Phytochemical and Antioxidant Activities of the Leaf Samples of Wonderful Kola (*Buccholzia coriacea)*

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Abstract **- Fresh and Freeze-dried samples of the leaf of wonderful Kola (*Bulcchozia coriacea)* were evaluated for their phytochemicals and antioxidant properties using standard methods. Both fresh and freeze-dried samples were extracted with distilled water, acetone and ethanol respectively. The ethanol and acetone leaf extracts showed tannin, flavonoid, terpenoids, saponins, alkaloids, steriods and phylobatamin with high antioxidant activities compared to the aqueous extract except the Flavonoid and Ferric reducing Antioxidant power (FRAP) that gave good activities in the aqueous and ethanol extracts. The fresh leaf extracts of aqueous, acetone and ethanol extracts contained: Total phenol (3.532 ±0.69, 13.164 ±0.21 and 10.975±0.34mg/g, Ferric Reducing Antioxidant Power (FRAP) (25.712 ±0.83, 6.378±0.40 and 18.850±0.74 mg/g, 1,1-diphenyl-2-picryl-hydrazyl (DPPH) (72.939 ±0.57, a 75.736 ±0.38 and 76.266±0.97 % and Fe^{2+} chelation (23.666 ±0.63, 55.126 ±0.13 and 57.022±0.38 % and Flavonoid (9.269±0.44, 4.792±0.87 and 7.590±0.02 respectively. The Freeze-Dried leaf extracts of aqueous, acetone and ethanol extracts contained; Total phenol (3.774 ±0.76, 4.937 ±0.69 and 14.176±0.46mg/g, Ferric Reducing Antioxidant Power (20.738 ±0.95, 10.984±0.71 and 15.174±0.39 mg/g, 1,1-diphenyl-2-picryl-hydrazyl (DPPH) (60.719 ±0.57, 82.627 ±0.15 and 74.971±0.89 % and Fe^{2+} chelation (22.612 ±0.72, 35.744 ±0.96 and71.278±0.29 % and Flavonoid (6.596±0.24,5.894±0.93 and 11.802±0.39 respectively. The results reveals that ethanol extract of the freeze dried wonderful kola leaf contained appreciable amount of phytochemicals with high antioxidant activity compared to the fresh leaves extracts. This suggests that the use of ethanol was more effective than other solvents for the extraction of phytochemicals from wonderful kola leaves. The leaves can be used as raw material in the chemical and pharmaceutical industry for the production of drugs due to its high antioxidant activity. The finding supports or validates the use of the seeds and leaves in the treatment of many ailments such as cardiovascular diseases as claimed by Traditional healers.**

***Keywords – Buccholzia coriacea*, Phytochemical, Freeze-dried, Antioxidant and Bioactive Compounds**

I. INTRODUCTION

Natural products from herbal plants are used in several parts of the world for therapeutic purposes because they are complex chemical storehouses which have good biological and antioxidant activities (Wang *et al.,* 2002, Sokmen *et al.,* 1999). *Buccholzia coricea* which belongs to the family *Capparaceae* commonly known as "Wonderful kola''is a perennial plant which grows as a tree. Its local names include; Uworo (Yoruba), Obi alata (Ekiti and Ondo), Owi (Edo), and Uke (Ibo) (Quarttrachu-Umbetto, 2007).

Bulcchozia coriacea like some medicinal plants is a good natural source of antioxidant, antimicrobial (antibacterial and antifungal) anti-diabetic activities which is attributed to its bioactive components which are mainly phytochemicals (Ezeigbo *et al*., 2016). Different part of the plant is used locally for treating many diseases such as, cough, chest pain, waist pain, irregular menstruation, internal pile, malaria, quick ejaculation, headache, hypertension, dysentery, premature aging, memory improvement, diabetics, rheumatism, migraine etc. (Kameswarao *et al*., 2003, Eleyinte 2012). The plant parts commonly eaten are the seeds which are either cooked or eaten raw. It is commonly known in the world as "memory nut" because it enhances the memory. It acts as cleanser of the blood, facilitates learning ability and strengthens the nervous system (Oseto, 2010, Fred- Jaiyesimi *et al.,* 2009).

The medicinal efficacy of the seed earned the plant its name wonderful kola, which has demonstrated potency against several disease conditions (Ezekiel and Onyeoziri, 2009, Ijarotimi *et al*., 2015). The seeds contain high percentage of carbohydrate which makes it a good source of energy for human nutrition. It has high saponins and phytates with low levels of oxalates and tannins. As such could be used as hypercholesterolemia due to its high saponin composition (Amaechi, 2009

Different parts of *Buccholzia coriacea* have been reported to have antimicrobial, antidiabetic, antidepressant, antiplasmodial, analgesic, anti-anxiety, anti-inflammatory, antioxidant and hyperlipidemia activities among others (Izah *et al*., 2018).

This study focused on the determination of phytochemicals present in the fresh and freeze dried leaves extracts of *Buccholzia coriacea* using different solvents and evaluating them for their antioxidant activity.

II. METHODOLOGY

Collection and Preparation of Samples

Mature leaves of *Buchholzia coriacea* were obtained from a farm settlement in Aaye Ekiti, Ido-Osi local Government area, Ekiti State, Nigeria and authenticated at the Department

of Crop, Pest and Soil management, the Federal University of Technology, Akure, Ondo State, Nigeria. The leaves were sorted, washed, chopped and divided into two parts. The first part was blended fresh using an electric blender and refrigerated at 4ºC .The second part was freeze dried, ground into a fine powder using a dry grinder and refrigerated at 4ºC prior analysis. Two hundred grams (200 g) each of the leaf sample was immersed in one litre (1L) of water, acetone and ethanol separately shaken intermittently for 72 h. The extracts were filtered, concentrated using rotary evaporator, transferred into air-tight sample bottles and refrigerated at 4^0C prior analysis.

Phytochemical Screening

The qualitative phytochemical analysis of the plant materials for secondary metabolites such as tannins, alkaloid, flavonoid, steroid, terpenoid, saponins, anthraquinone, were carried out using methods described by Harborne, (1973), Sofowora, (1993),Trease and Evans, (1989) and Edeoga*et al*., (2005).

Quantitative Phytochemical Analysis

Determination of Tannins

Accurately weighed 0.2 g of finely ground sample was poured into a 50 ml sample bottle. 10 ml of 70 % aqueous acetone was added and properly covered. The bottles were put in an ice bath shaker and shaken for 2h at 30 ^{0}C. Each solution was then centrifuged and the supernatant store in ice. 0.2 ml of each solution was pipetted into the test tube and 0.8 ml of distilled water was added. Standard tannic acid solutions were prepared from a 0.5 mg/ml of the stock and the solution made up to 1 ml with distilled water. 0.5 ml of Folinciocateau reagent was added to both sample and standard followed by 2.5 ml of 20 % Na_2CO_3. The solution were then vortexed, allowed to incubate for 40 min at room temperature, its absorbance was read at 725 nm against a reagent blank concentration of the same solution from a standard tannic acid . (Edeoga and Eriata, 2005).

Determination of Saponin

The spectrophotometric method of Brunner (1994) was used for Saponin determination. Two grams (2 g) of the finely ground sample was weighed into a 250 ml beaker and 100 ml of Isobutyl alcohol was added. Shaker was used to shake the mixture for 5 h to ensure uniform mixing. The mixture was filtered using No 1 Whatman filter paper into 100 ml beaker containing 20 ml of 40 % saturated solution of magnesium carbonate ($MgCO_3$). The mixture obtained again was filtered through No 1 Whatman filter paper to obtain a clean colourless solution. 1 ml of the colourless solution was taken into 50 ml volumetric flask using pipette, 2 ml of 5 % iron (iii) chloride ($FeCl_3$) solution was added and made up to the mark with distilled water. This was allowed to stand for 30 min for the colour to develop. The absorbance was read against the blank at 380 mm.

Determination of Alkaloid

The method of Harbone (1973) was used in this test. The sample (5g) was weighed into a 200ml beaker and 200ml of 10% acetic acid in ethanol was added and covered, and allowed to stand for 4hrs. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue, which was the alkaloids, was dried and weighed.

$$\% \text{ Alkaloid} = \frac{W_2 - W_1}{\text{wt ofsample}} \times 100$$

Where

W_1 = weight of dried empty filter paper

W_2 = weight of filter + alkaloid residue after drying.

Evaluation of Antioxidant Properties

Total phenol, total flavonoid, ferric reducing property, free radical scavenging ability (1, 1- diphenyl-2-picryhydrazyl) and Iron Chelation were determined using standard methods developed by; (Singleton *et al.*, (1999), Bao *et al.*, (2005), Dorman *et al.*, (2003), Pulido *et al.*, (2002), Gyamfi *et al.*, (1999) and Puntel,*et al.*, (2005) respectively.

Determination of Total Phenol

The total phenol content of the sample was determined by the method of Singleton *et al.*, (1999). 0.2 ml of the sample was mixed with 2.5 ml of 10 % FolinCiocalteu's reagent and 2 ml of 7.5 % sodium carbonate. The reaction mixture was subsequently incubated at 45ºC for 40 min, and the absorbance was measured at 760 nm in the spectrophotometer, garlic acid was used as standard phenol. The garlic acid standards in different concentrations were prepared to obtain the calibration curve.

Determination of Total Flavonoid

The total flavonoid content of the sample was determined using a colourimeter assay developed by Bao*et al.*, (2005). 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 rutin? equivalent.

Determination of Ferric Reducing Property

The reducing property of the sample was determined as described by Dorman *et al.*, (2003), 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 °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.

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*., (1999) 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.

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.

Determination of Fe^{2+} Chelation

The ability of the sample to chelate Fe^{2+} was determined using a modified method of (Carter, 1971). 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.

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 (1999). Freshly prepared sample (50 µl) was added to a reaction mixture containing 120 µl, 2 0 mm deoxyribose, 400µl, 0.1M phosphate buffer pH 7.4, 40 µl, 20mM hydrogen peroxide and 40µl, 500 µM $FeSO_4$, and the volume was made to 800µl with distilled water. The reaction mixture was incubated at 37 ^{O}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 be incubated in boiling water for 20min. The absorbance was measured at 532nm in spectrophotometer.

III. RESULTS AND DISCUSSION

The phytochemical screening of the extracts of both the fresh and the freeze-dried leaves of *Buccholzia coriacea* showed the presence of Tannin, Flavonoid, Saponin, Terpenoid, Alkaloid, Cardiac glycoside, Antraquinone and Steroid (Table1). Tannin was highly present in the freeze-dried extracts compared to the fresh ex tracts, although generally, the ethanol extract of the freeze-dried leaves had all the phytochemicals tested except cardiac glycoside. Also, anthraquinone which was reported absent in the leaves of *Buccholzia coriacea* (Ejikeugueu *et al*., 2014, Nwachukwu *et al*., 2014), was present in the ethanol extract of the freeze-dried and fresh leaves.

Table 1: Phytochemical screening of the extracts of wonderful kola leaf

Phytochemicals	**Acetone Extracts**		**Aqueous Extract**		**Ethanol Extract**	
	FL	**DL**	**FL**	**DL**	**FL**	**DL**
Tannin	++	++	+	+	++	++
Saponin	-	+	_	+	+	+
Steroid	_	+	_	+	+	+
Phylobatamin	+	+	+	+	+	+
Flavonoid	+	+	+	+	++	+
Alkaloid	+	+	+	+	+	+
Terpenoid	+	++	+	+	+	-
Cardiac glycoside	-	-	+	_	-	-
Antraquinone	-	-	-	-	+	+

++ = Highly present, + = Less present, - = Absence, FL = Fresh Leaf, DL = Freeze -Dried Leaf

Guided by the qualitative phytochemical screening, the quantitative analysis of both fresh and freeze-dried wonderful kola leaf reveals the presence of tannin, alkaloid, terpenoid and saponin in all the leaf extracts. The phytochemical content of the leaf displayed an appreciable amount with higher values in acetone and ethanol extracts. The value of alkaloid in fresh samples of the seed in aqueous, acetone and ethanol were 5.98, 3.60 and 5.26 % respectively while that of dried sample were 6.79, 8.27 and 7.91% respectively. Aqueous extract gave the highest value for alkaloid. The amount of alkaloid reported in the research was appreciably higher than that observed in the leaf of cola acuminate and cola nitida with 1.23 and 1.01% respectively as reported by Otoide and Olanipekun, (2018).

Table 2: Quantitative phytochemical contents of wonderful kola leaf

Phytochemicals	**Fresh FLA**	**Fresh** FLA1	**Fresh** FLE	**Dried** DLA	**Dried** DLA1	**Dried** DLE
Flavonoid(mg/g)	2.53±0.69	2.26 ±0.21	3.05± 0.34	3.77± 0.69	2.74± 0.76	4.16± 0.46
Alkaloid (%)	5.98±0.57	3.60±0.74	5.26± 0.62	6.79± 0.57	7.91± 0.89	8.27± 0.15
Saponin (mg/g)	3.66±0.63	2.16±0.13	2.07± 0.38	4.62± 0.72	5.04± 0.96	5.28± 0.29
Terpeno	1.94±0.	2.03±0.	1.80±	2.71±	3.02±	3.17±

id(mg/g)	71	95	0.74	0.83	0.40	0.39
Tannins (mg/g)	0.51±0.50	0.89±0.49	1.20±0.44	1.75±0.23	1.60±0.30	1.95±0.23

FLA= Fresh Leaf Aqueous, FLA1= Fresh Leaf Acetone, FLE= Fresh Leaf Ethanol, DL =Dried Leaf Aqueous, DLA1= Dried Leaf Acetone, DLE= Dried Leaf Ethanol

Results are mean ± standard deviation of triplicate values

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Tannin was highly present in the freeze-dried extracts compared to the fresh extracts, although generally, the ethanol extract of the freeze-dried leaves had all the phytochemicals tested except cardiac glycoside. Also, anthraquinone which was reported absent in the leaves of *Buccholziacoricea*(Ejikeugue*et al*., 2014, Nwachukwu*et al*., 2014),was present in the ethanol extract of the freeze-dried and fresh leaves.

Terpenoid which has been reported to be helpful in hastening healing of wound and inflamed mucus membrane is said to be responsible for the hot taste of *B. coriacea*leaf (Okwu, 2004). The values in fresh leaf aqueous, acetone and ethanol extracts are 1.94 and 2.03 and 1.80 mg/g respectively while higher values were recorded for the freeze dried samples (2.71, 3.02 and 3.17 mg/g). The level of tannin in the fresh leaf aqueous, acetone and ethanol extracts gave (0.15, 0.89 and 1.20 mg/g respectively. The dried leaf extracts also displayed higher level of tannin in aqueous, acetone and ethanol extracts with the values 1.75, 1.60 and 1.95 mg/g respectively. The high level of tannin suggests that the plants could be used as antifungal, antidiarrheal, antioxidant and antihaemorrhoidal agent (Asquit and Butter, 1986).The freeze dried leaf has higher level of tannin than in fresh sample.

The samples have an appreciable saponin content which was higher in aqueous, acetone and ethanol extracts of freeze dried extracts (4.62, 5.64 and 5.28 mg/g) than in fresh leaf extract of 3.66, 2.16, and 2.07 mg/g respectively. Some of the general characteristics of saponin include formation of foam in aqueous solution, hemolytic activity and bitterness (Trease and Evans 1989). The saponin content is higher than the saponin reported in Garcina cola 2.02mg/g as reported Adesuyi *et al;* 2012.

Antioxidant Activity

Total phenolic content in acetone and ethanol extract of fresh leaf were 13.164 mg/g and 10.975 mg/g) respectively and this was higher than value obtained in aqueous extract of fresh leaf sample. Also, the ethanol extract displayed the highest value of 14.176 mg/g in the freeze dried 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., (2016). Polyphenol compounds are commonly found in both edible and medicinal plants and they have been reported to have various biological effects including antioxidant activity.

Table 3: Antioxidant properties of the Leaf Extracts (Fresh and Dried)

Antioxidant Assay	**Fresh FLA**	**Fresh** FLA1	**Fresh** FLE	**Dried** DLA	**Dried** DLA1	**Dried** DLE
Total Phenol (mg/g)	3.532 ±0.69	13.164 ±0.21	10.975 ±0.34	4.937 ±0.69	3.774 ±0.76	14.176 ±0.46
DPPH (%)	72.939 ±0.57	75.736 ±0.74	76.266 ±0.97	60.719 ±0.57	82.627 ±0.15	74.971 ±0.89
Flavonoid (mg/g)	9.269 ±0.44	4.792 ±0.87	7.590 ±0.02	6.894 ±0.93	5.596 ±0.24	11.802 ±0.39
Iron Chelation (%)	23.666 ±0.63	55.126 ±0.13	57.022 ±0.38	22.612 ±0.72	35.744 ±0.96	71.278 ±0.29
FRAP (mg/g)	25.984 ±0.71	6.378 ±0.40	18.85 0 ±0.74	20.738 ±0.95	10.984 ±0.71	15.174 ±0.39
Hydroxyl Radical (%)	41.499 ±0.915	51.729 ±0.07	47.622 ±0.48	39.481 ±0.49	45.965 ±0.69	50.288 ±0.06
No Radical (%)	17.513 ±0.50	36.898 ±0.49	28.877 ±0.44	12.700 ±0.33	26.203 ±0.30	33.957 ±0.67

Fresh Leaf Aqueous = FLA, Fresh Leaf Acetone = FLA1, Fresh Leaf Ethanol = FLE, Dried Leaf Aqueous = DLA

Dried Leaf Acetone = DLA1, Dried Leaf Ethanol = DLE.

Flavonoid showed good antioxidant activity in aqueous and ethanol extracts of fresh sample with 9.269 mg\g, 7.590 mg\g and freeze dried 6.596 mg\g and 11.802 mg/g) respectively and the highest value of 11.802 mg\g observed was in ethanol extract of dried sample . The values of the acetone extract (fresh and dried) 4,792 &6.894 respectively were lower than values observed when distilled water and ethanol were used, 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 (Nickavor *et al.*, 2003).

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 sample showed the highest values for iron chelation of 57.022 and 71.278 mg/g respectively. The result obtained for leafextracts confirm that Fe^{3+} - Fe^{2+} transformation occurred in the presence of the extracts, thereby confirming their antioxidant potentials.

The result in Table 3 also shows that fresh and dried sampleshave higher antioxidant activity in the aqueous extracts of the samples using DPPH. The best free radical scavenging activity of 82.627 mg/g was observed in acetone extract of the freeze dried leaf 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 (Roginsk *et al.*, 2005). The result of Ferric reducing antioxidant power (FRAP) gave the highest values in fresh leaf aqueous extract of both fresh and freeze dried samples(25.712 and 20.738 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 aqueous extract of fresh leaf followed by the freeze dried sample with 25.712 and 20.738mg/g 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.

IV. CONCLUSION

The aqueous, acetone and ethanol extracts of wonderful kola leaf were investigated for their phytochemical and antioxidant properties. Phytochemicals screening reveals the presence of tannin, saponin, steroid, phylobatamin, flavonoid, alkaloid, terpenoid and anthraquinone in the ethanol extracts of the samples with better activity recorded in the freeze dried sample. All the extracts displayed high value of free scavenging ability in all the solvents used. The results suggest that the leaf is a good source of phytochemicals and natural antioxidant which validates the reason why the leaf 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.

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