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Comparative *In vitro* Antioxidant Activities of Aqueous Extracts of *Garcinia kola* and *Buchholzia coriacea* Seeds

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Abstract

Comparative *in vitro* antioxidant activities of aqueous extracts of *Garcinia kola* and *Buchholzia coriacea* seeds were carried out using 2, 2-diphenyl 1-picrylhydrazyl (DPPH) free radical scavenging activities, ferric reducing antioxidant power (FRAP) and inhibition of lipid peroxidation assays. Total phenolics, alkaloids, tannins, saponins and flavonoids constituents of the samples were also determined. *G. kola* seeds contained higher concentrations of secondary metabolites compared to *B. coriacea* seeds except flavonoids. Both *G. kola* and *B. coriacea* exhibited minimum percentage inhibition when compared with the gallic acid. However, *B. coriacea* exhibited significantly ($p < 0.05$) higher percentage DPPH scarvenging activities (44.66%) when compared with *G. kola* (43.62%) at maximum concentration of 100 µg/mL. *G. kola* on the other hand, showed higher percentage inhibition of lipid peroxidation when compared with *B. coriacea* at all tested concentrations. Percentage FRAP by *B. coriacea* was significantly ($p < 0.05$) higher than *G. kola* at 100 µg/mL (46.65%, and 38.35%, respectively) and 50 µg/mL (38.75%, and 46.05%, respectively), while lower percentage FRAP of the *G. kola* than *B. coriacea* were recorded at 25 µg/mL (24.26% and 38.24%, respectively) and 12.5 µg/mL (34.41% and 37.41%, respectively). Therefore, *Garcinia kola* and *B. coriacea* showed appreciable antioxidant activities at varying concentrations and this may be due to the presence of various phenolic compounds in both samples.

Keywords: *Buchholzia coriacea; Garcinia kola;* Antioxidant; DPPH free radicals; Secondary metabolites.

Introduction

Diseases that are caused by oxidative stress such as ischemia, anemia, arthritis, inflammations, neuro-degeneration, Parkinson's disease, ageing process, type 2 diabetes mellitus and perhaps dementias, are as consequences of accumulation of free radicals in the body (Aiyegoro and Okoh 2010). Free radicals generated from respired oxygen are called reactive oxygen species (ROS) which bring about damage to other molecules by extracting electrons from them in order to attain stability (Chanda and Dave 2009). The families of ROS are ions, atoms or molecules that have the ability to oxidize reduced molecules. ROS are various forms of activated oxygen, which include free radicals such as superoxide anion radicals ($\cdot O_2^-$) and hydroxyl radicals (·OH), as well as non-free radicals (H_2O_2) and singlet oxygen (Chanda and Dave 2009).

Antioxidants are both natural and synthetic compounds, capable of scavenging free radicals and to inhibit oxidative processes caused by free radicals (Hayat et al. 2010). Currently, many researches focus on finding natural antioxidants of plant origins. *In vivo* studies on bioactive components from medicinal plants and vegetables strongly support the fact that plant constituents with antioxidant activities are capable of exerting protective effects against free radicals in biological systems (Sini et al. 2010). The role of medicinal plants in disease prevention and control have been attributed to antioxidant effects of their phytoconstituents, usually associated to a wide range of amphipathic molecules, broadly termed polyphenolic compounds (Castilho et al. 2012). Examples of such constituents include; phenols, flavonoids, vitamin C and E, β-carotene, and α-tocopherol among others (Hamzah et al. 2018a, 2018b).

B. Coricea which belongs to the *Capparidaceae* family is an evergreen shrub found in some African countries including Cameroon, Central African Republic, Gabon, Congo, Angola, Nigeria, Ghana, and Liberia (Ijarotimi et al. 2015, Umeokoli et al. 2016). *B. Coricea* is a forest tree with large, glossy leaves and conspicuous cream white flowers in racemes at the end of the branches (Mbata et al. 2009). The plant is easily recognized by the compound pinnate leaves and the long narrow angular fruits containing large, usually aligned seeds. In Nigeria, *B. Coricea* has various local names, which include "Uwuro" (in Yoruba), "esson bossi" (in Central Africa), "Uke" (in ibo), "Ovu (in Birni) and Aponmu (in Akure). The medicinal efficacy of the *B. Coricea* seeds (Figure 1a) earned the plant its common name "wonderful kola" (Ijarotimi et al. 2015, Umeokoli et al. 2016).

Garcinia kola seeds (Figure 1b), generally known as 'bitter kola' in Nigeria, are produced by plants that belong to a family of tropical plants known as Guttiferae and it grows abundantly throughout the West and Central Africa (Hutchinson and Dalziel 1954). The plant grows wild and is also domesticated because of the wide medicinal values of the extract of its various components in folk medicine. *G. kola* nut is culturally and socially significant in some parts of south eastern Nigeria (West Africa) where the yellow nut is served for traditional hospitality in private, social and cultural functions. The plant is known to contain high contents of bioflavonoid compounds (Iwu 1986) with a general anecdotal effect in folk medicine in Africa (Adaramoye et al.2005). Its constituents include flavonoids (bioflavonoid), xanthenes and benzophenones and have shown anti-inflammatory, anti-parasitic, antimicrobial, and antiviral properties (Iwu et al. 1987). The seeds are edible and are consumed as adjuvants to the true kola (*Cola nitida*) and for medicinal purposes (Braide 1989). *Garcinia kola* plant is a wonder plant because every part of it has been found to be of medicinal importance. *Garcinia kola* is used in folklore remedies for the treatment of ailments such as liver disorders, diarrhoea, laryngitis, bronchitis and gonorrhoea (Adesina et al. 1995). The seed is masticatory and used to prevent and relieve colic, chest colds and cough and can as well be used to treat headache (Ayensu 1978). It is also used in the treatment of jaundice, high fever and purgative (Iwu et al. 1987), stomach ache and gastritis (Ajebesone and Aina 2004), cirrhosis and hepatitis (Okwu 2003).

At present, the most commonly used synthetic antioxidants are butylated hydoxyanisole (BHA), butylated hydoxytoluene (BHT), propylgallate (PG), and test butylated hydroquinone. However, these synthetic antioxidants have adverse side effects such as hepatic damage and carcinogenesis (Thangavelu and Thomas 2010). Thus, there is a need for screening plants containing natural antioxidants with less or no side effects, for use in foods or medicinal materials to replace synthetic antioxidants. The objective of this research work was thus, to evaluate the *in vitro* antioxidant properties of aqueous extracts of *Garcinia kola* and *Buchholzia coriacea* seeds.

Figure 1: Photographs of *Buchholzia coriacea* (a) and *Garcinia kola* (b) seeds.

Materials and Methods

Sample collections

G. kola and *B. Coriacea* seeds were purchased in the month of April 2019 at Gwari market located in Chanchaga Local Government Area Minna, Niger State, Nigeria. One hundred and twenty-five grams (125 g) of each *G. kola* and *B. Coriacea* seeds were purchased and wrapped in dried plantain leaves and gently stored and transported in a small bamboo basket. The seeds were authenticated by Dr. O.Y. Dawud in the Department of Plant Biology, Federal University of Technology, Minna. The coats of the seeds of each sample were removed, cut into pieces and dried at room temperature (28 ± 2 °C). The seeds were thereafter pulverized using electric blender (Philip model) and kept in air-tight container till further use.

Sample extraction

The method of Busari et al. (2014) with little modifications was adopted for the extraction of the samples. Briefly, forty grams (40 g) of each of the seed powder samples were weighed and macerated with 800 mL of distilled cold water for 72 hours with constant mixing. Thereafter, the mixture obtained was filtered using whatman filter paper size 1. The filtrate from each sample was concentrated under reduced pressure using rotary evaporator at temperature of 70 °C and the molten samples obtained were lyophilized. Exactly 15.57 g (39.25%) and 17.68 g (44.20%) of *G. kola* and *B. Coriacea* seeds extracts obtained were kept in sample containers until further use.

Determination of phenolics content

The phenolics content was estimated following the method of McDonald et al. (2001). Briefly, 0.5 mg/mL of each extract was dissolved in methanol and each of these solutions was mixed with 2.9 mL of 2% Na_2CO_3. The mixture was left to stand at room temperature for 2 minutes and then 0.1 mL of 0.2 N Folin-Ciocalteau reagent was added. The mixture was then incubated for 30 minutes at room temperature. The absorbance of the mixture was taken at 750 nm with a spectrophotometer (Shimadzu model; UV1800). Gallic acid (0.5 mg/mL in methanol) was used as standard. The total phenolic content of the plant extracts was expressed as milligram gallic acid equivalents (mg gallic acid/g extract). All samples were analyzed in triplicates.

Determination of flavonoid content

The flavonoids content was determined by the colorimetric method described by Barreira et al. (2008). A 0.5 mg/mL of the extracts was dissolved in methanol and then 250 µL of each of these solutions was mixed with deionized water (1.6 mL) and 100 µL of 5% Na_2CO_3. The sample was left to stand for 6 minutes; afterwards, 150 µL of 10% $AlCl_3.6H_2O$ solution was added and further incubated for 5 minutes at room temperature. To stop the reaction, 500 µL of 1 M NaOH was added and the tubes were left at room temperature for 15 minutes. The absorbance was read at 510 nm using a spectrophotometer. The absorbance of each blank, consisting of the same sample mixtures, but with deionized water in place of 10% $AlCl_3{\cdot}6H_2O$ solution, was subtracted from the test absorbance. Quercetin (0.5 mg/mL in ethanol) was used as standard. Flavonoids content was determined as milligram quercetin equivalents (mg quercetin/g extract).

Determination of alkaloids content

Alkaloids contents of the crude extracts was determined according to the method employed by Oloyede (2005). Briefly, 0.5 g of the crude extract was weighed and dissolved in 5 mL of mixture of 96% ethanol : 20% H_2SO_4 (1 : 1) and then filtered. 1 mL of the filtrate was then added to a test tube containing 5 mL of 60% H_2SO_4 and allowed to stand for 5 minutes. Thereafter, 5 mL of 0.5% formaldehyde was added and allowed to stand at room temperature for 3 hours. The absorbance was read at wavelength of 565 nm. Vincristine extinction coefficient (E_{296}, ethanol{ETOH} = 15136 $M^{-1}cm^{-1}$) was used as reference alkaloid.

Determination of saponins content

Saponins content of the crude extract was determined using the method of Oloyede (2005). Briefly, 0.5 g of the crude extract was weighed and dissolved in 20 mL of 1 N HCl and boiled in water bath at 80 °C for 4 hours. The reaction mixture was cooled and filtered. 50 mL of petroleum ether was added and the ether layer was collected and evaporated to dryness. Thereafter, 5 mL of acetone-ethanol (1:1), 6 mL of ferrous sulphate and 2 mL of concentrated sulphuric acid were added and allowed to stand for 10 minutes. The absorbance was taken at 490 nm. Standard saponin was used to prepare the calibration curve.

Determination of tannins content

The procedure used in determining the total tannin contents was adopted from Sofowora (1993). A 0.2 g of the extracts was weighed into a 50 mL beaker and 20 mL of 50% methanol was added. The beakers were covered with aluminium foil and placed in a water bath with shaker at 80 ºC for 1 hour. The extract was allowed to cool, filtered with double layered whatman No. 41 filter paper into a 100 mL volumetric flask and 20 mL of water was added followed by the addition of 2.5 mL Folin-Denis reagent and 10 mL of 17% Na_2CO_3. The mixture was made up to 100 mL with water and allowed to stand for 20 minutes for the development of a bluish-green colour after proper mixing and the absorbance was read against blank with spectrophotometer at 760 nm. The same procedure was followed for tannic acid for standard.

Determination of percentage DPPH radical scavenging activity

The method of Oyaizu (1986) was used for the determination of scavenging activity of 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical in the extract solution. A solution of 2 mL of 0.004% DPPH was prepared in methanol and 1.0 mL of this solution was mixed with 1 mL of extracts prepared in 50 mL of methanol containing 0.05 g of dry extract and gallic acid (standard) at various concentrations. The reaction mixtures were mixed thoroughly and incubated at 25 ºC for 30 minutes. The absorbance of the test mixtures were measured spectrophotometrically at 517 nm. All experiments were performed in triplicate. Percentage inhibition was calculated using the following expression:

$$\% \text{ Inhibition} = \left\{\frac{(\text{Ablank}-\text{Asample})}{(\text{Ablank})}\right\} \times 100$$

where: Ablank is the absorbance of DPPH radical + methanol; Asample is the absorbance of DPPH radical + sample extract or standard and % Inhibition is the percentage inhibition.

Determination of ferric reducing antioxidant power (FRAP)

The reducing properties of the extracts were determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by Oyaizu (1986). The ferric reducing antioxidant power of the extracts was determined by preparing different concentrations of plant extracts and gallic acid (12.5 – 100 µg/ mL) in 1 mL of distilled water. The prepared extracts were mixed with phosphate buffer (3.0 mL, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 mL, 1%). The mixtures were incubated at 50 ºC for 20 minutes. Then, 2.5 mL of trichloroacetic acid (10%) was added to the mixture, and then centrifuged for 10 minutes at 3000 rpm. A 2.5 mL from the upper layer of solution was mixed with 2.5 mL of distilled water and 0.5 mL, 0.1% of $FeCl_3$. The absorbance was taken at 700 nm against a blank with spectrophotometer.

Determination of percentage inhibition of lipid peroxidation

The method of Halliwell et al (1995) was used to determine percentage inhibition of lipid peroxidation of the phenolics using a modified thiobarbituric acid reactive substances (TBARS) assay. Briefly, egg homogenate (0.5 mL, 10% v/v) was added to 0.1 mL of extract or gallic acid (10 mg/mL) and made up to 1 mL with distilled water. Thereafter, 0.05 mL of $FeSO_4$ was added and the mixture was incubated for 30 minutes. After which, 1.5 mL of acetic acid was pipetted followed by 1.5 mL of thiobarbituric acid in sodium dodecyl sulphate. The resulting mixture was vortexed and heated at 95 °C for 60 minutes. After cooling, 5 mL of butan-1-ol was added and the mixture was centrifuged at 12,000 x g for 10 minutes and the absorbance of the organic upper layer was measured at 532 nm.

Percentage Inhibition of Lipid Peroxidation = (1 - E / C) x 100; where C = Absorbance of fully oxidized control and E = Absorbance in the presence of the sample.

Data analysis

The values of triplicate experiments (n = 3) were expressed as mean ± standard error of mean (SEM). The data were analyzed using one-way analysis of variance (ANOVA) and Duncan test was used for the post hoc treatment. Level of significance was considered at $p < 0.05$.

Results

The quantities of flavonoids, phenolics, tannins, alkaloids and saponins in both *G. kola* and *B. coriacea* seeds are presented in Table 1. The results showed significantly ($p < 0.05$) higher phenolics, tannins, alkaloids and saponins in *G. kola* seed extracts when compared with *B. coriacea* seed extracts, while no significant difference between flavonoids content of *G. kola* and *B. coriacea* seeds extracts.

Table 1: Secondary metabolites constituents of *G. kola* and *B. coriacea* seeds

Samples	Tannins mg/g	Flavonoids mg/g	Total Phenol mg/g	Alkaloids mg/g	Saponinns µg/g
B. coriacea	4.29 ± 0.02^{b}	2.44 ± 0.03^{a}	3.92 ± 0.11^{a}	4.81 ± 0.01^{a}	152.68 ± 0.39^{a}
G. kola	6.50 ± 0.13^{a}	2.32 ± 0.01^{a}	4.00 ± 0.01^{b}	12.86 ± 0.02^{b}	534.11 ± 0.34^{b}

Values are presented as mean ± standard error of mean (SEM) of triplicate. Values are mean ± standard error mean (SEM), n = 3; Values with different letters along the column are significantly different at $p < 0.05$.

Percentage DPPH radical scavenging activity of *G. kola* and *B. coriacea*

The DPPH radical scavenging activities of both extracts are shown in Figure 2. The results indicated a concentration dependent activity of the extracts against free radical species. Although the maximum radical scavenging activities values were recorded at 100 µg/mL, where *G. kola* and *B. coriacea* exhibited 43.62% and 44.66%, respectively, but both are not comparable with the gallic acid at all concentrations.

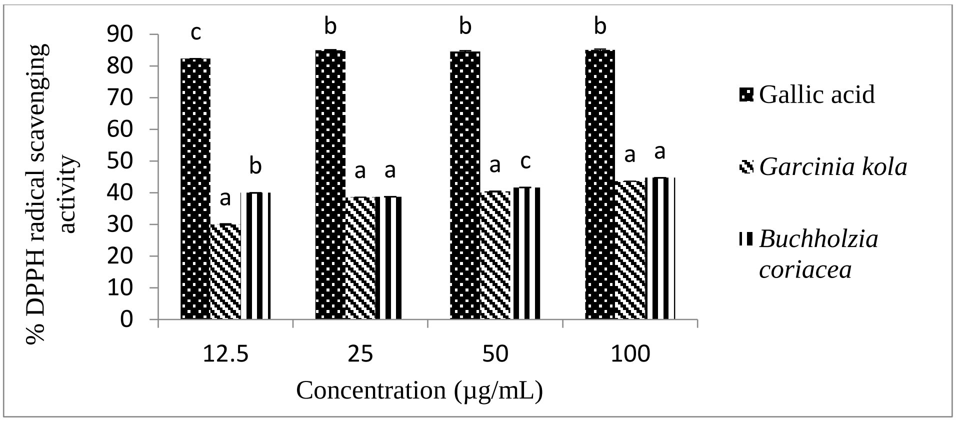

Figure 2: Percentage DPPH radical scavenging activity of *G. kola* and *B. coriacea*. Values are mean ± standard error of mean (SEM), n = 3; Values with different letters on the chart are significantly different at $p < 0.05$.

The percentage inhibition of lipid peroxidation of *G. kola* and *B. coriacea* seeds

The percentage inhibition of lipid peroxidation of *Garcinia kola* and *Buchholzia coriacea* seed extracts are shown in Figure 3. A concentration dependent activity was also observed in this case where both extracts showed the maximum percentage inhibition at highest concentration (100 µg/mL). The percentage inhibitions are in the following order: gallic acid > *G. kola* seed > *B. coriacea* seed extracts with 65.80%, 60.31% and 48.02% percentage lipid peroxidation inhibition, respectively. However, *G. kola* seeds exhibited significantly higher ($p < 0.05$) percentage inhibition at 25 µg/mL when compared to the gallic acid and *B. coriacea* seeds.

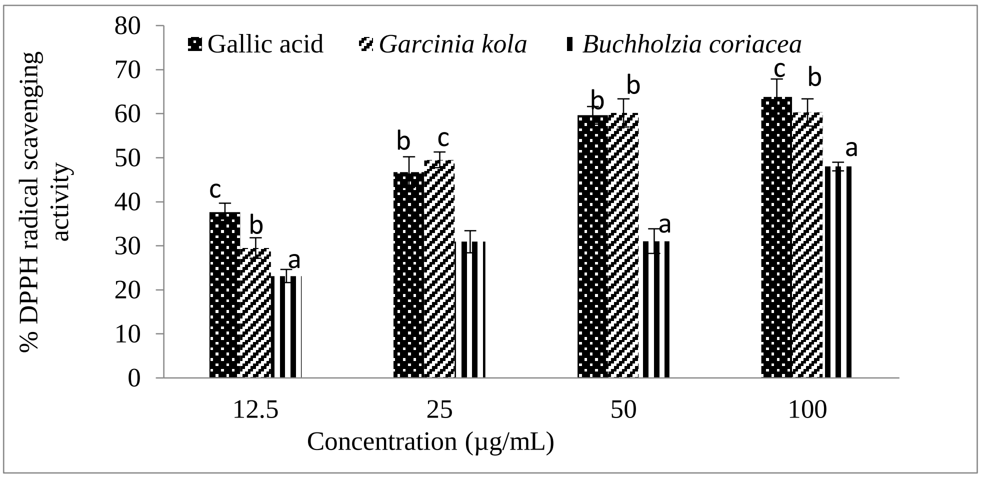

Figure 3: Percentage inhibition of lipid peroxidation of *G. kola* and *B. coriacea* seeds. Values are mean ± standard error of mean (SEM), n = ; Values with different alphabet on the chart are significantly different at $p < 0.05$.

Percentage ferric reducing antioxidant power (FRAP) of *G. kola* and *B. coriacea* seeds

The percentage ferric reducing antioxidant power of *G. kola* and *B. coriacea* seed extracts are shown in Figure 4. The results showed that the percentage FRAP by *B. coriacea* was significantly higher ($p \leq 0.05$) than that of *G. kola* at 100 µg/mL (46.65% and 38.35%, respectively) and 50 µg/mL (46.05% and 38.75%, respectively), while low percentage FRAP of the *B. coriacea* seed extract were recorded at 25 µg/mL (24.26%) and 12.5 µg/mL (34.41%) when compared with *G. kola* (38.24% and 37.41%, respectively).

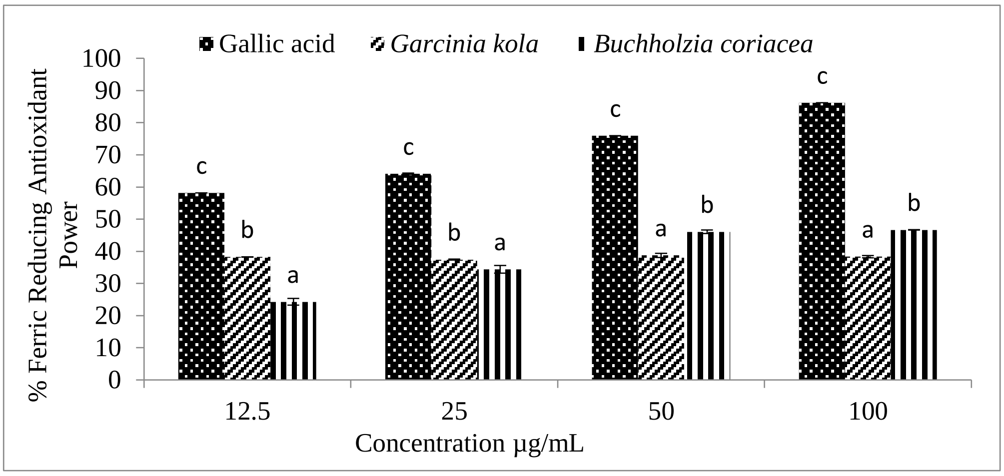

Figure 4: Percentage Ferric Reducing Antioxidant Power (FRAP) of *G. kola* and *B. coriacea* Seeds. Values are mean ± standard error of mean (SEM), n = 3; Values with different alphabet on the chart are significantly different at $p \leq 0.05$.

Discussion

The results showed that *G. kola* seeds contained higher amounts of tannins, alkaloids, saponins and total phenols, while *B. coriacea* contains high flavonoids. The presence of flavonoids in *G. kola* and *B. coriacea* are in agreement with the work of Adesuyi et al. (2012) and Okere et al. (2014), respectively, although without assessing antioxidant activities of both samples. The aforementioned secondary metabolites are known to be responsible for some of the medicinal properties in herbal plants. Tannins have been found to be useful in the treatment of inflamed or ulcerated tissues and they have notable activities in cancer prevention and treatment (Ruch et al. 1989, Aiyegoro and Okoh 2010). Alkaloids have also been associated with cytotoxicity effects (Nobori et al. 1994), analgesic effects, antispasmodic activity and antibacterial activities (Yadav and Agarwala 2011). Saponins are also secondary metabolites which are involved in plant defense systems due to their antimicrobial activities (Ayoola et al. 2008). Phenolics belong to a major class of compounds that act primarily as antioxidants (Hamzah et al. 2018a, 2018b). They have high redox potentials which allow them act as reducing agents, hydrogen donors and singlet oxygen quenchers (Kähkönen et al. 1999). Studies have shown that flavonoids exhibit numerous biological activities such as antioxidant, anti-inflammatory, antimicrobial, anti-angionic, anticancer and anti-allergic reactions (Anyasor et al. 2010, Chao et al. 2002, Igbinosa et al. 2009, Thitilertdecha et al. 2008). Therefore, aqueous extract of *G. kola* and *B. coriacea* may be found useful in the prevention and probably treatment of cancer and other oxidative stress related diseases.

DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activities in foods and complex biological systems (Esmaeili and Sonboli 2010). These findings are in agreement with previous studies on the DPPH radical scavenging activities of various plant extracts (Bajpai et al. 2015, Amari et al. 2014). The ability of these extracts in scavenging DPPH radicals may be attributed to the presence of phenolic compounds in them. Phenolic compounds have been shown in previous reports to scavenge free radicals in oxidative stress related diseases such as diabetes, liver damage and perhap cancer (Hamzah et al. 2018b). It therefore implies that, the seed extracts may be useful for treating radical-related pathological damages.

The reducing power of *B. coriacea* and *G. kola* seed extracts might be as a result of their ability to transform Fe^{2+} to Fe^{3+} that could be a result of the existence of hydrophilic polyphenolic compounds. Thus, these seed extracts can be rich sources of antioxidants and use in the prevention of many oxidative stress related diseases. Likewise, several plant extracts have also shown protective effects against Fe^{3+}-induced lipid peroxidation (Amari et al.2014, Geetha and Vasudevan 2004). The inhibition of lipid peroxidation by *G. kola* and *B. coriacea* could be due to Fe^{3+} chelation and its hydroxyl radical scavenging abilities. These abilities might be as a result of the presence of the secondary metabolites inherent in them especially flavonoids and other phenolic compounds.

Conclusion

Conclusively, *Garcinia kola* and *B. coriacea* seeds extracts showed appreciable antioxidant activities at varying concentrations. Therefore, *G. kola* and *B. coriacea* seeds extracts could be used in the development of a new drugs and antioxidant supplements that can subsequently used to prevent and treat diseases associated with oxidative stress.

Conflicting of Interest

Authors declare that no conflict of interests exists.

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