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Phytochemical and Chemical Properties of Raw, Cooked and Dried Seeds of *Buchholzia coriacea*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The qualitative phytochemical analysis of water and methanolic extracts of dried seed of *Buchholzia coriacea*, proximate composition and mineral contents of raw, cooked and dried seed samples were determined using standard methods. The phytochemicals obtained from both water and methanolic extracts of dried seed were alkaloids, tannins, flavonoids, saponins, cardiac glycosides, glycosides, reducing sugars, steroids, saponin glycosides, phenolics and terpeniods. The proximate contents of the raw, cooked and dried seed samples showed that the concentrations of the nutrients followed the order dried > raw > cooked. It was also observed that the carbohydrate content recorded the highest values in the three samples: raw (50.03 ± 0.035%), cooked (28.07 ± 0.007%) and dried (69.04 ± 0.007%). The mineral contents of the seed followed the order K>Na>Mg>P>Ca>Zn>Mn. It was also revealed that potassium content recorded the highest value in raw (6400.50 ± 0.70 mg/kg), cooked (8100.25 ± 0.35 mg/kg) and dried (28400.50 ± 0.70 mg/kg).

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Therefore, seed of *Buchholzia coriacea* is a good source of energy and can also be used as functional food.

Keywords: Phytochemical; chemical properties; seeds; food; energy; Buchholzia coriacea.

1. INTRODUCTION

Herbal medicine is an alternative form of medicine acceptable worldwide. It encompasses the use of plant materials in the diagnosis, prevention and treatment of physical, mental and social diseases [1]. Relevant information on the usefulness of herbal medicine have been traced to past experiences and observations documented since the creation of man [2]. Scientific validation of plant coupled with useful ethno-medicinal information is required for the development of alternative therapies to synthetic drugs [3].

Plants have basic nutritional importance by their content of protein, carbohydrates, fats and oils, minerals, vitamins and water responsible for growth and development in man and animals. In addition to vitamins and pro-vitamins in fruits and vegetables, the presence of bioactive plant components often called phytochemicals has been considered of crucial importance in the prevention of chronic diseases such as cancer, cardiovascular disease such as diabetics [4].

A wide range of plants derived dietary supplements, phytochemicals and pro-vitamins that assist in maintaining good health and combating diseases are now been described as functional foods, nutriceuticals and nutraceuticals. According to WHO more than 80% of the world population relies on traditional medicines for their primary health care needs [5]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body [6]. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds.

Wonderful kola is one of these plants that is claimed to have many bioactive compounds [7]. Buchholzia coriacea is locally and popularly called wonderful kola and it belongs to the plant family capparaceae [8]. The plant parts commonly eaten are the seeds which are eaten either cooked or eaten raw [9]. Wonderful kola is known worldwide as memory nut because it enhances the memory [7]. It acts as cleanser of the blood, facilitates learning absolutely and strengthens the nervous system and is also effective in the treatment of menstrual problems [7]. It is also useful in the treatment of hypertension and also prevents premature aging. Phytochemical, antispasmodic and antidiarrhoeal properties of the methanolic extract of the leaves of Buchholzia coriacea has been reported [10].

Therefore, since the seed of wonderful kola is commonly eaten raw or cooked, there is a need to evaluate the nutritive quality of the raw, cooked and dried seeds in order to know if heat treatment could alter the nutritive quality of the seed. Therefore, this research explored the phytochemical constituents of water and methanolic extracts of wonderful kola seed and also evaluate the proximate and mineral content of the raw, cooked and dried seeds.

2. MATERIALS AND METHODS

2.1 Collection of *Buchholzia coriacea* Seeds

Fresh seeds of *B. coriacea* were bought at Lusada Market, Ado-Odo Ota Local Government, Igbesa, Ogun State, Nigeria. The seeds were identified and authenticated by a Botanist in the Department of Biological Sciences, Crawford University, Faith City, Igbesa, Ogun State, Nigeria.

2.1.1 Processing of the seeds

The seeds were washed and divided into three parts - namely, raw, cooked and dried seeds. The raw seeds were chopped into pieces and grounded into paste using mortar and pestle. The seeds meant for cooking were chopped and cooked for 2 hours, while the seeds for drying were sliced and dried in a hot air oven at 50 ^{o}C for 48 hours. The dried seeds were then cooled in a desiccator and grounded into powder using mortar and pestle. The raw, cooked and dried samples were directly used for the proximate analysis and mineral content determination.

2.1.2 Extraction of plant materials

One hundred grams of *B. coriacea* powdered seed was separately placed in two conical flasks

containing 1000 cm^3 of water and methanol, followed by mixing and agitation for 6 hours before allowed to stand for 24 hours.

The mixtures were filtered using muslin cloth and filter paper and then concentrated into dry extracts by heating in water bath at 50℃. The dry extracts were scrapped off using spatula and then used directly for qualitative phytochemical screening.

2.2 Phytochemical Screening of Plant Extracts

2.2.1 Test for alkaloids

Exactly 0.5g of each extracts were stirred with 10 cm^3 of 10% hydrochloric acid and allowed to stand overnight. Two drops of Wagner's reagents were added to 1cm^3 of the extracts. A reddish brown precipitate observed indicated the presence of alkaloids [11].

2.2.2 Test for tannins

Few drops of $FeCl_3$ (10% w/v) solution were added to 3 cm^3 of the extracts in a test tube followed by shaking. A dirty green or dark blue coloration confirmed the presence of tannins [11].

2.2.3 Test for flavonoids

One millilitre of the extracts was treated with 1 cm^3 of dilute NaOH. The presence of a cloudy precipitate confirmed the presence of flavonoids [11].

2.2.4 Test for saponins

A 5 cm^3 of distilled water was added to the 2 cm^3 of the extracts in a test tube and shaken vigorously. The formation of foams or stable frothing following the shaking indicated the presence of saponins [11].

2.2.5 Test for cardiac glycoside

This test is called Keller-Killianis test. To 1 cm^3 of the extract, 2 cm^3 of 3.5% ferric chloride solution was added and allowed to stand for 1 minute. One centimeter cube (1 cm^3) of concentrated H_2SO_4 was carefully poured down the wall of the tube so as to form a lower layer. A reddish brown ring at the interface indicated the presence of cardiac glycoside [11].

2.2.6 Test for anthraquinones

Two milliliters of 10% hydrochloric acid was added to the extract in a test tube and boiled for about 2 minutes. Equal amount of chloroform was added to the test tube and vortexes twice. The chloroform layer was pipetted out and then equal volume of 10% ammonia was added. A pinkish red colour observed in upper layer indicated the presence of anthraquinones [11].

2.2.7 Test for glycosides

Exactly 2.5 cm^3 of 50% sulphuric acid was added to 5 ml of the extract in a test tube. The mixture was heated in boiling water for 15 minutes, cooled and neutralized with 10% NaOH. Then 5ml of Fehling's solution was added and mixture was boiled. A brick-red precipitate was observed which indicated the presence of glycosides [11].

2.2.8 Test for reducing sugars

One millilitre each of Fehling solution I and II were added to 2 cm^3 of the aqueous solution of the extract. The mixture was then heated in a boiling water bath for about 5 minutes. The production of a brick-red precipitate indicated the presence of reducing sugars [11].

2.2.9 Test for steroids

Exactly 2 cm^3 of acetic anhydride was added to 0.5 g water extract of each sample with the addition of 2 cm^3 H_2SO_4. A colour change from violet to blue or green indicated the presence of steroids [11].

2.2.10 Test for volatile oils

One (1 cm^3) of the fraction was mixed with dilute HCl. A white precipitate was formed which indicated the presence of volatile oils [11].

2.2.11 Test for saponin glycosides

To 2.5 cm^3 of the extract was added 2 cm^3 of Fehling's solution I and II. A bluish green precipitate showed the presence of saponin glycosides [12].

2.2.12 Test for phenolics

Half a gram (0.5 g) of the powdered dried seeds of each sample was boiled with 10 cm^3 of distilled water for 5 minutes and filtered white hot. Then 1 cm^3 of ferric chloride solution was added.

Formation of blue-black or brown coloration indicated the presence of phenol [11].

2.2.13 Test for terpenoids

Five centimetre cube (5 cm^3) of each extract was mixed with 2 cm^3 of chloroform. Three centimeter cube (3 cm^3) of concentrated H_2SO_4 was then added to form a layer. A reddish brown precipitate coloration at the interface formed indicated the presence of terpenoids [11].

2.3 Proximate Analysis

2.3.1 Moisture content determination

Two grams of the sample was weighed into reweighed aluminum drying dish. The sample was dried to a constant weight in an oven at 105℃ for 4 hours [13].

$$\text{Moisture content } (\%) = \frac{W_1 - W_2}{W_1 - W_0} \times 100 \quad (1)$$

Where W_o = weight of aluminum dish
W_1 = weight of fresh sample + dish
W_2 = weight of dried sample + dish

2.3.2 Total ash

Ash content was determined by putting 2 g of sample in a tarred crucible in a furnace and then igniting the furnace at about 450 ^oC for 2-3 hours. The furnace was switched off and opened after the temperature has reduced. The crucible was then transferred to a desiccator with the aid of crucible tongs and allowed to cool prior to weighing [13]. The percent ash content was calculated as follows:

$$\% \ \text{Ash} = \frac{W_3 - W_1}{W_2 - W_1} \times 100 \quad (2)$$

Where W_1= Weight of empty crucible
W_2= Weight of original sample
W_3= Weight of ashed sample

2.3.3 Crude fibre

Defatted sample of 3.5 g was transferred into a 500 cm^3 conical flask, 200 cm^3 of boiling 1.25% H_2SO_4 was added and mixture brought to boiling within a minute and allowed to boil gently for 30 minutes. The mixture was then filtered using filter paper by suction in conjunction with Buchner funnel. The separated material on the filter paper was rinsed well with hot distilled water and transferred back into the flask using spatula. 200 cm^3 of boiling 1.25% NaOH and few drops of antifoaming agent were added; the sample was then brought to boiling within a minute and allowed to boil for 30 minutes as done above. The sample was filtered and washed with hot distilled water four times and once with 10% HCl. The washing with distilled water was repeated, followed by methylated spirit petroleum ether. The residue is then transferred into the dried, weighed crucible and dried in the oven at 105℃. The dried residue was cooled in desiccators and weighed. The crucible was placed in muffle furnace at about 300℃ for 30 minutes. The furnace is switch off, the temperature allowed to go down and the crucible transferred into the desiccator. The crucible was then weighed [13]. The percent crude fibre was calculated as follows:

$$\% \ \text{Crude fibre} = \frac{W_2 - W_3}{W_1} \times 100 \quad (3)$$

Where W_1= Weight of sample used
W_2= Weight of crucible plus sample
W_3= Weight of sample crucible plus ash

2.3.4 Crude fat

Crude fat was determined using Soxhlet method. Pre-dried sample of 2 g was weighed into a pre-dried extraction crucible. The weight of pre-dried boiling flask was determined before petroleum ether was added. The extraction was carried out for about 4 hours. Boiling flask with the extracted and solvent was dried in an air oven at 60℃ to remove the solvent. The flask was then cooled in a desiccator and weighed [13]. The percent fat was calculated as follows:

$$\%\, Fat\, on\, dry\, weight\, basis = \frac{gram\, of\ fat\ in\, sample}{gram\, of\ dried\ sample} \times 100 \quad (4)$$

2.3.5 Protein

The folin-lowry method of protein assay was used [14]. Five centimeter cube (5 cm^3) of the alkaline solution was added to 1 cm^3 of the samples (already centrifuged at 4000 rpm for 15 minutes) and 1 cm^3 serial dilutions of standard protein solution of 0. 2mg/1 cm^3 the mixture was mixed thoroughly and allowed to stand at room temperature for 10 minutes. Half a centimeter cube (0.5 $cm^{3)}$ of diluted folin-ciocaltea reagent was rapidly added and mixed immediately. The

mixture in test tubes was left for 30 minutes and the absorbance read on spectrophotometer at 750 nm. The concentrations of protein in the sample were obtained from standard curve.

2.3.6 Carbohydrate

Phenol sulphuric acid assay was used [15]. The samples were centrifuged at 4000 rpm for 15 minutes and 1 cm^3 of supernatant was used for carbohydrate determination. Each of these samples and 1 cm^3 of serial dilutions of standard glucose solution of 0.1mg/cm^3 were mixed with 1 cm^3 of 5% w/v phenolic acid solution. Concentrated H_2SO_4 of 5 cm^3 was rapidly and directly added to the solution surface without touching the side of the tube. The solution was left undisturbed for 10 minutes before vigorously shaken. The absorbance was taking at 490 nm after 30 minutes using spectrophotometer.

2.4 Mineral Determination

The mineral content of the raw, cooked and dried seeds of *B. coriacea* was analysed with the Atomic Absorption Spectrophotometer (AAS) described by Association of Official Analytical Chemists [13]. The minerals analyzed were phosphorus, manganese, zinc, potassium, calcium, magnesium, sodium and iron. 1g of the seed flour in crucibles was oven dried for each of the elements to be determined. The crucibles were placed in the muffle furnace and the temperature was gradually increased to 200 ^oC. The samples were ashed until they turned white. The crucibles were removed and cooled in desiccators, followed by dissolution in 6M HCl. The mineral in each solution was determined by reading in the atomic absorption spectrophotometer (AAS).

3. RESULTS AND DISCUSSION

3.1 Qualitative Phytochemical Analysis of *B. coriacea* Seeds

Table 1 showed that both water and methanolic extracts of dried seed of *B. coriacea* contained phytochemicals such as alkaloids, tannins, flavoniods, saponins, cardiac glycosides, glycosises, reducing sugars, steroids, saponin glycosides, phenolics and terpenoids. This result agreed with similar study reported by Ibrahim and Fagbohun [16]. The absence of anthraquinones and volatile oils in the both extracts may be connected with difference in polarity and thus different extractability.

The role of these phytochemicals to well-being of plants and health of animals cannot be over emphasized. The presence of alkaloids in seed of *B. coriacea* could be partly responsible for its uses as pain relievers and antimicrobial agents according to Heikens et al. [17]. The presence of tannins in seed of *B. coriacea* may contribute to its function as anti-diarrheia and anti-heamorrhage agent [18]. The hot taste of *B. coriacea* seed may be due to the presence of tannin because of its astringent properties. According to Anowi et al. [10], the use of the extract of *B. coriacea* in the treatment of intestinal trouble in herbal medicine may be connected with the presence of flavonoids which help against allergies, inflammation, free radicals, ulcers, microbes, viruses and tumor as reported by Okwu [19]. According to Evans, [11], the use of glycosides as cardiac stimulants may not be unconnected to the reason why the seed of *B. coriacea* is used in treatment of cardiac failure and cardiac disease [20].

Table 1. Qualitative phytochemical analysis of *B. coriacea* seeds

Bioactive compounds	Water extracts	Methanolic extracts
Alkaloids	+	+
Tannins	+	+
Flavonoids	+	+
Saponins	+	+
Cardiac glycosides	+	+
Anthraquinones	-	-
Glycosides	+	+
Reducing sugars	+	+
Steroids	+	+
Volatile oils	-	-
Saponin glycosides	+	+
Phenolics	+	+
Terpenoids	+	+

Key: + = present, - = absent

3.2 Proximate Composition of Raw, Cooked and Dried *B. coriacea* Seeds

The results of the proximate composition of raw, cooked and dried seeds of *B. coriacea* in Table 2 showed that wonderful kola can be categorized as carbohydrate food. The highest value in moisture content of cooked seed sample (67.74 ± 0.007%) as against the raw (64.46 ± 0.002%) and dried seed sample (4.55 ± 0.004%) are similar to the work done by Nwachukwu et al. [21].

The low in moisture content of dried seed sample will definitely retard microbial growth and improve the shelf-life of the seed [21]. Apart from the moisture content which is very low (4.55 ± 0.004%), the dried seed sample recorded highest values in other nutrients such as crude fat (2.01 ± 0.002), ash (3.69 ± 0.001%), crude fibre (3.00 ± 0.001%), protein (12.04 ± 0.035%) and carbohydrate (69.04 ± 0.007%). This is followed by raw and cooked seed samples respectively. This could be due to the removal of moisture from the seed thereby making the nutrients to be concentrated. The low values observed in cooked seed sample in all the nutrients except moisture content, may be due to leaching out of nutrients during cooking.

Carbohydrates play an essential role in human nutrition as energy reserves. The high content of carbohydrate in raw (50.03 ± 0.035%), cooked (28.07 ± 0.007%), and dried (69.04 ± 0.007%) samples showed that seed of *B. coriacea* is a good source of energy for both animals and humans.

3.3 Mineral Content of Raw, Cooked and Dried *B. coriacea* Seeds

The amount of ash content in food material is related to minerals [22,23]. Minerals are required for tissue functioning and necessary human nutrition [21]. The results of mineral content of the seed of *B. coriacea* as given in Table 3 followed the order K> Na > Mg> P> Ca> Zn> Mn>Fe. The result of mineral content of the raw, cooked and oven dried sample of the seed revealed that potassium content was the highest in the raw (6400.50 ± 0.70 mg/kg), cooked (8100.25 ± 0.35mg/kg), and dried (28400.50 ± 0.70 mg/kg), followed by sodium (2294.50 ± 0.70 mg/kg) and magnesium (1960.50 ± 0.70 mg/kg) of the dried seed samples respectively. However, the oven dried seed sample was the highest in all the minerals analyzed followed by cooked and raw seed samples respectively. The low in minerals content of cooked sample may be due to loss of minerals through leaching during processing .The high content of potassium obtained was not in agreement with the work reported by Ibrahim and Fagbohun, [16], but was in agreement with the report of Aremu et al. [24] that potassium is the predominant mineral in Nigeria agricultural products. This means that seed of *B. coriacea* could be a good source of potassium to people who are deficient in potassium and are therefore infected with diarrhea, alkalosis and hypokalamia. Although, excess intake of potassium rich food could lead to hyperkalamia in Addison's disease, advanced chronic renal failure and dehydration shock. The appreciable amount of sodium in dry seed sample of *B. coriacea* could also help to regulate acid-base balance and preservation of normal irritability of muscles and permeability of the cells. The low level of manganese (raw- 5.51±0.01, cooked-15.05±0.07, and dried- 26.10±0.14) and

Table 2. Proximate composition of raw, cooked and dried *B. coriacea* seeds

Parameters	Raw sample	Cooked sample	Dried sample
Moisture content (%)	64.46±0.002	67.74±0.007	4.55±0. 004
Crude fat (%)	1.86±0.001	1.33±0.004	2.01±0.002
Protein (%)	10.01±0.014	7.05±0.014	12.04±0.035
Ash content (%)	2.63±0.002	1.23±0.003	3.69±0.001
Crude fibre (%)	2.67±0.033	1.82±0.003	3.00±0.001
Carbohydrate (%)	50.03±0.035	28.07±0.007	69.04±0.00 7

The values are represented as means of duplicate result ± S.D

Table 3. Mineral content of raw, cooked and dried *B. coriacea* seeds

Parameters	Raw sample	Cooked sample	Dried sample
Sodium (mg/kg)	0.00	51.10±0.14	2294.50±0.70
Potassium (mg/kg)	6400.50±0.70	8100.25±0.35	28400.50±0.70
Calcium (mg/kg)	140.50±0.70	200.50±0.70	210.40±0.57
Magnesium (mg/kg)	51.15±0.21	600.50±0.70	1960.50±0. 70
Zinc (mg/kg)	24.10±0.14	29.50±0.007	61.52±0.02
Iron (mg/kg)	0.00	0.00	0.00
Manganese (mg/kg)	5.51±0.01	15.05±0.07	26.10±0.14
Phosphorus (mg/kg)	314.31±0.57	27.51±0.01	451.42±0. 03

The values are represented as means of duplicate results ± S.D

absent of iron in the seed suggests that the seed cannot be used as a substitute for blood forming agent as it fell below RDA values [25]. It was also observed that there was low level of zinc (61.52 ± 0.02), calcium (210.40 ± 0.57) and phosphorus (451.42 ± 0.03) of the dried seed sample. This agreed with the work reported by Ibrahim and Fagbohun [16].

4. CONCLUSION

The study showed that water and methanolic extracts of *B. coriacea* seeds contained phytochemical substances that may be used for treatment of diseases and maintenance of good health. The results also showed that *B. coriacea* contains high percentage of carbohydrate, protein and fat when eaten raw, cooked and dried for medicinal values and this will make it a good source of energy. The mineral composition obtained was observed to be related to its low ash content and loss of nutrients through leaching was also observed during processing. The presence of potassium in the seed in appreciable amount could serve as a source of potassium for people who are deficient in potassium. The dried sample of the seed contained higher concentration of nutrients than raw and cooked sample. The seed of *B. coriacea* can therefore be used as functional food.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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