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Proximate, mineral and antioxidant activity of wonderful kola (*Buchholzia coriacea*) seed (fresh and freeze-dried)

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Abstract

Plants contain nutrient and phytochemicals, which serve as food and medicine to promote good nutritional and health wellbeing in human. *Buchholzia coriacea* is a perennial plant, and its seeds contain nutrient and medicinal properties. The present study, therefore, aimed at determining the proximate, mineral and antioxidant activity of fresh and freeze dried seed of *Buchholzia coriacea*. The *Buchholzia coriacea* seeds were processed as fresh and freeze dried using aqueous, ethanol and acetone extracts. The flour samples were analyzed for proximate, mineral and antioxidant activity using standard methods. From the results obtained in Mean ± SE, the moisture content (%) of fresh and freeze-dried seeds of Wonderful kola was 66.14±1.84 and 7.68±0.74, the Fat content (%) was 3.20±0.39 and 4.72±0.29, the Crude fiber content (%) was 7.09±0.08 and 5.63±0.28, the Crude Protein content (%) was 3.27±0.17 and 5.42±0.30, the Ash content (%) was 5.22±0.07 and 7.29±0.11, and the Carbohydrate content (%) was 15.08±0.90 and 69.26±1.15 respectively. The Sodium (Na) content of fresh seed and freeze dried seed was 10.46±6.01 and 14.80±0.50, Potassium (K) was 24.43±7.63 and 40.59±9.29, Calcium (Ca) was 7.56±0.98 and 12.33±0.60, Magnesium (Mg) was 2.68±1.41 and 8.24±0.58, Manganese (Mn) was 0.05±0.01 and 0.08±0.02, Iron (Fe) was 0.64±0.11 and 0.49±0.04, Copper (Cu) was 0.44±0.05 and 0.27±0.04, Zinc (Zn) was 0.44±0.04 and 0.50±0.13, Nickel (Ni) was 0.01±0.0 and 0.02±0.0, Cadmium (Cd) was 0.01±0.00 and 0.15±0.11, and Na/K was 0.43 and 0.36 respectively. Lead (Pb) was below detection limit in fresh seed, but was 0.02±0.01 in freeze dried seeds. All the extracts displayed high antioxidant activity and high value of free scavenging ability in all the solvents used. However, ethanolic extracts showed higher antioxidant activity compared to other solvents. The study concluded that the moisture content, fat, crude protein, ash and carbohydrate in freeze dried seed of *Bulchholzia coriacea* was higher compared to fresh seed. Comparatively, freeze dried seed had the highest values in Na, K, Ca, Mg, Mn, Zn, Ni and Cd than fresh seed samples. The proximate, mineral and antioxidant activity of wonderful kola seed was higher in freeze dried seed in comparison with the fresh seed.

Keywords: *Buchholzia coriacea*; Proximate; Minerals; Antioxidant; Freeze dried

1. Introduction

Wonderful kola also known as *Buchholzia coriacea* is a perennial plant of the Capparaceae family[1]. It is a small to medium-sized evergreen plant that may grow up to 20 meters in height and is found in Nigeria, Cameroon, Gabon, Central African Republic, Congo, Angola and Ghana, among other places [2]. The leaves are big and glossy, measuring 15-25 cm long and 5-7.5 cm wide [3], with prominent creamy white blossoms and medicinally valuable edible seeds. When fresh, the seeds are blackish, covered in purple aril, and have a harsh pungent flavor with a scorching spicy flavor [4]. The seeds have been given a variety of local names all over the world. It is known as 'Ndo' in Mende (Sierra Leone), 'Doe-fiah' in Kru-basa (Liberia), 'Eson-bese' in Akan-asante (Ghana), 'Banda' in Munga (West Cameroons), 'Esson bossi'

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in Central Africa, and 'Kola Pimente' in French. In Nigeria, it is called 'Owi' in Edo State, 'Okpokolo' in Igbo, 'Uwuro' and 'Aponmu' in Yoruba, 'Obi alata' in Ekiti and Ondo[5].

Buchholzia coriacea plants contain phytonutrients (protein, fiber, fat/oil, carbohydrate, minerals and vitamins) and phytochemicals, which promote good nutritional and health wellbeing in man [6]. Phytochemicals or bioactive plant components are naturally occurring chemical components that give plants color, flavor and smell, and are part of a plant naturally defense system for the plant (disease resistance)[7]. Phytochemical has been considered of crucial nutritional and health important in terms of preventing chronic disease such as cancer, cardiovascular disease and diabetics [8]. Plant-based foods contain biologically active components or phytochemicals, and that regular consumption of these phytochemicals has always been associated with health benefits [9]. The therapeutic efficacy of many indigenous plants for various diseases lies in some chemical substances that produce define physiological action in the human body [10]. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, and phenolic compounds [11].

Authors have variously reported that medicinal plants are plants whose one or more parts (including leaves, fruits, barks, stems, roots, flowers, latex /juice) have medicinal properties [12-14]. As such, plants are used for the treatment of several disease conditions. Medicinal plants are plants whose active ingredients and/or part are involved in the production of drugs [15]. Several plants species are found all over the world and are distributed into several families. Some plants are found in a particular region under specific environmental conditions. Plants are grown in soil, surface water (macrophytes) and another plant (epiphytes). As such, the use of plant for the treatment of disease conditions depends on its availability in particular region, education status of the users, and knowledge about its efficacy [13].

Despite the medicinal importance of Wonderful kola, 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*.[16] 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. To the best of our knowledge, most studies carried out on proximate, mineral and antioxidant activity of *Buchholzia coriacea* has focused on the fresh seed, bark and leaf of the plant; and no work has been reported on the freeze dried seed thus far. Hence, this study was carried out to determine the proximate, mineral and antioxidant activity of Wonderful kola (*B. coriacea*) seed (fresh and freeze dried).

2. Material and methods

2.1. Plant Collection and Authentication

The seeds of *Buchholzia Coriacea* were purchased from Bode market, Molete, Ibadan, Oyo-State, Nigeria and authenticated in the Department of Crop, Soil and Pest Management, at The Federal University of Technology, Akure, Ondo State, Nigeria.

2.2. Preparation of Seed Extract

The seeds were sorted, washed, chopped and divided into two parts. The first part was blended fresh using an electric blender and refrigerated at 4 ^{0}C. The second part was freeze dried, after which it was grounded into fine powder using a dry grinder and refrigerated at 4^{0}C prior analysis. The powder was used to prepare aqueous, ethanol and acetone extracts of fresh seed and freeze dried seed respectively. The extracts were prepared in different concentrations; 10 mg/ml, 20mg/ml, and 30mg/ml respectively [17].

2.3. Ethanol Extract Preparation

A Satoric AG Gottingen Electronic weighing scale was used to weigh 200 grams of pulverized Wonderful kola seed. The weighed sample was soaked in 1 litre of ethanol in a conical flask, mixed and left for 24 hours with interval stirring. The mixture was filtered using Whatman No.1 filter paper into a clean beaker and the ethanol was recovered using a Soxhlet apparatus and was evaporated to dryness using a steam bath at 100 ^{0}C [17]. The leaf sample was prepared same way as the seed as described above.

2.4. Aqueous Extract Preparation

Two hundred grams (200g) of the pulverized kola seed was weighed and macerated in 1 litre of distilled water. The mixtures were vigorously swirled. After the elapse of 24 hours with interval stirring, the mixture was filtered using

Whatman No.1 filter paper into a clean beaker, and the filtrate was concentrated to dryness by evaporation using the steam bath at 100 ^{0}C[17]. The leaf sample was prepared same way as the seed as described above.

2.5. Acetone Extract Preparation

The dried seeds were used to prepare the extract adopting the procedure described by Satyavani *et al*. (2011). 25 gms of the dried seed powder was mixed with 250 ml acetone and boiled (boiling point range 55.5-56.5^{0}C) in Soxhlet apparatus for 8hrs. The extract collected was stored at 4 ^{0}C for further use. The leaf sample was prepared same way as the seed as described above.

2.6. Proximate Analyses

Nutrient composition of the extract was determined using the standard procedures of Association of Official Analytical Chemists[18]. Triplicate samples were used for moisture content in a hot-air circulating oven (Galenkamp). Ash was determined by incineration (550 ^{0}C) of known weights of the samples in a muffle furnace (Hotbox oven, Gallencamp, UK, size 3) (Method No 930.05). Crude fat was determined by exhaustively extracting a known weight of sample in petroleum ether (boiling point, 40 to 60 ^{0}C) using TecatorSoxtec (Model 2043(20430001), 69, Slandegarupgade, DK-3400, Hilleroed, Denmark) (Method No 930.09). Protein content (N × 6.25) was determined by the micro-Kjeldahl method (Method No 978.04). Crude fiber was determined after digesting a known weight of fat-free sample in refluxing 1.25% sulfuric acid and 1.25% sodium hydroxide (Method No 930.10). Carbohydrate content was determined by difference, that is, addition of all the percentages of moisture, fat, crude protein, ash and crude fibre was subtracted from 100%. This gave the amount of nitrogen free extract otherwise known as carbohydrate[19].

% carbohydrate = 100 - (%Moisture + %Fat + %Ash + % Crude fibre + %Crude protein)

2.7. Mineral Analysis

The method described by Association of Official Analytical Chemists [18] was used for mineral analysis. Two grams (2 g) of each of the samples was digested with concentrated nitric acid and hydrogen peroxide, filtered and the filtrate in a 5 mL volumetric flask was loaded to Atomic Absorption Spectrophotometer, (model703 Perkin Elmes, Norwalk, CT, USA). Calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn), sodium (Na), potassium (K) were determined at wavelengths 317.9 nm, 285.2 nm, 259.9 nm, 324.7 nm, and 213.9 nm respectively. Sodium [Na] and Potassium [K] were determined using flame emission photometer (Sherwood Flame Photometer 410, Sherwood Scientific Ltd. Cambridge, UK), and NaCl and KCl were used as the standards. Phosphorus was determined using Vanodomolybdate method. The serially diluted phosphate standard solution was made acidic by addition of 2 ml nitric acid (2:1), 25ml of the Vanodo-molybdate reagent was added, the solution was diluted to the mark with distilled water, mixed thoroughly and allowed to stand for 10 minutes and the optical density was measured at 47mu. All values were expressed in mg/100 g[18].

2.8. Evaluation of Antioxidant Properties

2.8.1. Determination of Total Phenol

The total phenol content of the sample was determined by the method of Singleton *et al*.[20]. 0.2 ml of the sample was mixed with 2.5 ml of 10 % Folin Ciocalteu's reagent and 2 ml of 7.5 % sodium carbonate. The reaction mixture was subsequently incubated at 45 ^{0}C for 40 min, and the absorbance was measured at 760 nm in the spectrophotometer, using garlic acid as standard phenol. The garlic acid standards in different concentrations were prepared to obtain the calibration curve.

2.8.2. Determination of Flavonoid

The total flavonoid content of the sample was determined using a colourimeter assay developed by Bao *et al*.[21]. 0.2 ml of the sample was added to 0.3 ml of 5 % $NaNO_3$ at zero time. After 5 min, 0.6 ml of 10 % $AlCl_3$ was added and after 6 min, 2 ml of 1 M NaOH was added to the mixture followed by the addition of 2 ml distilled water. Absorbance was read at 510 nm against the reagent blank and flavonoid content was expressed as mg/g.

2.8.3. Determination of Ferric Reducing Property

The reducing property of the sample was determined as described by Dorman *et al*.[22] 0.25 ml of the sample was mixed with 0.25 ml of 200 mM of Sodium phosphate buffer pH 6.6 and 0.25 ml of 1 % KFC. The mixture was incubated at 50 ^{0}C for 20 min, thereafter 0.25 ml of 10 % Trichloroacetic acid was also added and centrifuged at 2000 rpm for 10 min, 1 ml of the supernatant was mixed with 1ml of distilled water and 0.2 ml of $FeCl_3$ and the absorbance was measured at 700 nm.

2.8.4. Determination of Free Radical Scavenging Ability

The free radical scavenging ability of the sample against DPPH (1,1-diphenyl-2-picryhydrazyl) using Gyamfi *et al*.[23] method. 50 µl of sample or control (water) and 450 µl of mmol/l Tris-HCL buffer (pH7.4) was pipetted into test tube and swirled. Then 1.0ml of 0.1mmol/l DPPH-methanol solution was added, the mixture was swirled and kept in a dark place for 30 min. After incubation period, absorbance was measured at 517nm with the mixture of water, buffer and methanol as blank solution.

2.8.5. Determination of NO (Nitrous oxide) Radical Scavenging Ability

Sodium Nitroprusside in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent. Scavengers of NO compete with oxygen, leading to reduced production of NO. Briefly 5 mM sodium nitroprusside in phosphate-saline was mixed with the sample, before incubation at 25 ^{0}C for 150 min. Thereafter the reaction mixture was added to Greiss reagent. Before measuring the absorbance at 546 nm, relative to the absorbance of standard solution of potassium nitrate treated in the same way with Greiss reagent[18].

2.8.6. Determination of Fe^{2+} Chelation

The ability of the sample to chelate Fe^{2+} was determined using a modified method of Carter[24]. 150 mM $FeSO_4$ was added to a reaction mixture containing 168 ml of 0.1M Tris-HCl pH 7.4, 218 ml saline and sample and the volume made up to 1 liter with distilled water. The reaction mixture was incubated for 5 min, before the addition of 13 ml of 1, 10-phenantroline the absorbance was read at 510 nm.

2.9. OH (Hydroxyl) Radical Scavenging Ability

The ability of the sample to prevent Fe^{2+}/H_2O_2 induced decomposition of deoxyribose was carried out using the method of Halliwell and Gutteridge [25]. Freshly prepared sample (50 µl) was added to a reaction mixture containing 120 µl, 20 mm deoxyribose, 400µl, 0.1M phosphate buffer pH 7.4, 40 µl, 20mM hydrogen peroxide and 40 µl, 500 µM $FeSO_4$, and the volume was made to 800 µl with distilled water. The reaction mixture was incubated at 37^{0}C for 30 min and the reaction was stopped by the addition of 0.5 ml of 2.8 % Trichloroacetic acid, this was followed by the addition of 0.4ml of 0.6% TBA solution. The tubes were subsequently incubated in boiling water for 20min. The absorbance was measured at 532nm in spectrophotometer.

2.10. Statistical Analysis

The data were analyzed using SPSS version 21.0. The mean and standard error of means (SEM) of the triplicate analyses were calculated. The analysis of variance (ANOVA) was performed to determine significant differences between the means. Values were significant at $p<0.05$.

3. Results

Table 1 showed the proximate analysis of fresh and freeze-dried seeds of Wonderful kola. From the results obtained in Mean ± SE, the moisture content (%) of fresh and freeze-dried seeds of Wonderful kola was 66.14±1.84 and 7.68±0.74, the Fat content (%) was 3.20±0.39 and 4.72±0.29, the Crude fibre content (%) was 7.09±0.08 and 5.63±0.28, the Crude Protein content (%) was 3.27±0.17 and 5.42±0.30, the Ash content (%) was 5.22±0.07 and 7.29±0.11, and the Carbohydrate content (%) was 15.08±0.90 and 69.26±1.15 respectively. There was significant difference ($p<0.05$) in the Moisture content and Carbohydrate content of fresh compared with freeze-dried seeds of Wonderful kola.

Table 2 showed the Mineral content of fresh and freeze-dried seeds of *B. coriacea* (Mg/Kg). From the results obtained, the Sodium (Na) content of fresh seed and freeze dried seed was 10.46±6.01 and 14.80±0.50, Potassium (K) was 24.43±7.63 and 40.59±9.29, Calcium (Ca) was 7.56±0.98 and 12.33±0.60, Magnesium (Mg) was 2.68±1.41 and 8.24±0.58, Manganese (Mn) was 0.05±0.01 and 0.08±0.02, Iron (Fe) was 0.64±0.11 and 0.49±0.04, Copper (Cu) was 0.44±0.05 and 0.27±0.04, Zinc (Zn) was 0.44±0.04 and 0.50±0.13, Nickel (Ni) was 0.01±0.0 and 0.02±0.0, Cadmium (Cd) was 0.01±0.00 and 0.15±0.11, and Na/K was 0.43 and 0.36 respectively. Lead (Pb) was below detection limit in fresh seed, but was 0.02±0.01 in freeze dried seeds. There was significant difference in the Calcium, Magnesium, Copper and Iron of fresh seed compared with freeze dried seed of *B. coriacea.*

Table 3 showed the antioxidant assay of fresh and freeze dried seeds of *B. coriacea*. The results obtained showed that at concentration of 10mg/ml and 20 mg/ml, there was no significant difference ($p>0.05$) in Total phenol content of fresh seed aqueous (FSAQ) extract, fresh seed acetone (FSAc) and fresh seed ethanol (FSE) compared with dried seed aqueous

(DSAQ), dried seed acetone (DSAc) and dried seed ethanol (DSE) extracts respectively. However, at 30 mg/dl, FSAc and FSAQ vary significant ($p<0.05$) with DSAc and DSAQ respectively. At concentration of 10mg/ml there was no significant difference ($p>0.05$) in Flavonoid content of fresh seed aqueous (FSAQ) extract, fresh seed acetone (FSAc) and fresh seed ethanol (FSE) compared with dried seed aqueous (DSAQ), dried seed acetone (DSAc) and dried seed ethanol (DSE) extracts respectively. Furthermore, there was no significant difference ($p>0.05$) in DPPH and OH radicals in all the extracts at concentration of 10, 20 and 30 mg/ml respectively. Similarly, at concentration of 10mg/ml and 20mg/ml, there was no significant difference ($p>0.05$) in FRAP in all the extracts studied, however, at 30mg/ml there was significant difference ($p<0.05$) in FRAP of fresh seed ethanol (FSE) compared with dried seed ethanol (DSE) extracts. Finally, at concentration of 10 mg/ml, 20mg/ml and 30mg/ml, there was no significant difference ($p>0.05$) in Fe^{2+}Chelation and NO radicals in all the extracts studied, except for Fe^{2+}Chelation were the values obtained at different concentrations (10 mg/ml, 20mg/ml and 30 mg/ml respectively) in FSAQ differ significantly ($p<0.05$) from other extracts.

Table 1 Proximate analysis of Fresh and Freeze-dried Seeds of B. Coriacea

Parameters	Fresh Seed	Freeze-Dried Seed
Moisture Content (%)	66.14±1.84a	7.68±0.74b
Fat (%)	3.20±0.39a	4.72±0.29a
Crude Fibre (%)	7.09±0.08a	5.63±0.28a
Crude Protein (%)	3.27±0.17a	5.42±0.30a
Ash (%)	5.22±0.07a	7.29±0.11a
Carbohydrate (%)	15.08±0.90a	69.26±1.15b

NB: Data were expressed as Mean ± SEM. Means with different superscript along the column differ significantly ($p<0.05$); * Means with different superscript along the column differ significantly ($p<0.05$)

Table 2 Mineral content of Fresh and Freeze-dried Seeds of *B. coriacea* (mg/kg)

Parameters (Metals)	Fresh Seed	Freeze Dried Seed
Sodium (Na)	$10.46±6.01^{B}$	$14.80±0.50^{B}$
Potassium (K)	$24.43±7.63^{B}$	$40.59±9.29^{B}$
Calcium (Ca)	$7.56±0.98^{B}$	$12.33±0.60^{C}$
Magnesium (Mg)	$2.68±1.41^{B}$	$8.24±0.58^{C}$
Manganese (Mn)	$0.05±0.01^{B}$	$0.08±0.02^{B}$
Iron (Fe)	$0.64±0.11^{B}$	$0.49±0.04^{C}$
Copper (Cu)	$0.44±0.05^{B}$	$0.27±0.04^{C}$
Zinc (Zn)	$0.44±0.04^{B}$	$0.50±0.13^{B}$
Nickel (Ni)	$0.01±0.0^{B}$	$0.02±0.0^{B}$
Cadmium (Cd)	$0.01±0.00^{B}$	$0.15±0.11^{B}$
Lead (Pb)	BDL	0.02±0.01
Na/K	0.43	0.36

NB: Data were expressed as Mean ± SEM. Means with different superscript along the column differ significantly ($P<0.05$)**.; Key: BDL = Below Detection Limit**

Table 3 Antioxidant Assay of Fresh and Freeze-dried Seeds of *B. coriacea* (Mg/Kg)

Total Phenol (mg/g)						
CONC.	**FSAQ**	**FSAc**	**FSE**	**DSAQ**	**DSAc**	**DSE**
10mg/ml	4.26±0.69^{A}	4.35±0.10^{A}	12.89±0.18^{A}	11.78±0.18^{A}	6.64±0.35^{A}	16.14±0.92^{A}
20mg/ml	8.15±0.09^{B}	6.29±0.12^{B}	16.38±0.17^{B}	13.32±0.45^{B}	8.63±0.09^{B}	23.19^{B}±0.21
30mg/ml	10.32±0.00^{C}	8.73±0.10^{C}	18.15±0.09^{C}	14.33±0.02^{B}	9.26±0.10^{B}	27.97^{C}±0.02
Flavonoid (mg/g)						
CONC.	**FSAQ**	**FSAc**	**FSE**	**DSAQ**	**DSAc**	**DSE**
10mg/ml	0.18±0.00^{A}	1.31±0.34^{A}	1.18±0.07^{A}	0.10±0.03^{A}	0.91±0.12^{A}	1.88±0.11^{A}
20mg/ml	0.23±0.03^{A}	2.52±0.46^{B}	2.86±0.38^{B}	0.11±0.01^{A}	1.48±0.03^{B}	3.72±0.04^{B}
30mg/ml	0.29±0.00^{B}	2.75±0.07^{B}	3.82±0.07^{B}	0.15±0.04^{C}	2.25±0.11^{C}	5.28±0.18^{C}
DPPH (%)						
CONC.	**FSAQ**	**FSAc**	**FSE**	**DSAQ**	**DSAc**	**DSE**
10mg/ml	60.81±0.85^{A}	70.54±0.15^{A}	80.86±0.53^{A}	57.92±0.44^{A}	68.70±0.18^{A}	85.72±0.03^{A}
20mg/ml	64.52±0.03^{B}	73.78±0.31^{B}	84.07±1.03^{B}	61.92±1.15^{B}	71.31±0.33^{B}	82.92±0.23^{B}
30mg/ml	70.67±0.12^{C}	78.95±0.34^{C}	86.63±0.18^{B}	68.96±0.12^{C}	77.02±0.41^{C}	77.30±0.03^{C}
OH Radical						
CONC.	**FSAQ**	**FSAc**	**FSE**	**DSAQ**	**DSA1**	**DSE**
10mg/ml	36.60±0.29^{A}	64.63±0.24^{A}	54.03±0.29^{A}	44.96±0.29^{A}	61.21±0.34^{A}	56.84±0.22^{A}
20mg/ml	45.97±0.43^{B}	71.22±0.24^{B}	63.40±0.43^{B}	54.61±0.43^{B}	68.27±0.08^{B}	68.59±0.86^{B}
30mg/ml	54.76±0.43^{C}	76.25±0.57^{C}	71.12±0.07^{C}	59.94±0.14^{C}	72.71±0.36^{C}	73.99±0.22^{C}
FRAP (mg/g)						
CONC.	**FSAQ**	**FSAc**	**FSE**	**DSAQ**	**DSA1**	**DSE**
10mg/ml	10.62±0.01^{A}	18.27±0.05^{A}	11.07±1.01^{A}	10.08±0.43^{A}	20.78±0.11^{A}	24.65±0.07^{A}
20mg/ml	20.18±0.60^{B}	21.34±0.06^{B}	26.82±0.08^{B}	17.27±0.65^{B}	22.64±0.27^{B}	26.85±0.20^{B}
30mg/ml	24.77±0.23^{C}	21.78±0.04^{C}	27.27±0.02^{B}	23.72±0.56^{C}	23.91±0.22^{C}	27.29±0.02^{B}
Fe^{2+}Chelation (%)						
CONC.	**FSAQ**	**FSAc**	**FSE**	**DSAQ**	**DSA1**	**DSE**
10mg/ml	64.47±3.79^{B}	30.41±0.06^{A}	51.57±1.52^{A}	84.13±0.28^{A}	32.77±0.39^{A}	9.55±0.70^{A}
20mg/ml	79.21±0.14^{C}	42.70±0.13^{B}	67.91±2.18^{B}	85.96±0.14^{B}	46.53±0.37^{B}	20.36±0.56^{B}
30mg/ml	84.27±1.12^{C}	54.97±0.34^{C}	88.41±0.63^{C}	89.82±0.21^{C}	58.15±0.32^{C}	89.82±2.60^{C}
	1.88	0.89	1.01	0.33	0.77	6.56
NO Radicals (%)						
CONC.		**FSAc**	**FSE**	**DSAQ**	**DSA1**	**DSE**
10mg/ml		33.20±0.48^{B}	39.97±0.40^{B}	22.06±0.40^{B}	30.92±0.34^{B}	44.92±0.27^{B}
20mg/ml		48.56±0.18^{C}	57.62±0.40^{C}	41.31±0.40^{C}	42.58±0.27^{C}	68.72±0.27^{C}
30mg/ml		60.35±0.07^{D}	72.86±0.40^{D}	50.67±0.40^{D}	56.87±0.54^{D}	77.54±0.27^{D}

NB: Data were expressed as Mean ± SE. Means with different superscript along the column differ significantly (P<0.05); **Keys:** Fresh seed Aqueous = FSAQ; Fresh seed Acetone = FSAc; Fresh seed ethanol = FSE; Dried seed Aqueous = DSAQ; Dried seed Acetone = DSAc; Dried seed ethanol = DSE

4. Discussion

Proximate analysis is one of the parameters used in assessing the nutritional value of edible plant. The moisture content (%) of fresh and freeze-dried seeds of Wonderful kola was 66.14±1.84 and 7.68±0.74. The value of moisture contents in the wonderful kola in the present study were similar to the report of other researchers [26-27]. The high moisture contents observed in fresh seed flour samples shows that the flour samples cannot be stored for long time in order to avoid spoilage. It is well known that the amount of moisture content in flour samples is a determinant of storage ability of the food sample. For instance, high moisture content in food sample facilitate the growth and multiplication of microorganisms, and that the activities of these microorganisms are the major factors responsible for the food spoilage [28]. However, the low moisture content of freeze dried seed flour of *Bulchholzia coriacea* showed that freeze drying is a better preservation method.

The Crude Protein content (%) of fresh and freeze-dried seeds of Wonderful kola was 3.27±0.17 and 5.42±0.30 respectively. The present study established that the protein content of freeze dried seed sample of *B. coriacea* was insignificantly higher ($p>0.05$) than that of fresh wonderful kola seed sample. This observation could be attributed to the various stages of fermentation processing, which involved heating and microbial activities. It is evident that fermentation increased the protein content of fermented food products over unfermented products [29-30]. This was attributed to microbial activities, which involve using crude fiber and carbohydrate as sources of energy and synthesizing structural proteins that are integral part of the microbial cells [31]. This present result agreed with other findings who reported on the increased in protein content of fermented food products [29]. In comparing the protein contents in this present study with other studies, the values obtained in our study (3.27 % and 5.47 %) were lower compared with the reports of Amaechi[32] for raw wonderful kola seeds (13.28 %), Akubugwo *et al.*[33] for *Solanum nigrum* var virginicum (17.63 %) and Ekop[34] for fluted pumpkin seeds (7.0 %). However, it was higher than the report of Odebunmi *et al.*[35] for *Cola nitida* (2.63 %) and *Cola nitida* (2.38 %). Hence, the seed can serve as an alternative source of plant protein.

The crude fibre content of the flour samples was 7.09±0.08 and 5.63±0.28 for fresh and freeze dried seed of *Bulchholzia coriacea*. These values were higher when compared with bitter kola (1.23 %)[36]. Nutritional study has shown that adequate fiber intake render some health benefits like lowering the serum cholesterol level, risk of coronary heart diseases, hypertension, constipation and diabetes[37]. Therefore, the high fiber content in wonderful kola seeds are of nutritional and health benefits.

The carbohydrate content (%) of fresh and freeze-dried seeds of Wonderful kola was 15.08±0.90 and 69.26±1.15 respectively. This finding showed that the carbohydrate content (%) of freeze-dried seed was significant ($p<0.05$) higher than fresh seed of wonderful kola. This finding is similar to the 69.83 % reported by Umeokoli *et al.*[38], 70.10 % reported by Okere and Ladeji[39] and 71.32 % reported by Ezekiel and Onyeoziri[40] and Okoli *et al.*[41] respectively.

The Ash content (%) of fresh and freeze-dried seeds of Wonderful kola was 5.22±0.07 and 7.29±0.11 respectively. This finding shows that the ash content of freeze dried seed was higher than the fresh seed. The 7.29 % of ash content of freeze dried seed of wonderful kola reported in this study is higher than the 5.20 % reported by Okere and Ladeji[39], 6.60 % reported by Ibrahim and Fagbohun[42], 3.69 % reported by Malomo *et al.*[43]. Similarly, the Fat content (%) of fresh and freeze-dried seeds of Wonderful kola reported in this study was 3.20±0.39 and 4.72±0.29 respectively. This finding shows that the fat content of freeze dried seed was higher than the fresh seed. The 4.72 % of fat content in freeze dried seed of wonderful kola reported in this study is higher than the 1.10 % reported by Okere and Ladeji[39], 2.30 % reported by Ibrahim and Fagbohun[42] and 2.01 % reported by Malomo *et al.*[43] respectively.

Medicinal plants are important source of natural antioxidant substances such as the polyphenols[44]. Total phenolic content in aqueous and ethanol extract of fresh seed at 30 mg/ml were 10.32 mg/g and 18.15 mg/g respectively and this was higher than value obtained in acetone extract of fresh seed sample. Also, the ethanol extract displayed the highest value of 27.97 mg/g at 30 mg/ml in the freeze dried seed sample (table 3) which is relatively high when compared with 0.054 mg/g observed in ethanol extract of a medicinal leaf *Hoslunndia opposite* Engl as reported by Bashir *et al.*[45] and 14.176 mg/g in the freeze dried leaf sample reported by Ajayi *et al.*[46]. There exist a strong correlation between the plant polyphenol contents and antioxidant activity they exhibit. Data from the current study show that aqueous, ethanol and acetone extracts of freeze dried seed of Wonderful kola is rich in polyphenols. Polyphenols have been associated with antioxidant activity and a vast number of other biological activities [44,47].

Flavonoid showed good antioxidant activity in aqueous and ethanol extracts of fresh sample with (0.29 mg/g and 3.82 mg/g) and freeze dried seed (0.15 mg/g and 5.28 mg/g) respectively and the highest value of 5.28 mg/g observed was in ethanol extract of freeze dried seed sample. The values of the acetone extract (fresh and freeze dried) was 2.75 and

2.25 mg/g respectively were lower than values observed when ethanol was used. This finding is in agreement with previous study on the flavonoid content of aqueous, ethanol and acetone extracts of leaf of wonderful kola reported by Ajayi *et al*.[46]. Flavonoids are the most common and widely distributed groups of plant spices phenolic. Flavonoid provides protection against diseases such as cancer, ageing, inflammation, neuro degenerative diseases by contributing along with antioxidant vitamins and enzymes to the total antioxidant defense system of the human body. They are potent water soluble super antioxidants that function in scavenging free radicals, inhibition of peroxidants and chelating transition metals [48].

The result also shows that Fe^{2+} chelation antioxidant property was higher in fresh and dried samples of ethanol extract than in aqueous and acetone extracts. Therefore, the ability of the extracts to chelate iron (II) ions was evaluated and expressed as Na_2EDTA/g extract. Ethanol extracts for both fresh and dried seed sample showed the highest values for iron chelation of 88.41 and 89.82 mg/g respectively. The result obtained for seed extracts confirm that Fe^{3+} - Fe^{2+} transformation occurred in the presence of the extracts, thereby confirming their antioxidant potentials. This finding is in agreement with previous study on the Fe^{2+} chelation antioxidant capacity of aqueous, ethanol and acetone extracts of the leaf of wonderful kola reported by Ajayi *et al.*[46].

The result as shown in table 3 also showed that fresh and freeze dried seed samples have higher antioxidant activity in the ethanolic extracts of the samples using DPPH. The best free radical scavenging activity of 85.72 mg/g was observed in ethanolic extract of the freeze dried seed though all the extracts displayed high values of free radical scavenging ability. DPPH radical scavenging assay provides easy, rapid and convenient method to evaluate antioxidants and radical scavengers [49]. This finding is in agreement with Ajayi *et al.*[46] who reported that the aqueous and ethanolic leaf extract of wonderful kola exhibited high values of free radical scavenging ability.

The result of Ferric reducing antioxidant power (FRAP) gave the highest values in ethanolic extract of both fresh and freeze dried seed samples (27.27 and 27.29 mg/g) than other solvents used. Iron (III) reduction is often used as an indicator of electron donating activity which is an important mechanism of phenolic antioxidant action. The iron (III) to iron (II) reducing ability is expressed as ascorbic acid equivalents. The reducing potential was found to also be very high in the ethanolic freeze dried seed extract at 30 mg/ml followed by acetone and aqueous seed extracts in both the freeze dried and fresh seed samples respectively. This shows that the extract of the samples had good potential to reduce the ferric ions into ferrous ions, which is a measure of antioxidant activity. Again, this finding is in agreement with Ajayi *et al.*[46] who reported high values of Ferric reducing antioxidant power (FRAP) in the aqueous extract of fresh and freeze dried leaf of wonderful kola.

Mineral compositions of wonderful kola seeds are depicted in table 2. Comparatively, freeze dried seed had the highest values in Na, K, Ca, Fe, Mg, Mn, Zn than fresh seed samples. This finding is consistent with previous study by Ijarotimi *et al.*[50] who reported that dried seed flour sample had the highest values for Mg, Fe, Na, K, Mn and Co than fresh seed flour samples. Potassium had highest concentration of the minerals and this agreed with the report of Aremu *et al*.[51], who established that potassium is the predominant mineral in plant products, whereas lead, cadmium and chromium were not detected in the flour samples. The molar ratio of Na/K was 0.43 in fresh seed and 0.36 in freeze dried seed. The Na/K of wonderful kola is within the recommended ranged values of <0.1 and >2.0 respectively; and this indicates that the samples would facilitate bone and teeth formation in children, prevent osteoporosis in adults and high blood pressure in man. Scientific research has shown that calcium in combination with phosphorous, magnesium and manganese facilitate bone and teeth formation in children and bone maintenance in adult [52].

5. Conclusion

In this study, the proximate composition of wonderful kola varies; this could be due to differences in processing and storage. The study conclude that the moisture content, fat, crude protein, ash and carbohydrate in freeze dried seed of *Bulchholzia coriacea* was higher compared to fresh seed. Comparatively, freeze dried seed had the highest values in nutritionally valuable minerals such as Na, K, Ca, Mg, Mn, Zn than fresh seed samples. All the extracts displayed high antioxidant activity and high value of free scavenging ability in all the solvents used. However, ethanolic extracts showed higher antioxidant activity compared to other solvents. The proximate, mineral and antioxidant activity of wonderful kola seed was higher in freeze dried seed in comparison with the fresh seed. The results suggest that the seed is a good source of phytochemicals and natural antioxidant which validates the reason why the seed is used in treatment of diseases such as cardiovascular diseases and many other ailments. Also, there is need for more detailed studies on the characterization of bioactive compounds of the plant extracts.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declared that no competing interests exist.

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