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Comparative phytochemical and antioxidant profile of crude seed powder, aqueous and methanolic seed extracts of *Buchholzia coriacea*

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Abstract

The increasing discovery of more medicinal plants have triggered increased scientific screening of their bioactivity in order to provide data that will help physicians and patients make wise decision before using them. This study was designed to elucidate comparative phytochemical and antioxidant properties of crude seed powder, aqueous and methanolic seed extracts of *Buchholzia coriacea.* The results showed that crude seed powder had the highest alkaloid and phenol content of 3.98±0.00% and 0.92±0.00%, while aqueous extract had the least alkaloid and phenol contentof 1.00±0.00% and 0.12±0.00%respectively. Methanolic extracts had the highest phytochemical components among the three extracts with flavonoids (12.03±0.0), saponins (1.99±0.01), terpenoids (2.00±0.00), tannin (0.10±0.00)and phytate(2.02±0.01) compositions, while aqueous extracts had the highest hydrogen cyanide (0.30±0.00) and glycoside (0.35±0.00). Antioxidant (DPPH) activities of *B. coriacea* showed that aqueous extract and crude seed powder had inhibition concentration (IC_{50}) of 4.65 mg/ml while methanolic extract had IC_{50} of 5.85 mg/ml. The result of the LD_{50} of the extracts showed the each extracts was well tolerated at a dose of 5000 mg/kg, an indication of high safety profile. The study therefore clearly demonstrated that methanolic extracts of *B. coriacea* have antioxidant, antihypertensive, hypocholesterolmic and anticarcinogenic properties owing to the presence of high levels of phytochemical components than the aqueous and crude seed powder.

Keywords: Phytochemical, Crude seed powder, Aqueous and methanolic extracts, *Buchholzia coriacea*

Introduction

The increasing discovery of more medicinal plants have triggered increased scientific screening of their bioactivity in order to provide data that will help physicians and patients make wise decision before using them [1]. Approximately 119 pure chemical substances extracted from higher plants are used in medicine throughout the world [2]. It has been reported that more than 80 % of the population in developing countries depends on plants for their medical needs [3] and about 2/3 of all the medicinal plant species are found in the tropics [4].

However, *Buchholzia coriacea* popularly known as wonderful kola is one of the medicinal plants that have been used in different ways as an alternative medication to promote people health in Nigeria, Africa and other parts of the world. It is a forest tree with large, glossy, leathery and conspicuous leaves at the end of the branches [5]. The common name of the plant is "magic kola" while the English name is "musk tree" [6]. In Nigeria, *B.coriacea* is known as "*Uworo", "Owi"* and *"Uke*" among Yoruba, Edo and Igbo tribes respectively [5]. Other common names include "*Ovu*" in Benin, and "*Aponmu"* in Akure [5]. Among central Africans, the fruit is known as "*esson bossi*" [7]. The plant is widely distributed in most African countries, and its parts have been used to treat a variety of sickness in most rural communities in Nigeria. Some of the diseases locally treated with wonderful kola include fever, gonorrhoea and gastrointestinal infections [5], [8], [9]. Topical application of wonderful kola leaves and fruits on the body are used to relieve fever in Sierra Leone while the seeds are applied on the stomach to manage difficult child-birth in Abidjan [10]. The use of wonderful kola plant parts in some traditional medicine practices have been credited to some bioactive substances which they contain in rich amounts [5][8]. It is therefore the aim of this study to elucidate comparative phytochemical and antioxidant screening of

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crude seed powder, aqueous and methanolic seed extracts of *B.coriacea.*

Materials and methods

Procurement of plant material

The seeds of *B.coriacea* were purchased from local markets around Nsukka metropolis, Nsukka Local Government Area of Enugu State, Nigeria. The seeds were identified [11] and authenticated by Mr. A.O. Ozioko of Bioresources Development and Conservation Programme (BDCP), Nsukka, Enugu State, Nigeria. Voucher specimen number (INTERCEDD/ 709) was deposited in the BDCP herbarium. The seed was deshelled, cleaned and cut into small sizes, air dried at room temperature until a constant weight was obtain (7 days)and pulverized into fine powder using a grinding machine (Honda: model 622, China).

Preparation of extracts

The method of extraction followed that of Okere *et al*. [12]. Two hundred gram (200 g) and 800 g of the powdered seeds were soaked in 500 ml of water and 700 ml of methanol respectively for 48 hours with intermittent shaking. The soaked substance were filtered using what man No. 1 filter paper and concentrated using rotary evaporator. The concentrated sample were further subjected to dryness using hot air oven set at 60 ^{0}C and then stored in an airtight bottle in a refrigerator for subsequent use. The crude powder was gotten by sieving the ground powder samples through muslin cloth (0.7 mm mesh size). The aqueous and methanolic extracts gave 19.18 and 5.17 % yield respectively.

Phytochemical analysis

Phytochemical analysis for the qualitative detection of biologically active compounds such as saponins, alkaloids, tannins, flavonoids, steroids, terpenoids, glycosides, cyanide and phytate on the crude powder, aqueous and methanolic seed extracts of *B. coriacea* were determined using the methods of Sofowara [13], Trease and Evans [14] and Harborne [15], while quantitative was determined using the methods of Pearson [16], Obadoni and Ochuko [17], Onwuka [18], Subhadhirasakul and Pechpongs [19], El-Olemy *et al*. [20] and Oberleas [21].

Determination antioxidant potential of crude powder, aqueous and methanolic seed extracts of *B. coriacea*

In order to evaluate the antioxidant potential through free radical scavenging by the test samples, the change in optical density of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals was monitored. This was determined using the method of Manzocco *et al*. [22]. The sample of each extract (0.2 ml) is diluted with methanol and 2 ml of DPPH solution (0.5 mM) is added. After 30 minutes, the absorbance is measured at 517 nm. The percentage of the DPPH radical scavenging is calculated using the equation:

% inhibition of DPPH radical = $[(A_{br} - A_{ar})]/ A_{br}) X100$,

where: A_{br} is the absorbance before reaction and A_{ar} is the absorbance after reaction has taken place.

Acute toxicity study

The lethal dose (LD_{50}) of the crude powder, aqueous and methanolic extracts was determined according to the method of Lorke [23]. A total of sixty three (63) mice were used for each for the study in two phases. For the first phase, twelve (12) mice were divided into 4 groups (A, B, C and D) of 3 mice per group, and treated with each extracts at the doses of 100, 200, 400 and 800 mg/kg respectively. The animals were observed for 2 hours, intermittently for 6 hours and over a period of 24 hours for signs of toxicity. For the second phase, nine (9) mice were divided into 3 groups (A, B and C) of 3 mice per group and treated with each extracts at the doses of 1000, 3000 and 5000 mg/kg respectively. They were observed for signs of toxicity for 24 hours. The numbers of animals dead within 24 hours after the administration were recorded for each group. The lethal dose was calculated as the arithmetic mean of the dose that killed the least number of animals and the one next to lower dose that did not kill any animal.

Statistical Analysis

Results obtained were presented as mean ± S.E.M and statistical evaluation of the quantitative phytochemicals of different extracts used was done using one-way analysis of variance (ANOVA) while Duncan's Multiple Range Test (DMRT) was used to test the significant differences among the extracts and level of significance was set at $p< 0.05$ [24].

Results

Qualitative and quantitative phytochemical composition of the crude seed powder, aqueous and methanolic seed extract of *B. coriacea*

Table 1 and 2 showed qualitative and quantitative phytochemical composition of aqueous, methanolic and crude seed powder of *Buchholzia coriacea*. Qualitatively, it was observed that crude seed powder had the highest alkaloid content followed by similar alkaloid contentsin methanolic aqueous extracts. Flavonoids were high and similar in all extracts. Furthermore, saponins, terpenoids, tannins, phenols and phytate were higher in methanolic than in crude powder and aqueous extracts. Steroids were absent in all the extracts. Hydrogen cyanide was present only in the aqueous

extract that also had higher level of cardiac glycoside than methanolic extract and crude powder of *B.coriacea* (Table 1).
Quantitatively, it was observed that crude seed powder had the highest alkaloid content followed by methanolic and aqueous extracts. The methanolic extract had significantly the highest flavonoid content, while the crude seed powder had the least flavonoid content, although, all the extracts had high concentrations of flavonoid (Table 2). For saponin, all the extracts were significantly different from each other, with aqueous extract having the least value, while the methanolic extract had the highest value (Table 2). The terpenoid content of methanolic extract was significantly higher (P<0.05) than the aqueous and crude powder extracts which had the similar terpenoid content. There was no significant difference (P>0.05) between the tannin content of aqueous and methanolic extracts of *B. coriacea*, but were significantly higher (P<0.05) than that of crude powder (Table 2). The phenolic content of crude seed powder of *B. coriacea* was highest while the aqueous extract was lower and the phenolic content of the three extract are significantly difference (P>0.05) from each other (Table 2). Methanolic extract has the highest phytate content while the aqueous extract had the lowest and they were significantly different (P<0.05) from each other. There were no significant difference (P>0.05) among steroid content of the three extract, although the levels were very low. The aqueous extracts had the highest cyanide content, while the methanolic extract had the least cyanide content, and were significantly different from one another. Similarly, the glycoside content of aqueous extracts was the highest while that of crude seed powder was the lowest and they were significantly different from one another (Table 2).

Table-1: Qualitative phytochemical screening of the crude seed powder, aqueous and methanolic seed extract of *B. coriacea*

S.No	Phytochemicals	Aqueous	Methanol	Crude powder
1	Alkaloids	++	++	+++
2	Flavonoids	+++	+++	+++
3	Saponins	+	+++	++
4	Terpenoids	+	+++	+
5	Tannin	+	++	+
6	Phenol	+	++	++
7	Phytate	+	+++	++
8	Steroids			
9	Hydrogen cyanide	+	-	-
10	Cardiac glycoside	++	+	+

+++ = High concentration, ++ = Moderate concentration, + = Trace concentration, -= Absent

Table-2: Quantitative phytochemical screening of the crude seed powder, aqueous and methanolic seed extract of *B. coriacea*

S. No	Phytochemicals	Composition (%)		
		Aqueous Extracts	Methanolic Extracts	Crude powder
1	Alkaloids	1.00±0.00^{a}	1.49±0.01^{b}	3.98±0.01^{c}
2	Flavonoids	8.03±0.01^{c}	12.03±0.01^{b}	6.10±0.06^{a}
3	Saponins	0.50±0.00^{a}	1.99±0.01^{c}	0.99±0.01^{b}
4	Terpenoids	0.50±0.00^{a}	2.00±0.00^{b}	0.50±0.00^{a}
5	Tannin	0.09±0.00^{a}	0.10±0.00^{a}	0.02±0.00^{b}
6	Phenol	0.12±0.00^{a}	0.68±0.00^{b}	0.92±0.00^{c}
7	Phytate	0.26±0.00^{a}	2.02±0.01^{c}	0.75±0.00^{b}
8	Steroids	0.02±0.00 ^{a}	0.01±0.00 ^{a}	0.02±0.00^{a}
9	Hydrogen cyanide	0.30±0.00 ^{c}	0.06±0.00 ^{a}	0.08±0.00 ^{b}
10	Glycoside	0.35±0.00 ^{c}	0.11±0.01 ^{a}	0.14±0.00 ^{b}

Values represent mean±SEM of 3 observations. Values with the same letter superscript on the same row are not significantly different (P>0.05), Values with different letter superscript on the same row are significantly different (P<0.05).

Antioxidant activity of crude seed powder, aqueous and methanolic seed extract of *B. coriacea*

The result of the antioxidant activities of crude seed powder, aqueous and methanolic seed extracts of *B. coriacea* indicated that the aqueous extracts and crude seed extracts of *B. coriacea* had similar inhibition concentration (IC_{50}) of 4.65 mg/ml that were lower than that of methanolic extracts (5.85 mg/ml).

Table-3: Antioxidant activity of crude seed powder, aqueous and methanolic seed extract of *B. coriacea*

S. No	Conc. (mg/ml)	DPPH radical scavenging activities (%)		
		BCAE	BCME	BCCP
1	1.00	14.38	10.59	23.14
2	2.00	26.61	13.11	29.15
3	3.00	33.92	24.21	39.63
4	4.00	37.71	24.97	45.20
5	5.00	42.37	36.74	47.38
IC_{50} (mg/ml)		4.65	5.85	4.65

BCAE=*Buchholzia coricea* aqueous extract, BCME= *Buchholzia coricea* methanolic extract, BCCP= *Buchholzia coricea* crude powder

Acute toxicity studies of crude seed powder, aqueous and methanolic seed extract of *B. coriacea*

There was no mortality at different doses (100, 200, 400 and 800 mg/kg) of aqueous extract, methanol extract and crude seed powder of *B. coriacea* after 24 hours in phase one (Table 4). Also, at dose levels of 1,000 mg/kg, 3,000 mg/kg and 5,000 mg/kg, there was no death recorded, although, cage side behaviours such as shivering, bulging of eyes, sedation, depression and dullness were observed in the animals.

Table-4: Acute toxicity of crude seed powder, aqueous and methanolic seed extract of *B. coriacea*

Doses (mg/kg)	Mortality recorded		
Phase 1	Aqueous extract	Methanolic extract	Crude seed powder
100	0/3	0/3	0/3
200	0/3	0/3	0/3
400	0/3	0/3	0/3
800	0/3	0/3	0/3
Phase 2			
1000	0/3	0/3	0/3
3000	0/3	0/3	0/3
5000	0/3	0/3	0/3

Discussion

Phytochemicals

The curative properties of plant derived medicine are due to the presence of bioactive components of varied composition in one or more parts of the plants. The phytochemical screening of aqueous extract, methanol extract and crude seed powder of *B. coriacea* indicated the presence of some phyto-compounds such as alkaloids, tannins, flavonoids, steroids, saponins, glycosides, phytate, cyanide and terpenes. These are the most important bioactive constituents of plant [25]. Alkaloids, as a component, have therapeutic activity as it is a primary natural phyto-component of plants. The *B. coriacea* extracts had moderate concentration of alkaloids indicated that it may be used as sedative, pain-relieving drug, anaesthetic, analgesic etc. [26][27]. Alkaloids also have medical, pharmacological and veterinary importance. Alkaloids belonging to beta-carboline group have been reported to possess antimicrobial, anti-HIV and antiparasitic activities [28]. Alkaloids have a wide range of pharmacological activities including antimalarial (e.g. quinine), anticancer (e.g. homoharringtonine), antiasthma (e.g. ephedrine)[29], cholinomimetic e.g. galantamine) [30], antiarrhythmic (e.g. quinidine), vasodilatory (e.g. vincamine), analgesic (e.g. morphine) [31], antibacterial (e.g. chelerythrine) [32] and antihyperglycemic (e.g. piperine) [33]. Manyhave found use in traditional or modern medicine, or as starting points for drug discovery. Other alkaloids possess psychotropic (e.g.psilocin) and stimulant activities (e.g. theobromine, cocaine, nicotine, caffeine) and have been used as recreational drugs[34].

Alkaloids can also be toxic (e.g. tubocurarine, atropine) [35]. Although alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly evoke a bitter taste [36] and in some cases may cause serious illness, injury or even death. The manner of poisoning with plants alkaloids can be divided into unintentional ingestion of plant material, intentional ingestion of plant material, and ingestion of abused plant material [37].

The flavonoid contents were in abundance in all the extracts. The presence of flavonoids in the extract showed that the plant have antioxidant properties; this could enhance the body's defense systems against pathologically induced free-radical generation as well as modify the body's reactions to allergens and viruses [38]. Flavonoids have been demonstrated to have a wide range of biological and pharmacological functions in *in-vitro*

studies. These include anti-inflammatory [39][40], anti-allergic [39], antioxidant [38][40], anti-microbial [41][42][43][44], anti-cancer [40][45] and anti-diarrheal activities [46]. Flavonoids have also been shown to inhibit topoisomerase enzymes [47][48] and to induce DNA mutations in the mixed-lineage leukaemia gene in *in-vitro* studies [49]. However, for most of the cases cited above, no follow up *in-vivo* or clinical research has been carried out thus leaving it impossible to affirm if these activities have any detrimental or beneficial effect on human health.

Saponins and phenols in food medicine and mastic ants contribute to the low rate of atherosclerosis and coronary heart disease [50]. Similarly, saponins from soybean have been shown to have hypocholesterolmic as well as anticarcinogenic effects [51]. Saponins consumption often times causes deleterious effects such as haemolysis and permeability of the intestines [52]. Thus, the methanolic extract and crude seed powder of *B. coriacea* that had abundant and moderate amount of saponins indicated the possibility of using these extracts in the management of cardiovascular diseases [50].

Tannins have biological properties that may favour the prevention and management of many ailments [53]. However, tannins may decrease protein quality by reducing palatability and digestibility; they have good antimicrobial and anti-inflammatory activities and thus are used as alternative to antimicrobial growth promoter factors in poultry [54]. Excess tannins may be toxic because tannins as metal ion chelators can decrease the bioavailability of iron which often leads to anaemia [55]. Therefore, the presence of tannin in the plant extract showed that the extract especially the methanolic extracts that had moderate amount may play a very important therapeutic role in the field of medicine. Thus, *B. coriacea* could be used in the management of viral, bacterial and fungal diseases.

Phytic acid has been reported to lower the nutritional value due to its limiting effects on the bioavailability of dietary minerals and essential trace elements (e.g. iron, zinc, calcium) in human intestine [56]. They equally possess antioxidant, anticarcinogenic and hypoglycaemic properties [57]. The high and moderate levels of phytate in the methanolic extracts and crude seed powder of *B. coriacea* showed that it could be used in the treatment of cancer and diabetes mellitus.

Steroids to some extent maintain hormonal balance by serving as a precursor or potent material in the synthesis of sex hormone [58]. Thus, the aqueous, methanolic extracts and crude seed powder of *B. coriacea* having trace amount of steroids is an indication that the plant could not be used in management of hormonal imbalance. More so, the aqueous seed extract *B. coriacea* contains very low cyanide content while cyanide was absent in methanolic extract and crude seed powder, which means that *B. coriacea* seed is not poisonous, and could serve as food or used as a herbal medicine.

Terpenoids and phenols have antioxidant properties and protect cells from oxidative damage [59]. Therefore, the presence of both terpenoids and phenols in all the extracts especially the methanolic extracts that have reasonable quantity of terpenoids, *B. coriacea* seed extracts could be used as an antioxidant in the management of oxidative damage.

Cardiac glycosides are found as secondary metabolites in several plants and are used in the treatment of congestive heart failure and cardiac arrhythmia. Furthermore, several cardiac glycosides such as peruvoside have been used in cancer control, especially ovary cancer and leukaemia [60].Aqueous extract of *B. coriacea* has moderate amount of cardiac glycoside while methanolic and crude seed powder have trace quantity. Thus, the plant could be used in the management of heart failure, cancer and leukaemia.

Antioxidant activities

Natural antioxidants that are present in plants are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. In the present study, we have evaluated the free radical scavenging activities of crude seed powder, aqueous and methanolic extracts of *B. coriacea* seed. However, among the three extracts tested for *in-vitro* antioxidant activity using the DPPH method, it was observed that aqueous extracts and crude seed powder of *B. coriacea* had equal and greater inhibition concentration (IC_{50}) of 4.65 mg/ml than that of methanolic extract which has 5.85 mg/ml. It is obvious that the components like tannins, saponins and other phyto-constituents of the extracts may be responsible for such activity. Several of such compounds are known to possess potent antioxidant activity [61]. Antioxidant properties of medicinal plants have been documented by several researchers [22], [38], [59], [61]. The present research however, corroborates that of Nwaehujor *et al.* [62] on the antioxidant properties of *B. coriacea* fruits. The study has therefore clearly demonstrated that methanolic extracts could have antioxidant, antihypertensive, hypocholesterolmic and anticarcinogenic effects thus; high medicinal properties owing to the presence of higher phytochemical components and antioxidant activities than the aqueous and crude seed powder.

Acute toxicity

The results of the toxicity study showed that all the *B. coriacea* extract used for the research had no lethal effect on the tested experimental animals, since no dearth was recorded at the dose of 5000 mg/kg, thus the plant has a wide safety margin in mice and may be save for human consumption. The finding of this study is similar to earlier reports that no death was recorded in the acute toxicity study carried out with *B. coriacea* seed extract [62][63][64]. Also, this work also agrees with Adisa *et al.* [6] who observed that no death was recorded after the toxicity study and therefore concluded that LD_{50} of *Buchholzia coriacea* is greater than 30,000 mg/kg body weight.

Conclusion

Buchholzia coriacea extracts are rich sources of phytochemicals such as alkaloids, flavonoids, saponins, terpenoids, tannin, phenol, phytate, steroids, hydrogen cyanide and glycoside. The crude seed powder had the highest alkaloid content followed by methanolic and aqueous extracts. The methanolic extract had the highest flavonoid content, while the crude seed powder had the least flavonoid content, although, all the extracts had high concentrations of flavonoid. The extracts have antioxidant properties and as such can be used in combating or preventing the deleterious consequences of oxidative stress in animals exposed to it. The extracts were non-toxic as such the seed can be used as food and as medicine. *B. coriacea* extracts have been demonstrated to have a wide range of biological and pharmacological activities thus: anti-inflammatory, anti-allergic, antioxidant, anti-microbial, anti-cancer, anti-diarrheal, anti-leukaemia, anti-topoisomerase, induction of DNA mutations, contribute to the low rate of atherosclerosis and coronary heart disease, are hypocholesterolmic as well as anticarcinogenic effects, used in the management of cardiovascular diseases among many others. These are the reasons why it is nicknamed “wonderful kola”.

Conflicts of Interests

Non to declare

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