its use as stimulant.

Kola nut generate a strong sense of euphoria and well-being, enhances alertness and physical energy, its elevate mood, increase tactile sensitivity, restrain appetite and hunger; it is also use as aphrodisiac. Kola nut is used to treat whooping cough and asthma because the caffeine present in it acts as a bronchodilator expanding the bronchial air passage (Odebunmi et al., 2009).

Garcinia cola (bitter kola) also called wonder nut belongs to the family Clusiaceae/Guttiferae. It grows mostly in coastal rain forest in south Western and south Eastern part of Nigeria. Bitter kola also contains caffeine and threobromine. It is also an aphrodisiac. It is believed that bitter kola cleans the digestive system, without side effect; hence, it is use in the treatment of abdominal problems (Odebunmi et al., 2009). Bitter kola contains flavonoids and other phenolics compounds. It is used as an anti-inflammatory agent; it also serves as raw material in pharmaceutical industries (Mazi et al., 2013).

Buchholzia coriacea (wonderful kola) was named after R.W Buchholz who first collected the plant in Cameroon in the late 19th century. The seeds of the plant earn it a common name "wonderful kola" because of its wide usage in traditional medicine. It is an evergreen, small to medium-sized tree growing up to 20 m tall (Erhirhie et al., 2015). It belongs to the family capparaceae juss. It grows mostly in Cameroon, Ivory Coast and other part of the world. Wonderful kola has numerous medicinal benefits and each part of the plant is use for treatment of ailments and diseases such as ulcer, hypertension, diabetes, malaria, cough to mention but a few (Owonikoko et al., 2015).

Seeds of kola nut (*Cola nitida*), bitter kola (*Garcinia cola*) and wonderful kola (*B. coriacea*) are well appreciated in Africa, for their socio-cultural, nutritive and medicinal values. However, these three species of kola has numerous pharmacological activities such as antioxidant, anti-cancer, anti-diabetic, anti-inflammatory, anti-hypertensive, anti-viral, cardiovascular activity, antimicrobial just to mention but a few (Zailani et al., 2016; Buba et al., 2016; Izah et al., 2018). And the numerous health benefits of these plants is due to the amount of important chemical compounds found in them such as phenolic acids, flavonoids and others (Duru et al., 2012; Ibrahim and Fagbohun, 2014). Some of these phenolic acids include caffeic acid, chlorogenic acid, gallic acid, ferulic acid and the flavonoids are Quercetin and Rutin. In a previous study, (Nyamien et al., 2017) reported that cola nitida (kola nut), contain caffeic acid, chlorogenic acid and others compounds, while quercetin and other compounds were reported in *G. kola* (*Bitter kola*) (Buba et al., 2016). However, there is paucity of

data on the presence of these phenolics in *B. coriacea* (wonderful kola), secondly the amount of these compounds present in each of this kola are not documented. This study is design to quantify quercetin, caffeic acid, gallic acid, ferulic acid, rutin and chlorogenic acid in *C. nitida*, *G. kola* and *B. coriacea* to determine the amount of these compounds presents in each of the species and to compare the quantity of the compounds among the species using a high-performance liquid chromatography (HPLC).

EXPERIMENTAL

Sample collection, identification and preparation

The seeds of *C. nitida*, *G. kola* and *B. Coriacea* were purchased from Karmo market in Abuja, Federal capital territory, Nigeria. The seeds were identified and authenticated in the Herbarium unit, department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria. The nuts were sliced into pieces to enhance drying. The sliced pieces were air dried for two weeks and crushed into powder using mortar and pestle.

Preparation of extract solutions

The dried powder seeds of *C. nitida* (26.0895 g), *G. kola* (25.3748 g) and *B. coriacea* (24.0872 g) were macerated with 100 mL of absolute ethanol each for the period of 48 hours. The sample solutions were filtered through Whatmann No. 1 filter paper. After which filtrate was transferred into a clean beaker and allow to evaporate to dry at room temperature. 0.2 g of the dried extracts was accurately weighed, transferred in to a volumetric flask and made up with 10 mL absolute ethanol. The final solution was passed through a 0.45 µm membrane filter prior to use. An aliquot of 10 µl of each sample solution was injected into the HPLC system for analysis.

Preparation of standard solutions

Reference compounds of quercetin, caffeic acid, gallic acid, ferulic acid, rutin and chlorogenic acid were all purchased from Sigma-Aldrich Germany. Suitable amount of the six reference compounds were accurately weighed, then dissolved in ethanol. The mixed stock solution contained 500 µg/ml of caffeic acid, 250 µg/ml of chlorogenic acid, 250 µg/ml of gallic acid, 250 µg/ml of quercetin, 500 µg/ml of rutin and 550 µg/ml of ferulic acid. After filtered, the reference substance solution was directly injected into the HPLC.

Apparatus and chromatographic conditions

The HPLC analysis was carried out on a Shimadzu HPLC system comprising Ultra-Fast LC-20AB prominence equipped with SIL-20AC autosampler; DGU-20A3 degasser; SPD20A UV-diode array detector (UV-DAD); column oven CTO-20AC, system controller CBM-20Alite and Windows LC solution software

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Table 1. The calibration curve.

Compound	Linear equation	Correlation coefficient (R ²)	Linear range (µg/ml)
Caffeic acid	Y = 11103x-15442	0.999	25-500
Chlorogenic acid	Y = 5586.5x+196030	0.998	7.812-250
Gallic acid	Y = 9248.1x+39417	0.999	10-250
Ferulic acid	Y = 6301x+29944	0.998	10-550
Quercetin	Y = 12819x+37596	0.996	60-250
Rutin	Y = 7892x+67898	0.997	3-500

Table 2. Precision and reproducibility.

Compound	Precision n=6		Repeatability n=6	
	Concentration (µg/ml)	%RSD	Concentration (µg/ml)	%RSD
Caffeic acid	500	0.64	500	0.77
Chlorogenic acid	500	1.63	500	1.05
Gallic acid	500	0.44	500	1.23
Ferulic acid	275	1.55	275	2.21
Quercetin	500	6.65	500	3.77
Rutin	500	4.67	500	1.98

(Shimadzu Corporation, Kyoto Japan); column, VP-ODS 5µm and dimensions (150 × 4.6 mm). A binary gradient elution system composed of 0.1% formic acid in acetonitrile as solvent A and 0.1% formic acid in HPLC grade water as solvent B. (Adamu et al., 2018). The gradient elution is as follows: 0-10 min, 18-20% A; 10-25 min, 20-30% A; 25-45 min, 30-70% A; 45-46 min, 70-80% A; 46-50 min, 80% A. The mobile phase flow rate was 0.8 ml/min, and column temperature was maintained at 40 °C. The DAD detector was set at 254 nm.

Validation of HPLC method

The HPLC method was validated in terms of linearity and precision according to ICH guidelines (ICH Topic Q2B, 1996). The calibration curve was constructed by running several mixed standards of different concentrations in triplicate. The correlation coefficient was determined using a linear regression model. The precisions and reproducibility of HPLC peak are measurements of quercetin, caffeic acid, gallic acid, ferulic acid, rutin and chlorogenic acid were calculated as the relative standard deviations (RSDs) of six repeated runs and six replicate runs.

RESULTS AND DISCUSSION

UPLC method validation of quantitative analysis

Linearity and detection limit

The linearity for six phenolics was established by plotting the peak area (Y) versus concentration (x) of each which was expressed by the equation given in Table 1. The linearity of all the calibration curves showed good linear

regression coefficients between ($r^2 = 0.996-0.999$) within test ranges.

Precision and reproducibility

Method precision and reproducibility were evaluated by the analysis of six injections of the same sample solution and injection of six replicates of the same sample, respectively. The percentage relative standard deviations (%RSDs) of peak area (PA) of characteristic peaks in the precision test were found in the range of 0.44-6.65%, whereas in the reproducibility test the RSDs of PA were also between 0.77 and 3.77%, respectively. All results indicated that the method of HPLC fingerprint analysis was valid and satisfactory.

As shown in Table 2, the precisions and reproducibility based on peak area and retention time measurements of six components were found to be satisfactory for all the target phenolics. All these illustrated that UPLC-DAD method was precise, and sensitive enough for simultaneous quantitative determination of quercetin, caffeic acid, gallic acid, ferulic acid, rutin and chlorogenic acid. The entity and content of bioactive components of medicinal plants are always highly variable. Considering the variety of the components in the seeds of kola nut (*C. nitida*), bitter kola (*G. cola*) and wonderful kola (*B. coriacea*) and in order to give the most chemical information of these plants, HPLC provide a suitable technique which can perform both qualitative and quantitative analysis of the bioactive compounds in these

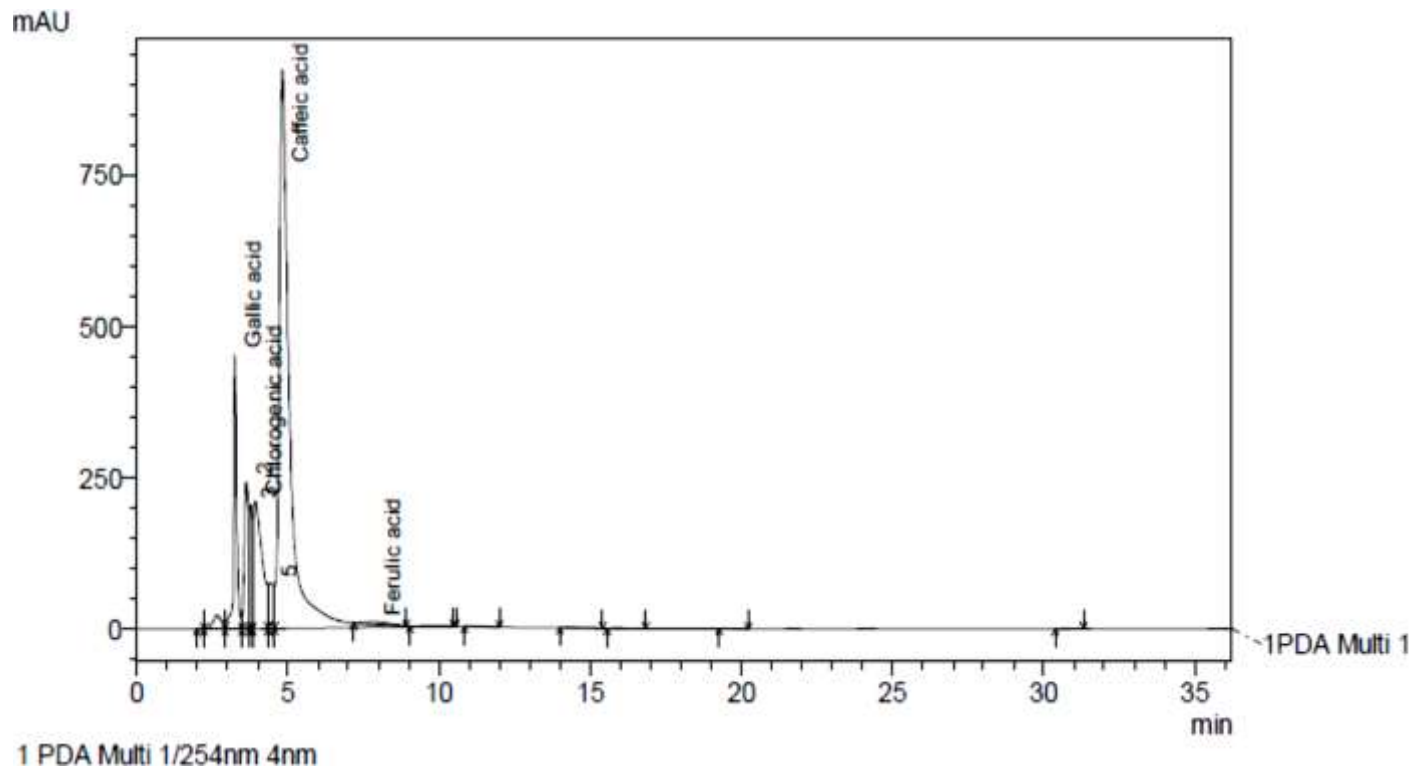


Figure 1. HPLC chromatogram of *C. nitida*.

plants. The HPLC profile of all the components in the seeds of kola nut (*C. nitida*), bitter kola (*G. cola*) and wonderful kola (*B. coriacea*) was established. Bioactive compounds which are previously reported to show good antioxidant properties including: quercetin, caffeic acid, gallic acid, ferulic acid, rutin and chlorogenic acid were quantified in each of the samples.

To provide information of the bioactive compounds in these kolas and for best separation in the chromatograms, the mobile phase and its flow rate, conditions for elution, column temperature and detection wavelength were investigated in this study. Variation in the ratio of water to acetonitrile in the mobile phase provided improvement in separation. In a previous study, Xu et al. (2009), observed that acetonitrile/water mobile phase system achieved better resolutions for phenolic acids. In addition the retention behavior of phenolic acids on the reversed-phase HPLC column was significantly affected by the pH of the mobile phase. So the addition of 0.1% formic acid into mobile phases helped to achieve a good baseline and satisfactory resolution of the phenolics. The detection wavelength was set at 254 nm because most of the characteristic components have satisfactory sensitivity at this UV wavelength. The HPLC conditions developed in this study produced insight into the fingerprint pattern of the different kolas. The chromatographic peaks were identified by comparing their retention time and UV spectrum with that of each

reference compound, which was eluted in parallel with a series of mobile phases.

Quantitative analysis of the six phenolics in the samples

Quercetin, caffeic acid, gallic acid, ferulic acid, rutin and chlorogenic acid are known phytochemical constituents proven to be good natural antioxidants. It could be seen from Figures 1 to 3 that the investigated phenolics and other compounds in these kolas were separated using the developed UPLC-DAD method. A total of 7, 12 and 15 characteristic peaks were identified in the HPLC profile of *C. nitida*, *G. cola* and *B. coriacea* respectively. Six of the characteristic peaks were assigned by comparing the UV spectra and their retention time with those of the reference compounds. In *C. nitida* peak 1, 4, 6, and 7 were identified as gallic acid, chlorogenic acid, caffeic acid and ferulic acid. Peaks 1, 5, 7, 8 and 10 were identified as gallic acid, caffeic acid, rutin, ferulic acid and quercetin in *G. cola* and for *B. coriacea* chromatogram (Figure 3) peak 2, 4, 5, 7 and 9 were identified as gallic acid, chlorogenic acid, caffeic acid, rutin and ferulic acid respectively.

The quantitative analysis data (Table 3) showed that the quantity of these compounds in *C. nitida* is caffeic acid (101.24 mg/g), chlorogenic acid (36.35 mg/g), gallic

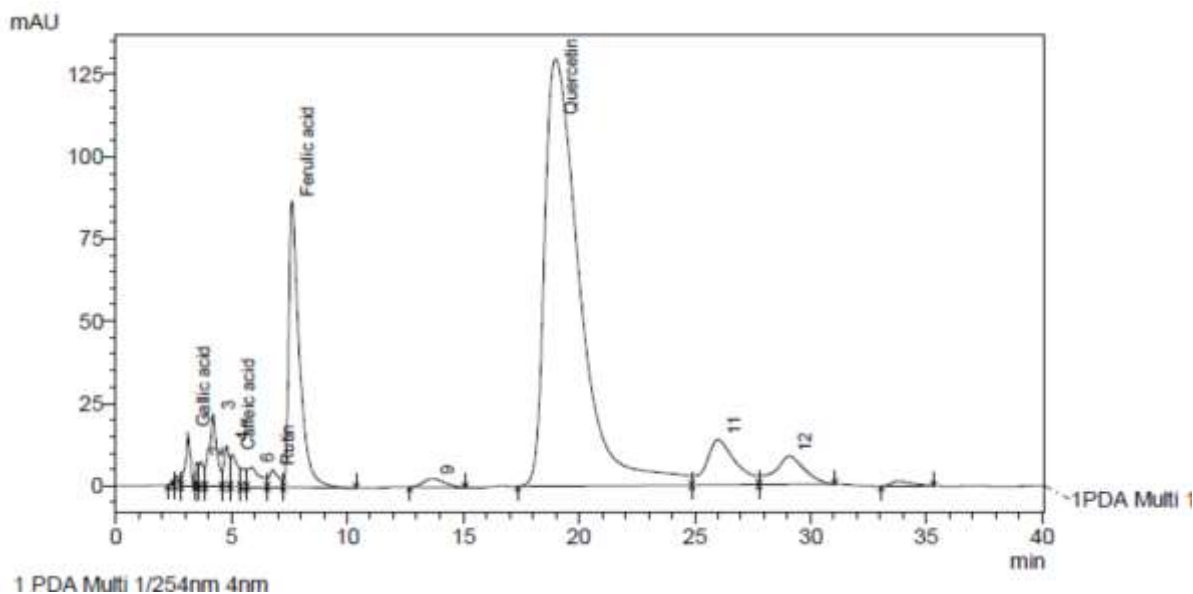


Figure 2. HPLC chromatogram of *G. kola*.

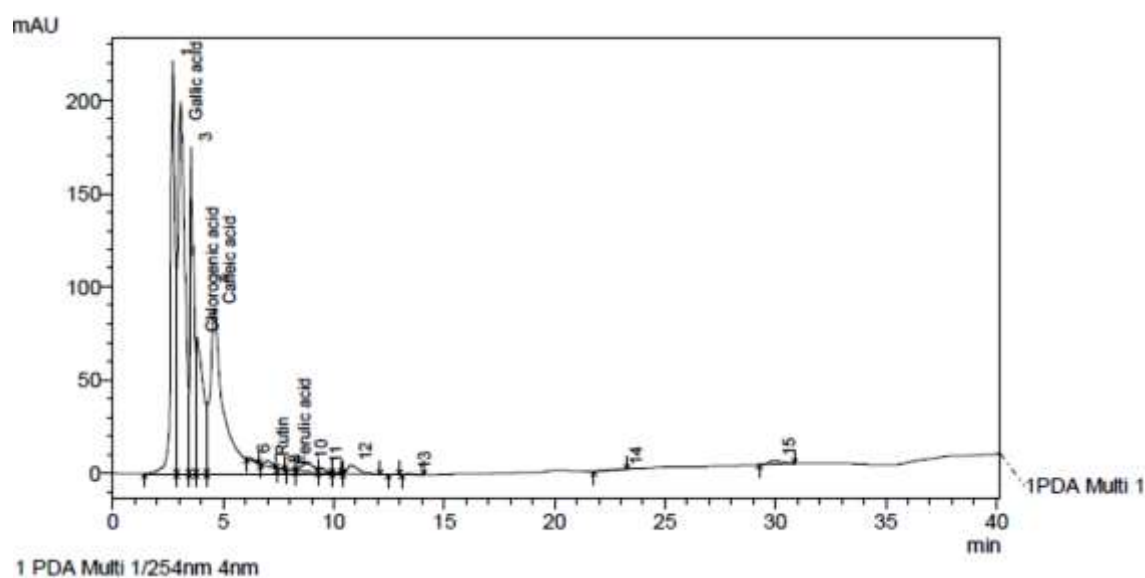


Figure 3. HPLC chromatogram of *B. coriacea*.

acid (16.99 mg/g) ferulic acid (1.47 mg/g) while quercetin and rutin were not detected. In *G. kola* caffeic acid is (0.84 mg/g), gallic acid (1.02 mg/g), ferulic acid (21.83 mg/g), quercetin (53.24 mg/g), rutin (0.49 mg/g) and chlorogenic acid was not detected. *B. coriacea* has caffeic acid (1.03 mg/g), chlorogenic acid (0.33 mg/g), gallic acid (1.07 mg/g) while ferulic acid, quercetin and rutin were not detected.

Table 3 also showed that the most abundant compound

in the three species of kolas is caffeic acid in *C. nitida* follow by quercetin in *G. kola*. Chlorogenic acid was equally as abundant as caffeic acid in *C. nitida* but very minute in *B. coriacea* and absent in *G. kola*. This shows that the levels of these compounds present in individual samples varied considerably and the concentration of each compound in different samples was significantly different. Although *C. nitida* is the most widely and regularly consumed, because of its varied socio-cultural

Table 3. The compounds in the three kola species (mg/ml).

Compound	<i>C. nitida</i>	<i>G. kola</i>	<i>B. coriacea</i>
Caffeic acid	101.24	0.84	1.03
Chlorogenic acid	101.24	ND	0.33
Gallic acid	16.99	1.02	1.07
Ferulic acid	1.47	21.83	ND
Quercetin	ND	53.24	ND
Rutin	ND	0.49	ND

ND= not detected.

importance. This study has also shown that *C. nitida* contains high amounts of phenolics compounds as compare to the other species of kola seeds investigated in the study.

The seeds of these kolas have received great interest due to their social significance and pharmacological properties. Phenolic compounds in synergy with the other compounds investigated in this study are responsible for their health benefits. Studies have shown that caffeic acid possesses anti-inflammatory, anti-mutagenic, anti-bacterial and anti-carcinogenic properties (Genaro-Mattos et al., 2015). Chlorogenic acid is an ester of caffeic acid have antioxidant properties which are propose to play a crucial role in protecting food, cell and many organs from oxidative degenerative. It has been discovered from research that diet that contain CGA compounds plays a vital role in preventing various diseases and ailments associated with oxidative stress such as cancer, cardiovascular, aging, diabetes, neurodegenerative diseases, anti-bacterial and anti-inflammatory activities (Ayelign and Sabally, 2013). Gallic acid has been proved to have potential preventive and therapeutic effects in many diseases, where the oxidative stress has been implicated including cardiovascular diseases, cancer, neurogenerative disorders and in aging.

Gallic acid has been reported to possess good pharmacological activities and could be exploited lead compound for new drug development (Nayeem et al., 2016). Ferulic acid is an antioxidant found in many staple foods, such as fruits, vegetables, cereals, coffee and in many other plants, exhibiting a wide range of pharmacological activities such as antioxidant, neuroprotective, anticancer, anti-diabetic, cardio-protective, anti-inflammatory and other activities (Gohil et al., 2012). Quercetin is present in most plants and is known for its biological activities such as antioxidant, antiviral, anticancer, antimicrobial, and anti-inflammatory and others (Maalik et al., 2014). Rutin is a flavonol, richly found in plants, such as passion flower, buckwheat, tea, and apple. It has demonstrated a number of pharmacological activities which includes: antioxidant, vaso-protective, anti carcinogenic, neuroprotective and

cardio protective (Ganeshpurkar and Saluje, 2017). The result from the study suggests that *C. nitida* with high phenolic acid contents could exhibit a better antioxidant activity than *G. kola* and *B. coriacea*.

Conclusion

HPLC method shows peaks with reasonable heights and fair resolutions were assigned as “characteristic peaks” for identification of these kolas. Using this analytical method, some phenolics and flavonoids compounds were quantified. It can be concluded from the results that all the three species of kola contain phenolics compounds but only *Garcinia kola* contain the two flavonoids compounds (quercetin and rutin) investigated in this study. The most abundant compound in the three species of kola is caffeic acid in *C. nitida* follow by quercetin in *G. kola*. *C. nitida* is the most widely and regularly consumed, because of its varied socio-cultural importance. This study has also shown that it contains high amounts of phenolics compounds compared to the other two species of kola investigated in the study. The method described offers a good quantitative analytical approach to phenolic acids determination in plant extracts.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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