In vitro antioxidant activities of hydroethanolic extract of defatted wonderful kola (*Buchholzia coriacea*) seeds and its safety evaluation in murine models

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ABSTRACT

Introduction: *Buccholzia coriacea* seed possesses a vast number of therapeutic applications in African traditional medicine. However, there are still limited information on its bioactive constituents, pharmacological actions and safety profile. This study was designed to prepare hydroethanolic extract of (defatted) *Buccholzia coriacea* seeds (HEBCS), evaluate its total phenols and flavonoids, *in vitro* antioxidant activity and its safety in rats and mice.
Methods: Seeds were defatted with hexane and the residue extracted with 80% ethanol (EtOH). The total phenols and flavonoid content of the lyophilised HEBCS was determined followed by in vitro antioxidant activity against stable free radicals ($DPPH^{\bullet}$, $ABTS^{\bullet+}$) and reactive oxygen species ($^{\bullet}OH$, NO and H_2O_2). Limit test (at 5000 mg/ kg body weight (bw) and acute toxicity test (at doses of 62.5, 125, 250 and 500 mg/kg bw) was carried out in rats followed by sub chronic toxicity study (at doses of 125 and 250 mg/ kg bw) in mice. biomarkers of hepatic function (ALT, AST, ALP and total bilirubin) and renal function (urea and creatinine) were assessed.
Results: The total phenols and flavonoids content of HEBCS was found to be 31.76 µg GAE/ mg and 22.82 µg QE/ mg respectively. The half-maximal inhibitory concentration (IC_{50}) value for HEBCS when tested against $DPPH^{\bullet}$, $ABTS^{\bullet+}$, $^{\bullet}OH$, H_2O_2 and NO were found to be 257.85, 496.73, 883.68, 475.68 and 1786.42 µg/mL respectively. The Median Lethality Dose (LD_{50}) for HEBCS in rat was greater than 5000 mg/ kg body weight. HEBCS did not show any significant ($P = .05$) hepatic or renal toxicity.
Conclusion: Data obtained from the present study highlight the safety of HEBCS and its potential role as a source of natural antioxidants.

Keywords: Buchholzia coriacea; Total Phenols and Flavonoids, DPPH and $ABTS^{\bullet+}$ scavenging activity; Hepatic and Renal Function biomarkers Rat and Mouse

1. INTRODUCTION

In recent times, the therapeutic applications of Herbal Medicines are rapidly gaining attention due to their potentials in the prevention and treatment of acute and chronic diseases (1,2). African traditional medicine has been regarded as one of the oldest, and about the most assorted of all therapeutic systems (3). Africa is very rich in cultural as well as botanical diversity in healing practices. Moreover, reports have shown that African herbal medicines have potentials in the treatment of many diseases (4).

Despite the medicinal importance of these herbs, they are yet to be widely applied as much as western drugs. This can be attributed to limited information regarding their bioactive constituents, pharmacological mechanisms, and safety profile. Yuan *et al.* (5) described the importance of traditional medicine as being too valuable to be ignored in the development of modern drugs. Therefore, further research is required to validate the safety and efficacy of the medicinal plants' extracts and other natural products applied in traditional medicine.

Buchholzia coriacea is known as Wonderful Kola and its indigenous names in Nigeria are 'Obi Iyanu' or 'Obi ata' in Yoruba and 'Okpokolo' in Igbo. It is a forest tree with large, glossy leaves and white flowers at the end of the branches (6). *B. coriacea* possesses a vast number of medicinal values and wide medicinal applications in traditional medicine (7). In Folk medicine, the seed is used for the treatment of fever, diabetes mellitus, hypertension, rheumatism, cough, and catarrh and also in stimulating the body's immune system. It is also applied as an anthelmintic agent, elevates mood, suppress appetite and hunger, and clean the digestive system. Nutritional and Phytochemical evaluation some solvent extracts of *B coriacea* show that it contains minerals and important class of phytochemicals like alkaloids, glycosides, saponin, steroids, tannin, flavonoids, terpenes, and phenols (8-10). In a recent study, its aqueous extract was found to contain some phenolic acids and flavonoids including catechin, caffeic acid, *p*-coumaric acid, quercetin and apigenin (11).

The present study was designed to prepare the hydroethanolic extract of defatted *Buchholzia coriacea* seeds and evaluate the total phenols and flavonoids content as well as the *in vitro* antioxidant potentials against stable free radicals and selected reactive oxygen species. Part of the objective of this study was also to evaluate its toxicity potentials in rat and mouse models.

2. MATERIAL AND METHODS

2.1 Chemicals

Chemicals used in this research include *p*-nitrophenylphosphate (p-NPP), Folin-Ciocalteu reagent, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salts radical cation ($ABTS^{\bullet+}$), 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$), ethanol, methanol, ascorbic acid, gallic acid, quercetin, aluminium chloride, sodium acetate, sodium carbonate (Merck, Germany). All other reagents used were of analytical grade.

2.2 Assay kits

Assay kits for alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, urea and creatinine are products of Randox® diagnostics limited, Antrim, UK.

2.3 Plant Material

Samples of *B. coriacea* seeds were collected from a farm site in Osi, Kwara State, Nigeria and authenticated at the Department of Biology, University of Ilorin in Kwara State, Nigeria. A voucher specimen (UIH 1067) was deposited in the Herbarium of the Department. The name of the plant was also checked with the plant list database at http://www.theplantlist.org.

2.4 Experimental Animals

Albino rats were obtained from the experimental animal breeding house, College of Basic Medical Sciences, University of Ibadan, Nigeria. Albino mice were procured from a private animal breeding facility in Iwo, Osun State, Nigeria. They were kept in wire-meshed cages and fed with commercial chow diet and water ad libitum. Animal procedures were carried out in accordance with the International Guidelines on laboratory animal handling (12). For each experiment, animals were acclimatized to laboratory conditions, one week prior to the commencement of study.

2.5 Preparation of the plant extract

The seeds were washed in clean water, cut into thin slices with knife and air-dried. The seeds were ground to powder and defatted by extracting exhaustively with n-hexane. The residue was air-dried and re-extracted with 80% ethanol (Figure 1). The resulting 80% ethanol extract was filtered, concentrated under reduced pressure, lyophilised and kept refrigerated until used.

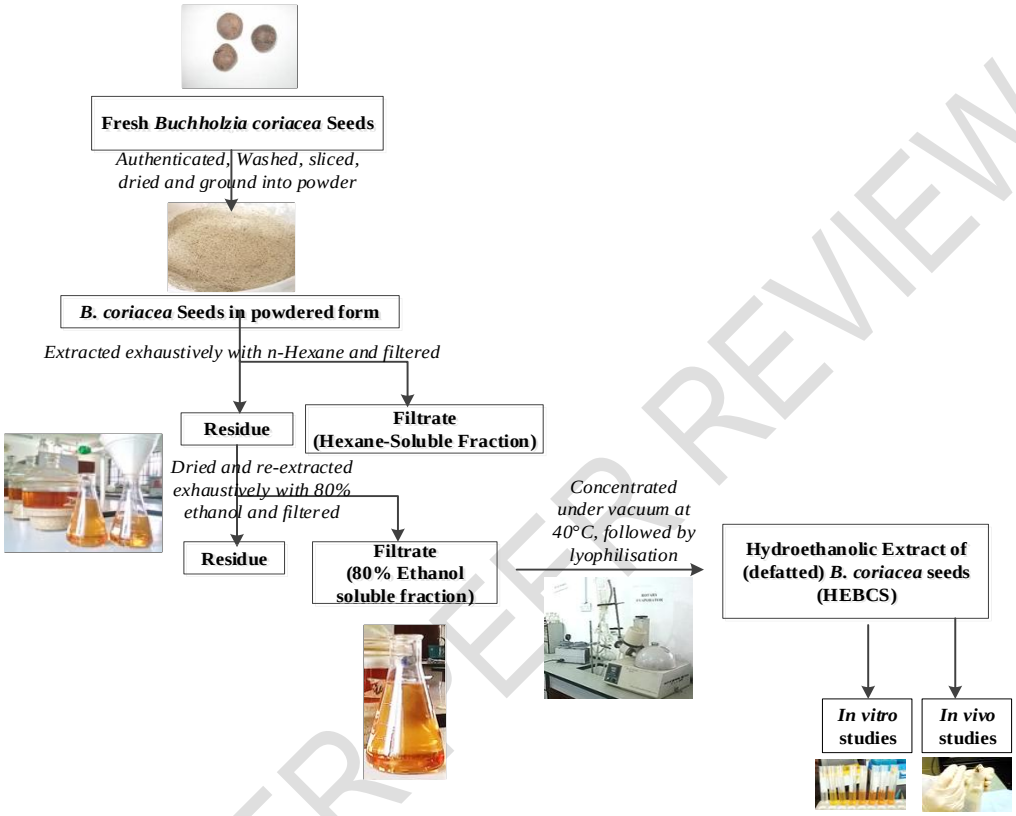

Figure 1. Steps in the preparation of HEBCS.

2.6 Total Phenols Assay

The total phenols content of HEBCS was determined according to Chandra *et al.* (13) with slight modifications. 0.2 mL of HEBCS (150 µg/mL) or standard solutions of gallic acid (10 µg/mL, 20 µg/mL, 50 µg/mL, 100 µg/mL and 150 µg/mL) was mixed with 0.6 mL of water and 0.2 mL of Folin-Ciocalteu's phenol reagent (1 : 1). After 5 min, 1 mL of sodium carbonate solution (8% w/v in water) was added to the mixture and the volume was made up to 3 mL with distilled water. The reaction mixture was kept in the dark for 30 min and followed by centrifugation at 3000 rpm for 5 min. The absorbance of blue coloured supernatant was measured at 765 nm against reagent blank. The phenols content was calculated as µg gallic acid equivalents/ mg dried extract based on the standard curve of gallic acid (Y = 0.0014x − 0.0482, R^2 = 0.9915). All determinations in sample and standard were carried out in triplicate.

2.7 Total Flavonoids Assay

Total flavonoid content of HEBCS was determined according to Camarena-Tello et al. (14) with minor modifications. 250 µL of HEBCS (150 µg/mL in 80% methanol) or standard quercetin solutions (10 µg/mL, 20 µg/mL, 50 µg/mL, 100 µg/mL and 150 µg/mL) were added to 1.250 µL of dH_2O, and 75 µL of $NaNO_2$ (5% w/v) in separate tubes. After 6 min, 150 µL of 10% w/v $AlCl_3$ was added. After another 5 min, 500 µL of 1 M NaOH and 275 µL of H_2O were added. The absorbance of the resulting solution was measured at 510 nm in a spectrophotometer. The total flavonoids in HEBCS was calculated from the calibration plot for quercetin (Y = 0.0013x + 0.065, R^2 = 0.9695) and expressed as µg quercetin equivalent (QE)/ mg of dried extract. All the determinations (sample and standard) were carried out in triplicate.

2.8 Evaluation of DPPH Radical Scavenging Activity

The free radical scavenging activity of HEBCS (in terms of hydrogen donating or radical scavenging ability) was measured according to Subba *et al.* (15), with minor modifications using the stable free radical, DPPH. 3 mL of HEBCS or ascorbic acid at various concentrations (10 µg/mL, 20 µg/mL, 50 µg/mL, 100 µg/mL and 150 µg/mL) or control (without the test compound but with an equivalent amount of methanol) were taken in separate test tubes. 1 mL of DPPH solution (0.1 mM DPPH in methanol) was added gently, stirred and kept at room temperature for about 30 min. The absorbance of sample, control, and standards was measured at 517 nm using a spectrophotometer. Free radical scavenging activity is based on a decrease in the absorbance of the reaction mixture. The percentage inhibition of DPPH radical was calculated using the formula:

$$\%\ DPPH^{\bullet}\ Scavenged = \frac{A_c - A_t}{A_c} \times 100$$

Where $A_c = absorbance\ of\ control$; $A_t = absorbance\ of\ test\ (sample\ or\ standard)$

The half-maximal inhibitory concentration (IC_{50}) was calculated for HEBCS and standard (ascorbic acid).

2.9 $ABTS^{\bullet+}$ radical cation scavenging activity

The method described by Birasuren et al. (16) with minor modifications was used to determine the $ABTS^{\bullet+}$ scavenging activity of HEBCS. This assay was based on the ability of different substances to scavenge the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salts radical cation ($ABTS^{\bullet+}$). 0.1 mL of HEBCS or ascorbic acid solutions at various concentrations (10 µg/mL, 20 µg/mL, 50 µg/mL, 100 µg/mL and 150 µg/mL) was mixed with 2.9 mL of diluted $ABTS^{\bullet+}$ (1:88 v/v in ethanol) solution. After incubation at room temperature for 20 min, the absorbance was measured at 734 nm in a spectrophotometer. A lower absorbance value indicates higher ABTS radical scavenging activity. The activity was expressed as percentage ABTS radical scavenged:

$$\%\ ABTS^{\bullet+}\ scavenged = \frac{A_c - A_t}{A_c} \times 100$$

Where

$$A_c = absorbance\ of\ control; A_t = absorbance\ of\ test\ (sample\ or\ standard)$$

The half maximal inhibitory concentration (IC_{50}) was calculated for HEBCS and standard (ascorbic acid).

2.10 Nitric Oxide Radical ($NO^{\bullet}$) Scavenging Activity

The method described by Jagetia et al. (17) was used to determine the $NO^{\bullet}$ scavenging activity of HEBCS. Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction (18). Sodium nitroprusside (5 mM) in standard phosphate buffer saline

solution (0.025 M, pH:7.4) was incubated with different concentrations of HEBCS or ascorbic acid at various concentrations (10 µg/mL, 20 µg/mL, 50 µg/mL, 100 µg/mL and 150 µg/mL), and incubated at room temperature for 5 hr. Control experiments without the test compounds but equivalent amounts of buffer were carried out in a similar manner. After 5 hours, 0.5 mL of incubation solution was removed and mixed with 0.5 mL of Griess reagent (1% sulphanilamide, 2% O-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride). The absorbance of the chromophore produced was measured at 546 nm with a spectrophotometer. All the experiments were performed in triplicates and percentage inhibition of nitric oxide radical was calculated by using the formula:

$$\%\ NO^{\bullet}\ scavenged = \frac{A_c - A_t}{A_c} \times 100$$

Where

$$A_c = absorbance\ of\ control;\ A_t = absorbance\ of\ test\ (sample\ or\ standard)$$

The half maximal inhibitory concentration (IC_{50}) was calculated for HEBCS and standard (ascorbic acid).

2.11 Hydroxyl Radical (•OH) Scavenging Activity

The hydroxyl radical-scavenging activity of HEBCS was determined using 2-deoxyribose method as described by Afsar et al. (19) with minor modifications. 100µL HEBCS or ascorbic acid at various concentrations (10 µg/mL, 20 µg/mL, 50 µg/mL, 100 µg/mL and 150 µg/mL) was mixed with assay mixture containing 2.8 mM 2-deoxyribose, 20 mM ferrous ammonium sulphate solution, 100 µM EDTA in a total volume of 1 mL of buffer (0.2 M phosphate buffer saline, pH 7.4). The reaction was initiated by the addition of 100 µL of 20 mM H_2O_2 and incubated at 37 °C for 15 min. Then, thiobarbituric acid solution (1 ml, 1%, w/v) and trichloroacetic acid solution (1 ml, 2%, w/v) were added. The mixture was boiled in a water bath for 15 min and cooled in ice, and its absorbance was measured at 532 nm. All experiments were done in triplicates. The •OH scavenging activity was calculated as:

$$\%\ \bullet\mathrm{OH}\ scavenged = \frac{A_c - A_t}{A_c} \times 100$$

Where

$$A_c = absorbance\ of\ control;\ A_t = absorbance\ of\ test\ (sample\ or\ standard)$$

The half maximal inhibitory concentration (IC_{50}) was calculated for HEBCS and standard (ascorbic acid).

2.12 Hydrogen Peroxide (H_2O_2) Scavenging Activity

Hydrogen peroxide scavenging activity was measured by the method described by Sroka and Cisowski (20). 100 µL of sample or ascorbic acid at various concentrations (10 µg/mL, 20 µg/mL, 50 µg/mL, 100 µg/mL and 150 µg/mL) were added to 100 µL of 0.002% H_2O_2. Then 800 µL of 0.1 M phosphate buffer (Na_2HPO_4:KH_2PO_2; pH) and 100 mM NaCl were added. The reaction mixture was preincubated for 10 min at 37ºC. Then 1000 µL of 0.2 mg/mL phenol red dye with 0.1 mg/mL horseradish peroxidase in 0.1 M phosphate buffer was added. After 15min 50 µL of 1 M NaOH were added and absorbance was measured at 610 nm. All measures were repeated three times. The hydrogen peroxide scavenging activity was calculated according to the following equation:

$$\%\ H_2O_2\ scavenged = \frac{A_c - A_t}{A_c} \times 100$$

Where

$$A_c = absorbance\ of\ control;\ A_t = absorbance\ of\ test\ (sample\ or\ standard)$$

The half maximal-inhibitory concentration (IC_{50}) was calculated for HEBCS and standard (ascorbic acid).

2.13 Acute oral toxicity study (OECD[425])

The Limit Dose Test was carried out according to the OECD guidelines 425 (21). 5000 mg/kg bw of HEBCS was administered orally by gavage to three male albino rats (260 ± 5g) that have been fasted overnight. Thereafter, animals were observed for 14 days for symptoms of toxicity.

2.14 Sub-acute Oral Toxicity Study in Rats

Thirty (30) male Albino rats (160–190 g) were assigned into five groups (n=6/ group). Group I (control) received dH_2O, while animals in groups II, III, IV, and V received HEBCS at doses of 62.5, 125, 250 and 500 mg/kg b.w. respectively. HEBCS was dissolved in dH_2O and administered orally once daily for a period of seven days. Twenty four (24) hours after the final dose, blood sample were collected, allowed to stand for 30 minutes and subjected to centrifugation at 4000 rpm. for 5 min to obtain serum. Protein contents of serum samples were determined by the biuret method (22).

Activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin level in serum samples were determined using assay kits (Randox®, Antrim, UK), following the manufacturer's protocol. Alkaline phosphatase (ALP) activity was determined by the method of Wright *et al.* (23). Renal function was assessed by determining the concentration of urea and creatinine in the serum of rats using assay kits, according to the manufacturer's procedure.

2.15 Sub-Chronic Oral Toxicity Studies of HEBCS in Mice

Fifteen male Albino mice (32–35 g) were assigned into three groups (n=5/ group). Group I (control) received dH_2O, while animals in groups II, and III received HEBCS at doses of 125 and 250 mg/kg b.w. respectively. HEBCS was dissolved in dH_2O and administered orally once daily for a period of six (6) weeks. Blood samples were collected via the retro orbital vein for evaluation of hepatic and renal function 24 hours after the last dose. Blood samples were allowed to stand for 30 minutes and the subjected to centrifugation at 4000 rpm. for 5 min to obtain serum. Protein contents of serum samples were determined by the biuret method (22). Activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin level in serum samples were determined using assay kits (Randox®, Antrim, UK), following the manufacturer's protocol. Alkaline phosphatase (ALP) activity was determined by the method of Wright *et al.* (23). Renal function was assessed by determining the concentration of urea and creatinine in the serum of mice using assay kits, according to the manufacturer's procedure.

2.16 Statistical analysis

Results were expressed as mean ± SEM for *in vitro* experiments and mean ± SD for animal studies. Statistical analysis and graphical constructions were done with Graphpad® Prism 6.0.1 (Graphpad Software, La Jolla, CA). Statistical significance ($P = .05$) of the differences between experimental groups were determined by one-way analysis of variance (ANOVA) and complemented with Tukey's test.

3. RESULTS

3.1 Total Phenolics and Flavonoid content of HEBCS

Table 1 shows the total phenolics and flavonoids content of HEBCS. As presented in the table, total phenols and flavonoids content of HEBCS are 31.76 µg GAE/ mg and 22.82 µg QE/ mg respectively.

Table 1. Total phenolic and flavonoid contents of HEBCS

Total phenolics (µg GAE/ mg dried extract)	Total Flavonoids (µg QE/ mg dried extract)
31.76 ± 2.63	22.82 ± 5.42

3.2 *In vitro* Antioxidant Activity of HEBCS

Figure 2 shows the antioxidant activities of HEBCS against DPPH$^{\bullet}$, ABTS$^{\bullet+}$, $^{\bullet}$OH, H_2O_2 and NO. The antiradical activity of HEBCS against DPPH is presented in Figure 2a. The DPPH scavenging activity increased with increasing concentrations of HEBCS. IC_{50} value (the concentration of antioxidant substance required to scavenge 50% of DPPH) of HEBCS was found to be 257.85 µg/ mL (Table 2) with the standard (ascorbic acid) having an IC_{50} value of 77.29 µg/mL.

The antiradical activity of HEBCS against ABTS$^{\bullet+}$ is shown in Figure 2b. The scavenging activity was increased with the increasing concentrations of HEBCS. IC_{50} value (the concentration of antioxidant substance required to scavenge 50% of ABTS$^{\bullet+}$) of HEBCS was calculated as 496.73 µg/ mL and that of the standard as 262.62 µg/mL (Table 2).

Figure 2c shows the activity of HEBCS against NO$^{\bullet}$. The NO scavenging activity of HEBCS increased with the increasing concentrations of HEBCS up to a concentration of 50 µg/ mL. After this concentration, the NO$^{\bullet}$ scavenging activity no longer increase relative to concentration of HEBCS. As presented in Table 2, the IC_{50} value (the concentration required to scavenge 50% of NO$^{\bullet}$) for HEBCS was 1786.42 µg/ mL and that of the standard (ascorbic acid) 743.52 µg/mL.

The anti-$^{\bullet}$OH activity of HEBCS is presented in figure 2d. The $^{\bullet}$OH scavenging activity increased rapidly at lower concentrations of HEBCS, after which the curve fattens for higher concentrations. As shown in Table 2, the IC_{50} value (the concentration required to scavenge 50% of $^{\bullet}$OH) for HEBCS was found to be 883.68 µg/ mL and that of ascorbic acid 151.61 µg/mL.

Figure 2e shows the H_2O_2 scavenging activity of HEBCS and ascorbic acid. The scavenging activity of HEBCS increased with the increasing concentrations up to 50 µg/ mL, and thereafter the curve flattens. The IC_{50} value (the concentration required to scavenge 50% of H_2O_2) for HEBCS was found to be 475.68 µg/ mL (Table 2). On the other hand ascorbic acid possesses IC_{50} value of 145.75 µg/mL.

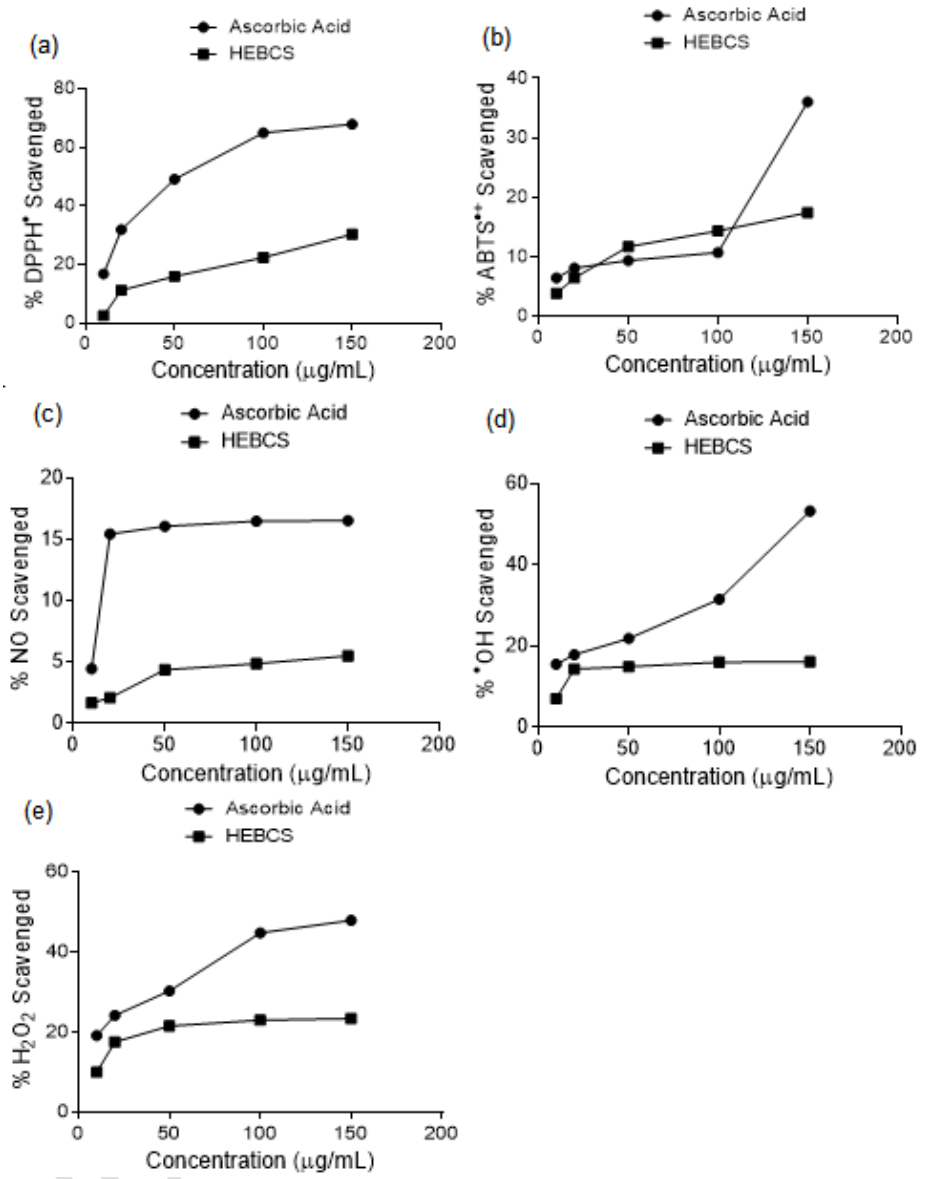

Figure 2. Antioxidant activity of various concentrations of HEBCS (10 - 150 µg/mL) tested against (a) DPPH, (b) $ABTS^{\bullet+}$, (c) NO, (d) $^{\bullet}OH$ and (e) H_2O_2. Each point represent the mean ± SEM from three independent determinations.

Table 2. Half maximal inhibitory concentration (IC_{50}) values for Ascorbic acid and HEBCS

Assay → Antioxidant/ Extract ↓	**DPPH•**	**$ABTS^{\bullet+}$**	**$^{\bullet}OH$**	**H_2O_2**	**NO**
Ascorbic Acid (IC_{50} in µg/mL)	77.29	262.62	151.61	145.75	743.52
HEBCS (IC_{50} in µg/mL)	257.85	496.73	883.68	475.68	1786.42

3.3 Acute Oral Toxicity

In the limit dose test carried out, the rats did not show any sign of toxicity and no death was recorded. Observations from the single dose acute toxicity study show that the Median Lethality Dose LD_{50}) of HEBCS is greater than 5000 mg/ kg bw (>5000 mg/ kg bw).

3.4 Subacute oral toxicity profile of HEBCS in rats

Figure 3 shows the effects of HEBCS on biomarkers of hepatic function in rat. Compared to the control, the doses of HEBCS cause a slight elevation in the serum activities of AST, ALT and ALP; although these increases are not statistically significant *(P = .05)*. In a similar manner, serum levels of bilirubin increased slightly in the HEBCS groups compared to control; however, the increases are not statistically significant. The effects of the various doses of HEBCS on biomarkers of renal function is shown in Figure 4. Various doses of HEBCS caused a slight increase in the serum levels of urea and creatinine compared to control. However, the observed increases were not statistically significant *(P = .05)*.

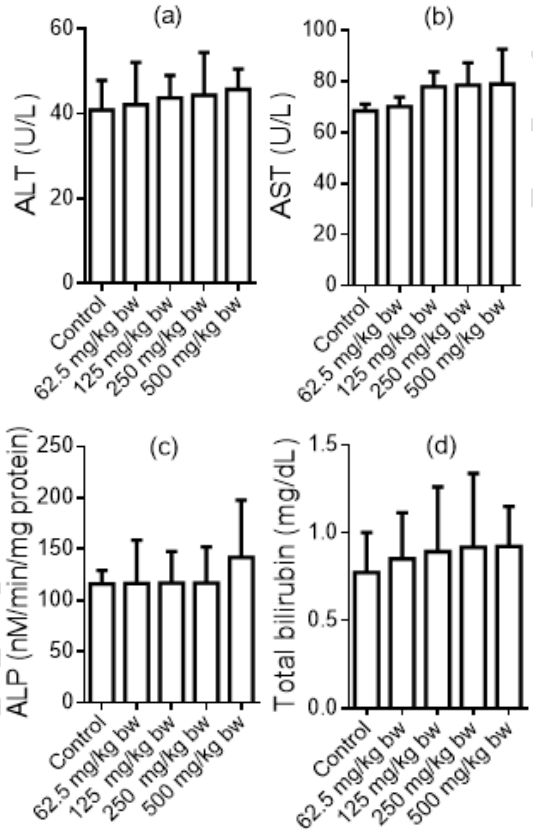

Figure 3. Effects of oral administration of HEBCS for seven days on serum (a) ALT activity, (b) AST activity, (c) ALP activity and (d) total bilirubin level in rats. *Each bar represents the mean ± SD (n = 6).*

3.5 Sub chronic toxicity profile of HEBCS in mice

Figure 5 shows the effects of HEBCS on biomarkers of hepatic function in mice. Compared to the control, the two doses of HEBCS investigated cause a slight increase in the serum activities of AST, ALT and ALP; although not statistically significant *(P = .05)*. In a similar manner, serum levels of bilirubin increased slightly in the HEBCS groups compared to control; also not statistically significant *(P = .05)*. Figure 6 shows the effects of two doses of HEBCS on biomarkers of renal function in mice. The two doses of HEBCS evaluated caused

a slight increase in the serum levels of urea and creatinine compared to control. Although, these increases were not statistically significant *(P = .05)*.

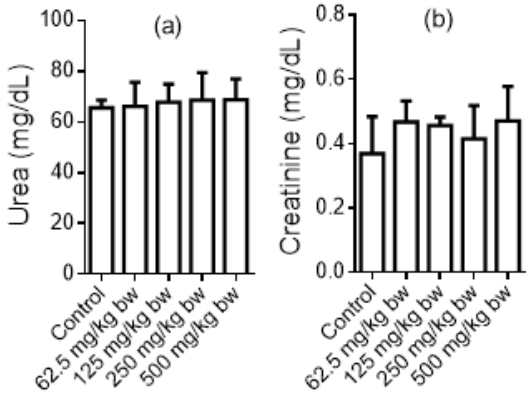

Figure 4. Effects of oral administration of HEBCS for seven days on serum levels of (a) urea and (b) Creatinine in rats. *Each bar represents the mean ± SD (n = 6).*

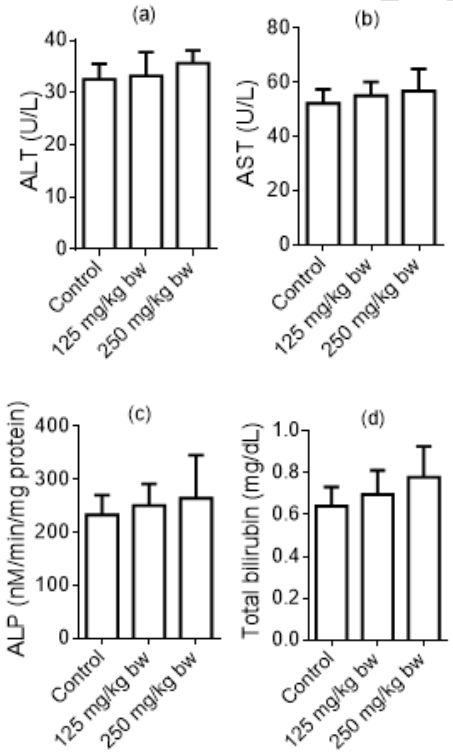

Figure 5. Effects of oral administration of HEBCS for six weeks on serum (a) ALT activity, (b) AST activity, (c) ALP activity and (d) total bilirubin level in mice. *Each bar represents the mean ± SD (n = 5).*

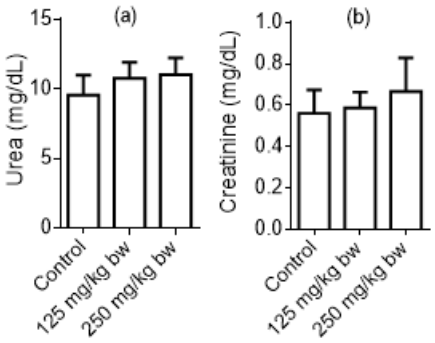

Figure 6. Effects of oral administration of HEBCS for six weeks on serum levels of (a) urea and (b) Creatinine in mice. *Each bar represents the mean ± SD (n = 5).*

4. DISCUSSION

In recent years, research efforts have focused on harnessing the therapeutic potentials of natural pharmacological agents (24). Food scientists and nutritionists are often interested in polyphenolic content and antioxidant capacity of plant-derived extracts among other therapeutic potentials offered by these extracts (25). Interests in antioxidants derived from their ability to prevent oxidative stress and protect against diseases with pathophysiology involving free radicals and oxidative damage (26). *B. coriacea* seed has been widely applied in traditional medicine to treat a number of health conditions (27). Several solvent extracts of the seed have been evaluated for their phytoconstituents and therapeutic potentials (27). In the present study, we prepared the hydroethanolic extract of defatted *B. coriacea* seeds, evaluated the total polyphenolic content, antioxidant activity in vitro and its toxicity potential in rats and mice.

Medicinal plants are important source of natural antioxidant substances such as the polyphenols. Plant phenols and flavonoids are vital components of the human diet due to their antioxidant activity and ability to alleviate oxidative stress-induced damage often associated with certain chronic diseases (28, 29). There exist a strong correlation between the plant polyphenol contents and antioxidant activity they exhibit (30). Data from the current study show that hydroethanolic extract of (defatted) *B coriacea* seeds (HEBCS) is rich in polyphenols. Polyphenols have been associated with antioxidant activity and a vast number of other biological activities (28,29). Our data also show that the total phenols and flavonoids contents of HEBCS are comparable with some previous report on aqueous extract of *B coriacea* seeds (11).

Free radicals are important factors in the development of most acute and chronic diseases. In the present study, we investigated the effect of HEBCS against selected free radicals and reactive oxygen species *in vitro.* Our data show that HEBCS possess a good anti-radical activity against DPPH, ABTS and OH radicals. This is in agreement with those reported earlier for aqueous extract (11) and methanol extract (31) of *B. coriacea*. The observed antioxidant activity of HEBCS in terms of DPPH, ABTS and OH scavenging potentials may be related to the high quantity of phenols and flavonoids it contains.

The extract also shows antioxidant activity against two reactive oxygen species (ROS): H_2O_2 and NO. Under physiological condition, H_2O_2 is produced from the action of superoxide dismutase (26). NO, on the other hand, is vital to the control of vasodilation, signalling and inflammatory response (32). NO scavengers compete with oxygen and inhibit the production of nitric oxide. Under physiological conditions, superoxide radical and H_2O_2 react to produce other forms of ROS such as hydroxyl radical and singlet oxygen (26). From the IC_{50} values

obtained in this study, it may be speculated that HEBCS can protect against the damaging effects of ROS under cellular conditions.

The safety of a potential drug material is vital to its potential applications as a therapeutic agent. The limit dose evaluation of HEBCS in rats show that its LD_{50} value is greater than 5000 mg/kg. This is in agreement with the values obtained in previous studies on other solvent extracts of *B coriacea* seeds (33-35). This is an indication of the high safety profile of HEBCS and its wide application in traditional medicine. Furthermore, subacute toxicity evaluation in rat and subsequent subchronic toxicity study in mice also showed no statistically significant hepatic or renal toxicity. This indicates that HEBCS at the doses evaluated may be well tolerated during its use in the treatment of diseases.

5. CONCLUSION

In conclusion, our data show that HEBCS is rich in polyphenols and the extract also showed relevant antioxidant property against free radicals and reactive oxygen species. In addition, data from this study shows that HEBCS is relatively safe with respect to hepatic and renal function. These provide experimental support for the applications of *B coriacea* in traditional medicinal. Further studies including molecular docking studies on HEBCS may open up new insight into its potential therapeutic application.

COMPETING INTERESTS

The authors declare no conflict of interest.

ETHICAL APPROVAL

The study was approved by the Faculty of Natural Sciences Ethical Review Committee, Ajayi Crowther University (FNS/ERC/201700016).

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ABBREVIATIONS

$ABTS^{\bullet+}$:2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salts radical cation;
ALP: alkaline phosphatase
ALT: alanine aminotransferase
AST: aspartate aminotransferase;
bw: body weight;
dH_2O: distilled water;
DPPH: 1,1-diphenyl-2-picrylhydrazyl;
EtOH: ethanol;
GAE: gallic acid equivalent;

H_2O_2: hydrogen Peroxide;
HEBCS: hydroethanolic extract of Buchholzia coriacea seeds;
IC_{50}: half-maximal inhibitory concentration;
LD_{50} :median lethality dose;
NO: nitric oxide;
$^{\bullet}OH$:hydroxyl radical;
p.o.: per oral;
p-NPP: p-nitrophenylphosphate;
QE: quercetin equivalent; rpm: radian per minute;
SD: standard deviation
w/v: weight per volume