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Evaluation of Phytochemical and Proximate Composition of *Garcinia kola* Seeds and *Vernonia amygdalina* Leaves Sold In Abuja

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ABSTRACT
This study was aimed at evaluating the phytochemical and proximate composition of *Garcinia kola* (Bitter kola) seeds and *Vernonia amygdalina* (Bitter leaf) sold in Abuja, Nigeria. Bitter leaf and bitter kola were collected from various markets in Abuja. Some of the bitter leaves were collected from various gardens as bitter leaf widely grows in most parts of Nigeria. In evaluation of phytochemical constituents, Flavonoids and Saponins were the most abundant in both *G. kola* and *V. amygdalina* (4.54 ±0.35: 6.07 ±6.05) mg/100g. Flavonoid contents of *G. kola* (3.62 ±2.54) mg/100g and *V. amygdalina* (21.04 ±9.72) mg/100g represented the highest phytochemical contents. There was, however, no significant difference ($p > 0.05$) in the Saponins and Flavonoids content of *G. kola* and *V. amygdalina:* Cal. t. = 0.132 and 0.546 < Tab. t. 0.05, df.8 = 2.306. From this study, percentage moisture content of *G. kola* and *V. amygdalina* (45.07 ±13.13: 80.35 ±8.28) and total carbohydrate (38.08 ±4.97: 21.79 ±2.36), represented the highest constituent in both plants respectively. The elemental composition of our test plants showed that Carbon (456.28 ±68.84ppm) and Hydrogen (46.94 ±11.69) were lesser in *G. kola* compared to *V. amygdalina*, with abundant Carbon (576.07), Potassium (72.38 and Phosphorus (8.10 ±8.83 ppm). Vitamin C and E were the most abundant vitamins with *G. kola* yielding 12.26 ±1.31: 11.03±3.09 mg/100g and *V. amygdalina* yielding 2.21 ±0.41: 1.89 ±1.38mg/100g. This study demonstrated a high prospect of availability of highly beneficial phytochemicals which demonstrate antioxidative, antiemetic and even antimicrobial qualities in large quantities. It is advocated that optimal consumption of these plants is of immense benefits and with the rich amount of data available, they can be used as good alternatives to synthetic therapeutic agents.
Keywords: PHYTOCHEMICAL, VITAMINS, GARCINIA, VERNONIA, ABUJA, NIGERIA.

I. INTRODUCTION

Garcinia Kola is a medium sized tree of West and Central Africa origin, particularly popular in parts of Nigeria as a tree of the rain forests (Iwu, 1993). *Garcinia kola* is highly valued for its edible nuts and traditionally used by African medicine men who believed that it had purgative, anti-parasitic, and antimicrobial properties (Adegboye et al., 2008). It has been identified as a potent antibiotic, effective in the treatment of many diseases Adesuyi et al., 2012). The fruit, seeds, nuts and bark of the plant have been used for centuries in folk medicine to treat ailments from coughs to fever, diarrhea, tuberculosis and other bacterial infections. The seed, commonly known as Bitter Cola, is a masticatory agent and is a major kola substitute offered to guests at home and shared at social ceremonies.

Bitter Leaf; *Vernonia amygdalina* is used in traditional medicine as a tonic and remedy against constipation, fever, high blood pressure and many infectious diseases (Iwalokun *et al.,* 2006). The leaves are consumed either as a vegetable (macerated leaves in soups) or aqueous extracts as tonics for the treatment of various illnesses**.** *Vernonia amygdalina,* Bitter Leaf, a shrub; 10 meters tall; is much branched; and densely pubescent. Leaves are alternate, blade ovate-elliptical to lanceolate, cuneate or rounded at base, terminal Inflorescence Flowers; style hairy, brown to black, crowned by the much longer pappus bristles (Schippers, 1997; Beentje, 2000; Ekpo and Etim, 2009; Kullar *et al.*, 2011).

Garcinia kola seed and *Vernonia amygdalina* leaf are both traditional plant parts; which are eaten as whole as kola, food or as medicine. They are also used as medicine especially across the generation in Nigeria and West Africa. Both plants have high appeal as food condiments, especially as they are used widely in Nigeria as condiments and medicine (Ekpo and Etim, 2009). The local use of natural plants as primary health remedies due to their medicinal properties is quite common in Africa (Tula *et al.,* 2011) and is widespread throughout the world. This phenomenon is referred to as traditional or herbal medicine (Tula *et al.,* 2011). Medicinally, the phytochemicals obtained from the seeds have been used for many years in treating infectious diseases (Uzoigwe and Agwa, 2011).

II. MATERIALS AND METHODS

PREPARATION OF PLANT MATERIAL

Fresh ripe pods of Bitter Cola were purchased from Zuba, Abuja fruit market. They were then taken in polythene bags to Medicinal Plant Research Department, National Institute for Pharmaceutical Research and Development Idu Abuja, where it was properly identified before analysis. Seeds were removed from the pods. The hull was carefully removed by peeling with a scalpel blade. The seeds were then, first chopped into tiny pieces with a stainless steel knife. This was to increase the surface area needed for quick drying. The chopped pieces were completely sundried for 5 days. The sample was blended in a blender previously washed with sterile distilled water and cleaned with methylated spirit. The powder was packed in clean cellophane pouches and labelled.

On the other hand, healthy fresh leafy branches of *Vernonia amygdalina* used for this study were obtained from the same market and some from NIPRD garden located at Idu Abuja, FCT Nigeria. Fresh leaves were obtained from the stem and prepared for use in the proximate composition analysis according to standard methods (Adesuyi *et al.*, 2012).

EXTRACTION OF *Vernonia amygdalina* LEAVES AND OF *Garcinia kola* SEEDS

Three hundred grams (300g) of the milled *G. kola* seed powder was added into solvents: distilled water, ethanol and methanol respectively. This was mixed for ten minutes, left to stand for 24 hours and filtered through a giant funnel with a collector below, according to Momoh, 2010. The extracts of the seeds were prepared in accordance with the method of Basri and Fan (Nwaokorie *et al.,* 2010). Briefly, one hundred grams of the powdered seeds were steeped in 500 mL of ethanol, methanol and distilled water for 24 h with shaking at 140 rev/min. The resulting extracts were filtered using Whatman No 1 Filter paper. The extracts were further concentrated to dryness under reduced pressure at 37 °C using a rotary evaporator to remove the solvents. The various extraction solvents were used according to the procedure below:

Extraction of Bitter Leaf

Solvent Used	Composition
Metanol Extraction	300g of crushed leaves +2.3 litres of Methanol
Ethanol Extraction	300g of crushed leaves +2.3 litres of Ethanol
Aqueous Extraction	300g of crushed leaves +2.3 litres of Distilled water

Extraction of Bitter Cola

Solvent Used	Composition
Methanol Extraction	300g of crushed seeds + Methanol
Ethanol Extraction	300g of crushed seeds + Ethanol
Aqueous Extraction	300g of crushed seeds + Distilled water

Aqueous extracts were subjected to evaporation in an oven at 40^{o}C after which the resultant residue was collected and freeze-dried (lyophilized) using a freeze drying machine. The filtrates from methanol and ethanol were concentrated on a rotary evaporator and the concentrated extracts were dried using a hot water bath.

PRIMARY PHYTOCHEMICAL ANALYSIS OF *Garcinia kola* AND *Vernonia amygdalina*

Portions of the *G. kola* seed and *V. amygdalina* leaf extract powder were subjected to phytochemical analysis, using Trease and Evans methods to test for alkaloids, tannins, flavonoids, Saponins, and cardiac glycosides as adopted from Onwuliri et al., (2009). The intensity of the coloration determined the abundance of the compound.

PROXIMATE ANALYSIS OF *Garcinia kola* AND *Vernonia amygdalina*

The standard methods of the Association of Official Analytical Chemists (AOAC), (2012) as adopted by Eroarome, 2012 were used in the determination of the moisture, dry matter, crude protein, crude fat total carbohydrate, lipid, dry matter, crude fibre and ash contents of each sample as adapt**ed** by (Eroarome, 2012). Each analysis was carried out in triplicate.

ELEMENTAL AND MINERAL CONTENT

This was adopted with modifications from Asaolu (2012) and Sodamade (2013). The mineral contents of each sample were determined by spectrophotometry after dry ashing of the samples. Each ash sample was transferred quantitatively into a conical flask and dissolved in 10 ml of 3NHCl. The mixture was heated on a hot plate. The solution was then filtered into a 100 ml volumetric flask and made up to the mark with distilled water. The mineral contents (K, Ca, Mg, Fe, Zn, Mn and P) of the solutions were determined using atomic absorption spectrophotometer.

DETERMINATION OF VITAMINS

Vitamins A, B1,B2,C, and E were determined using methods as adapted by Ejoh et al., 2005.

III. RESULTS

TABLE 1: PHYTOCHEMICAL CONSTITUENTS OF *GARCINIA KOLA* SEEDS AND *VERNONIA AMYGDALINA* LEAVES

	Garcinia kola			*Vernonia amygdalina*		
Phytochemical	**Aqueous Extract**	**Ethanol Extract**	**Methanol Extract**	**Aqueous Extract**	**Ethanol Extract**	**Methanol Extract**
Tannins	+	+	+	+	+	+
Glycosides	+	+	+	+	-	+
Saponins	+	_	_	+	+	+
Alkaloids	+	+	+	+	-	-
Terpenes	_	_	_	-	-	-
Sterols	_	+	+	+	+	+
Phenols	+	_	_	+	-	-
Flavonoids	+	+	+	+	+	+
Anthraquinones	_	_	_	+	-	-
Carbohydrates	+	+	+	+	+	+
Resins	+	+	+	+	+	+
Oil	_	+	+	-	-	-

Key: (+) = presence of; (–) = absence of

TABLE 2: PHYTOCHEMICAL AND ANTI-NUTRITIVE COMPOSITION OF *GARCINIA KOLA* SEEDS (BITTER COLA) AND *VERNONIA AMYGDALINA* BITTER LEAF EXTRACT

Variables	Alkaloids	Saponins	Tannins	Flavonoids	Phenol	Cyanide	Cardiac glycoside	Phytate	Oxalate
	(mg/100g)								
Bitter Cola									
Mean	2.24	4.54	0.05	3.62	0.49	0.27	0.60	1.75	0.06
±SD	±0.05	±0.35	±0.04	±2.54	±0.53	±0.05	±0.05	±0.09	±0.03
Bitter Leaf									
Mean	0.14	6.07	0.02	21.04	0.73	0.13	0.13	0.36	0.11
±SD	±0.02	±6.05	±0.07	±9.72	±0.22	±0.17	±0.16	±0.24	±0.15

SD: Standard error of mean of five readings

Tab. $t_{0.05}$, df.8 = 2.306 < Cal. t = 2.608 - 88.61. Therefore there is a significant difference in the Alkaloids, Tanins, Phenol, Cyanide Content, Vitamin C., Glycoside, Phytate and Oxalate content of *G. kola* and *V. amygdalina* ($p < 0.05$).
There is, however, no significant difference in the Saponins and Flavonoids content of *G. kola* and *V. amygdalina:* Cal. t. = 0.132 and 0.546 < Tab. t. $_{0.05}$, df.8 = 2.306 ($p > 0.05$)

TABLE 3: PROXIMATE COMPOSITION OF *GARCINIA KOLA* SEEDS (BITTER COLA) AND *VERNONIA AMYGDALINA* BITTER LEAF EXTRACT

Variables	Moisture	Crude fat	Crude protein	Crude fibre	Total carbohydrate	Lipid	Dry matter	Ash
	Percentage (%)							
Bitter Cola								
Mean	45.07	1.99	4.75	10.85	38.08	0.47	35.75	2.55
±SD	±13.13	±4.14	±1.85	±0.64	±4.97	±0.33	±5.46	±1.91
Bitter Leaf								
Mean	80.35	8.54	15.44	10.31	21.79	0.43	15.44	10.74
±SD	±8.28	±1.99	±4.73	±1.98	±2.36	±0.23	±5.76	±1.01

SD: Standard error of mean of three readings

Cal. t = 2.776 > Tab t. 0.05 at df.8 = 2.306 (Moisture, Crude Fat, Crude Protein, Crude Fibre , Total Carbohydrate , Lipid , Dry Matter content of *G. kola* and *V. amygdalina*
There is no significant difference in the: Cal. t. (-1.02 – 0.793) < Tab. t 0.05; $df._4$ = 2.776. Ash content, ($p <$ 0.05): Cal. t. = 4.311 > Tab. $t._{0.05}$; df.8 = 2.776.

TABLE 4: ELEMENTAL CONSTITUENTS OF *GARCINIA KOLA* (BITTER COLA) AND *V. AMYGDALINA*

Variables	Potassium K	Calcium Ca	Magnesium Mg	Iron Fe	Zinc Zn	Phosphorous P	Sodium Na	Carbon C	Nitrogen, N	Hydrogen, H
						(ppm)				
Bitter Cola										
Mean	5.27	3.51	1.63	0.49	1.63	8.10	6.36	456.28	9.10	46.94
±SD	2.66	1.84	0.38	0.10	1.16	8.83	1.67	68.84	2.56	11.69
Bitter Leaf										
Mean	72.38	0.93	0.54	0.21	0.14	0.03	0.48	576.07	8.45	8.88
±SD	±13.20	±0.39	±0.42	±0.32	±0.09	±0.03	±0.16	±178.53	±1.32	±3.21

SD: Standard error of mean of five readings

Cal. t. = (2.314, 6.158, 14.737, 3.4656.682 and 46.457) > Tab. t 0.05; df.8 = 2.306. :. There is a significant difference ($p < 0.05$) in the Calcium, Magnessium, Iron Fe, Zinc, Sodium (Na), H_2: Vitamin C content of *G. kola* and *V. amygdalina*. However, no significant difference in the Potassium (K), Phosphorus and Nitrogen content of *G. kola* and *V. amygdalina*: Cal. t = (1.171, 0.328, 0.010, 0.248) < Tab. $t_{0.05}$; df.8 = 2.306 ($p > 0.05$).

TABLE 5: VITAMIN COMPOSITION OF *GARCINIA KOLA* (BITTER COLA) AND *VERNONIA AMYGDALINA* (BITTER LEAF)

Variables	Vit. A (IU)	Vit.B1	Vit. B2	Vit. B3	Vit. C	Vit. E (IU)
			Mg/100g			
Bitter Cola						
Mean	1.18	0.70	0.38	1.62	12.26	2.21
±SD	±0.15	±0.13	±0.15	±0.23	±1.31	±0.41
Bitter Leaf						
Mean	1.76	0.72	0.14	1.47	11.03	1.89
±SD	±0.65	±0.17	±0.01	±0.55	±3.09	±1.38

SD: Standard error of mean of five readings

There is no significant difference in the vitamin B1, B3, C and E content of *G. kola* and *V. amygdalina:* Cal. t. = (1.33, 1.339, 0.346, 0.489) < Tab. t 0.05 df.8 = 2.306 ($p > 0.05$). However, there is a significant difference in the vitamin A and B2 contents of *G. kola* and *V. amygdalina.* Cal .t = 4.173 and 2.4 0 > Tab. t. 0.05 df.8 = 2.306 ($p < 0.05$)

IV. DISCUSSION

In this study, the phytochemical components of *G. kola* and *V. amygdalina* showed high concentrations of alkaloids, Saponins and flavonoids, moderate concentrations of phytate and oxalate. Phenol, cyanide and glycoside were present in low quantities in both plants, although the flavonoid in *V. amygdalina* was much

higher. This disagrees with some studies; which reported low concentrations of alkaloids, Saponins, flavonoids in both plants (Almagoul *et al.,* 1985; Mboto *et al.,* 2009; Odiongenyi *et al.,* 2009). Moderate concentrations of phenols, glycoside and tannins in both *G. kola* and *V. amygdalina* have been reported (Mboto *et al.,* 2009). Many studies have already reported that saponins, tannins, flavonoids and phenolic compounds contain antimicrobial properties (Subrahmanyam *et al.,* 2001; Osman *et al.,* 2003). Antibacterial activity of *V. amygdalina* against some Gram-negative and Gram-positive bacteria has also been reported, with suggestions that Bitter Leaf could be effective on drug resistant microorganisms, and in wounds dressing (Iwalokun *et al.,* 2003; Tula *et al.,* 2011; Uzoigwe and Agwa, 2011). *G. kola* on the other hand has been attributed with good antimicrobial and antiviral properties (Iwu, 1993). The seeds are used in the treatment of bronchitis and throat infections. Similarly in a recent study, crude extract of *G. kola* exhibited *in vitro* antimicrobial activities against both gram-positive and gram-negative organisms comparable to streptomycin and tetracycline (Adegboye *et al.,* 2008).

In both *Garcinia kola* and *Vernonia amygdalina*, carbon was the most abundant element of all the mineral constituents. In this study, the Potassium (72.38 ±13.20ppm) content of *V. amygdalina* is very high compared to the value obtained in *G. kola* (5.27 ±2.66). This finding agrees with Gafar *et al.* (2011) who reported 61.5 ±0.38 in *V. amygdalina*, 6.42 mg/100g in *G. kola*. On the contrary, the high Potassium content in *V. amygdalina* and low *G. kola* content only confirms the importance of these plants as used in health care. Recommended adult daily dosage of Potassium of 2000mg means that both plants may contribute only small amounts of dietary Potassium (Iwu, 1993; Iwalokun *et al.,* 2006).

The Calcium contents of *G. kola* (3.51 ±1.84ppm) and *V. amygdalina* (0.93 ±0.39ppm) in this study compares favorably with the Calcium content from *Vernonia calvoana* (1.44 ±0.06), plant of the same family (Hassan and Umar, 2004, 2006). On the contrary, some studies have reported higher calcium content in *V. amygdalina* leaf concentrates, including (Ejoh *et al.,* 2007) with 151.6 ±1.40 mg/100g. This then indicates that *G. kola* and *V. amygdalina* serve as a significant source of dietary calcium which is needed for growth, the maintenance of good, strong bones, teeth and muscles (Okaka and Okaka, 2001; Oko and Agiang, 2009). In the same vein, Magnesium constituent in *G. kola* (1.63 ±0.38ppm) and *V. amygdalina* (0.54 ±0.42ppm) were low compared to high values obtained by Nwaogu *et al.,* (2000) in *V. amygdalina* leaves (96.5 ±0.96 mg/100g). This suggests that *G. kola* is a better source of magnesium compared to *V. amygdalina* plant even though they were in small quantities.

As Zinc is a vital cellular growth regulator, it also plays important functions in gene expression and as a co-factor of enzymes that are responsible in carbohydrate, proteins and nucleic acid metabolism (Gafar *et al.,* 2011). In this study, the presence of Zinc in both *G. kola* (1.63 ±1.16) and *V. amygdalina* (0.14 ±0.09) compares favorably with other low content in other studies where Zinc concentration of about 1mg/100g was recorded in *Vernonia amygdalina* leaf (Hassan and Umar, 2004). This means that Zinc constituent in both plants are in safe proportion for human consumption and use for food or in drug formulation. We observed Iron content for *G. kol*a and *V. amygdalina as* 0.49 ±0.10 and 0.21 ±0.32 respectively. This conforms favorably with the Iron content of some cultivated vegetables such as spinach (1.6mg/100g) lettuce (0.7 mg/100g) and cabbage (0.3 mg/100g) in similar soil type and area (Gruda, 2005). On the contrary; the values in this study is low compared to the Iron content obtained by Sodamade *et al.,* (2011) in *Vernonia amygdalina* (2.3 ±0.42mg/100g).

In addition, Sodium composition of *G. kola* (6.36 ± 1.67mg/100g) is higher than in *V. amygdalina* (0.48 ±0.16mg/100g). However, it is generally low compared to the values obtained in *Vernonia amygdalina* leaf (57.5 ±0.34 mg/100g) in earlier studies of Bitter Cola and Bitter Leaf (Okwu and Josiah, 2006; Njoku and Akumefule, 2007; Oko and Agiang, 2009). This low content is beneficial as only small amount of Sodium intake is naturally required (excess often lead to high blood pressure). It is therefore of a great advantage that test plants could serve as snacks and food for hypertensive conditions. This is also similar to the small amount recorded for Manganese in *Vernonia amygdalina* (0.1 ±0.14), or 11.6 mg/100g reported for Basalm apple (Hassan and Umar, 2006). Manganese, though an important in-activator of many enzymes; is also one of the elements required in small amount in humans.

The Vitamins C contents of our *G. kola* (12.26 ±1.31), *V. amygdalina* (11.03 ±3.09) mg/100g and Vitamin E (2.21 ±0.41: 1.89 ±1.38) IU, were the most abundant. The vitamin C content in this study was low compared to the value (197.5mg/100g) obtained for *V. amygdalina* by Ejoh *et al.,* (2005). Vitamin B3 (1.62 ±0.23) mg/100g in *G. kola* and Vitamin A in *V. amygdalina* were in moderate quantities. Compared to Vitamin B2 detected in smaller amount (0.38 ±0.15: 0.14 ±0.01) mg/100g for *G. kola* and *V. amygdalina*, respectively. Vitamin A value in *V. amygdalina* (1.76 ±0.65 IU) was higher than that in *G. kola* (1.18 ±0.15 IU) and comparable to Ejoh *et al.,* (2005). The presence of these vitamins further supports the therapeutic importance of these two plants. For instance, due to the vitamin A content, small amounts of vegetable in food has been recommended for prevention of river blindness.

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