



RESEARCH PAPER

COMPARATIVE PROXIMATE, PHYTOCHEMICAL AND MINERAL CONTENTS OF FREEZE-DRIED AND AQUEOUS EXTRACTS OF *VERNONIA AMYGDALINA* DEL. LEAVES AND *GARCINIA KOLA* HECKEL SEEDS

***¹Bolajoko, E.B., ^{2,3}Onyeaghala, A.A., ¹Akinosun, O.M., ⁴Attah, A.F., ⁵Moody, J.O., ⁶Khine, A.A.**

¹Department of Chemical Pathology, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Nigeria; ²Department of Chemical Pathology, University College Hospital, Ibadan; ³Department of Medical Laboratory Science, Lead City University, Ibadan, Nigeria; ⁴Department of Pharmacognosy & Drug Development, Faculty of Pharmaceutical Sciences, University of Ilorin, Nigeria; ⁵Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Nigeria; ⁶Division of Chemical Pathology, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg Academic Laboratory, National Health Laboratory Service Cape Town, South Africa.

*Corresponding author email: bolajokoelizabeth@gmail.com; eb.bolajoko@mail1.ui.edu.ng

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ABSTRACT

Medicinal plants have been used for centuries to meet primary healthcare needs. This study compared proximate, phytochemicals, micronutrient and toxic metal concentrations in freeze-dried and aqueous extract samples of *Vernonia amygdalina* (VA) leaves and *Garcinia kola* (GK) seeds. Fifty gram of freeze-dried samples and 50 mL of freshly prepared aqueous extracts of VA and GK each were used for the analysis. Proximate compositions of VA and GK were assessed using standard analytical methods. Phytochemicals were quantified spectrophotometrically, vitamins by High Performance Liquid Chromatography (HPLC) and mineral compositions by Atomic Absorption Spectrophotometry (AAS). Proximate compositions were significantly elevated in freeze-dried samples of VA and GK compared with aqueous extracts. Phytochemicals: tannins, polyphenols and flavonoids, were higher in freeze-dried samples of VA and GK compared to aqueous extracts ($p < 0.05$). Terpenoids and alkaloids were only observed in VA ($p < 0.05$) but not detected in GK. Similarly, micronutrients including manganese, iron, copper, zinc, vitamins A, C and E were more concentrated in freeze-dried samples compared to aqueous extracts ($p < 0.05$); while, toxic metals such as lead and cadmium were not detected in both freeze-dried and aqueous extracts of VA and GK. The findings suggested that freeze-dried samples of *Vernonia amygdalina* leaves and *Garcinia kola* seeds could possess good medicinal and therapeutic properties.

Keywords: Freeze-dried samples, Aqueous extracts, Micronutrients, Phytochemicals, Proximate

INTRODUCTION

The use of plants in traditional medicine (TM) or complementary medicine (CM) has a long history in health maintenance and in disease prevention as well as treatment, particularly for chronic diseases (WHO, 2013). Across the globe, use of plant has either served as a complement in meeting primary healthcare needs or is the mainstay of healthcare as practiced in most developing countries (Adesuyi *et al.*, 2012; WHO, 2013). Many plants used in African TMs, to alleviate symptoms of illnesses, have been reported to have medicinal importance. These indigenous plants are known to contain chemical substances including alkanoids, tannins, flavonoids, phenolic compounds, steroids, saponins and glycosides. These have been



known to produce definite physiological actions in the body. Therefore; they are either used for treatment purposes or for production of herbal remedies (Sofowora, 1999; Udochukwu *et al.*, 2015).

Vernonia amygdalina (VA) Del. leaf is well known for its bitter taste. The vegetable is used in preparing traditional soup in Nigeria. It belongs to the Asteraceae family (Crellin *et al.*, 1989; Farombi and Owoeye, 2011). This plant is reported to be exceptionally rich in proteins, vitamins and mineral elements such as iron, phosphorus, calcium, potassium, zinc, copper, folic acids and ascorbic acids (Agbogidi and Akpomorine, 2013). Premised on this, extracts of VA leaves and roots have been used in the treatment of fever, hiccups, kidney problems, stomach discomfort, diabetes mellitus, dysentery and other gastrointestinal tract problems, bacteria and parasitic infections, malaria and cancer amongst others (Crellin *et al.*, 1989; Kupchan *et al.*, 1969; Schippers, 2000; Erasto *et al.*, 2005; Ezuruike and Prieto, 2014; Udochukwu *et al.*, 2015). Similarly, it is very potent in scavenging free radicals and chelating metal ions due to the antioxidant properties of the flavonoids and its phenolic contents (Winslow and Krol, 1998; Seef *et al.*, 2001).

Garcinia kola (GK) Heckel, also known as “bitter kola”, is used in Nigeria for different occasions. In the Eastern part of Nigeria, it is used in welcoming guests; while in the West, for traditional wedding and naming ceremonies. It is an angiospermae belonging to the family Clusiaceae or Guttiferae (Farombi and Owoeye, 2011; Adesuyi *et al.*, 2012). The medicinal importance of bitter kola is based mainly on the phytochemical components of the plants obtainable from its roots to its leaves (Iwu *et al.*, 1990). The fruit, seeds, nuts and bark of the plant have been used for centuries in TMs to treat ailments such as bronchitis, throat infections, colic, head or chest colds and cough. In addition, the extracts of the seed have been reported to possess several pharmacological activities. These include anti-inflammatory, analgesic, molluscidal, anti-atherogenic, antioxidant, anti-parasitic, anti-microbial and hepato-protective activities (Farombi *et al.*, 2002; Okunji *et al.*, 2002; Eleyinmi *et al.*, 2006; Madubunyi, 2010; Adesuyi *et al.*, 2012).

In most traditional medicine and scientific reports, the medicinal properties of the extracts of VA leaves and GK seeds have been well documented. However, there is paucity of data on the use of whole plant (freeze-dried samples) of VA leaves and GK seeds. To bridge this gap, we postulate that using the whole plant may have better health indication than the aqueous extracts. This study compared the proximate, phytochemicals, micronutrient and toxic metal concentrations in the freeze-dried (whole plant) and aqueous extract (partial plant) samples of VA leaves and GK seeds.

MATERIALS AND METHODS

Sample Preparation: *Vernonia amygdalina* leaves and GK seeds were obtained, identified and authenticated by Mr. Esimekhuai Donatus, a taxonomist of the Department of Botany, University of Ibadan. The voucher specimen numbers were assigned for *Vernonia amygdalina* (UIH-22612) and *Garcinia kola* (UIH22611) and deposited in the herbarium. The leaves of VA were kept from sunlight and washed thoroughly with distilled water, while the seeds of the GK were peeled to remove the seed coat covering the pulp, rinsed and grated into smaller pieces.

Freeze-dried samples: Onekilogram of VA leaves and 500 g of grated seeds were freeze-dried using a pressure freeze-drying machine at temperature of -65°C and -40°C (machine and sample temperature respectively) to a constant weight. The dried leaves and pulps were then blended into powder using a blender, sieved with laboratory sieve with a mesh size of 0.5 mm. The resulting sieved powdery samples were separately poured into an air-tight brown container and were stored at -20°C for 14 days. To obtain the aqueous extracts, 50 g of the freeze-dried samples of VA leaves and GK seeds each were dissolved in 100 mL of distilled water and 50 mL collected.

Aqueous samples: Five hundred gram of VA leaves were washed and blended with 100 mL of distilled water and sieved. Two hundred gram of GK seeds were rinsed and grated into smaller pieces and then blended with 100 mL of distilled water.

Laboratory determinations of the components of VA leaves and GK seeds: About 50 g each of freeze-dried samples of VA leaves and GK seeds were used for the analysis of the proximate contents such as moisture, crude protein, crude fat, crude fibre, total ash, carbohydrates and pH; while 50 mL of the freshly prepared aqueous extracts of VA leaves and GK seeds each were used for the analysis of phytochemicals (tannins, polyphenols, terpenoids, flavonoids and alkaloids), micronutrients (manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), vitamins A, C and E) and toxic metals: cadmium (Cd), and lead (Pb).



Proximate Assay: The standard methods of the Association of Official Analytical Chemists (AOAC) were used to determine the moisture, crude protein, crude fibre, crude fat, carbohydrate and total ash contents of each sample. Moisture content was determined by heating 2.0 g of each sample to a constant weight in a crucible placed in an oven maintained at 105°C. The dry matter was used in the determination of the other parameters. Crude protein (% total nitrogen x 6.25) was determined by the Kjeldahl method as described by the AOAC, using 2.0 g samples (AOAC, 1990). Crude fibre was obtained by digesting 2.0 g of sample with 1.25% (w/v) H₂SO₄ and 1.25% (w/v) NaOH. The fibre was filtered, dried at 120°C and weighed. The residue was incinerated in a muffle furnace maintained at 550°C for 5hrs. Crude fat was obtained by exhaustively extracting 5.0 g of each sample in a Soxtec 2050 equipment maintained at 135°C for 1 hour using petroleum ether (boiling point range 60-80°C) as the extractant. Ash was determined by the incineration of 1.0 g samples placed in a muffle furnace maintained at 550°C for 3 hours. Total carbohydrate was obtained from the difference between the total weight of the food and the other constituents in the food including protein, fibre, fat, alcohol/water and ash. Each analysis was carried out in duplicate (AOAC, 1990).

Determination of tannin contents: The tannins were determined by Folin – Ciocalteu method (Miean and Mohamed, 2001; Marinova *et al.*, 2005; Afify *et al.*, 2012; Singh *et al.*, 2012). In this method, 10 mL volumetric flask containing 7.5 mL of distilled water and 0.5 mL of Folin-Ciocalteu phenol reagent was added 0.1 mL of the sample extract. Also added to the mixture in the flask is 1 mL of 35% sodium carbonate (Na₂CO₃) solution. The total volume was made up to 10 mL with distilled H₂O. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of tannic acid (20, 40, 60, 80 and 100 µg/mL) were prepared in the same manner as described earlier. Absorbance for test and standard solutions was measured against the blank at 725 nm with an UV-Vis spectrophotometer 1000 series (CECIL Instruments Limited, Milton Technical centre Cambridge CB24 6AZ, England). The tannin content in VA leaves and GK seeds were expressed in terms of mg of tannic acid equivalent (TAE)/100g of extract.

Determination of polyphenols: The total phenolic contents of VA leaves and GK seeds were determined spectrophotometrically using Folin-Ciocalteu reagents with analytical grade gallic acid as the standard. An aliquot of 1mL extracts or standard solution of gallic acid (20, 40, 60, 80 and 100 mg/L) was added to 25 mL volumetric flask containing 9mL of distilled water. One millilitre of Folin-Ciocalteu phenol reagents was added to the mixture and shaken. After 5 minutes, 10 mL of 7% sodium carbonate (Na₂CO₃) solution was added to the mixture. The solution was diluted to 25 mL with distilled H₂O and mixed. A reagent blank using distilled H₂O was prepared. After incubation for 90 minutes at room temperature, the absorbance against prepared reagent blank was determined at 750 nm, which is the wavelength for all phenolic compounds (Marinova *et al.*, 2005) with UV-Vis spectrophotometer 1000 series (CECIL Instruments Limited, Milton Technical centre Cambridge CB24 6AZ, England). The total phenolic contents of VA leaves and GK seeds were expressed as mg gallic acid equivalents (GAE)/100g of fresh weight (Singleton *et al.*, 1999; Chaovanalikit and Wrolstad, 2004; Ghasemzadeh *et al.*, 2010; Nasir *et al.*, 2011).

Terpenoids: Terpenoids was measured by the method described by Ferguson (1956). An aliquot of 1mL extracts or standard solution was added into a conical flask containing 10 mL of petroleum ether. The mixture was left for 15 min followed by intermittent shaking. At the end of 15min, it was filtered and the resulting absorbance was measured at 420nm using UV-Vis spectrophotometer 1000 series (CECIL Instruments Limited, Milton Technical centre Cambridge CB24 6AZ, England). The intensity of colour developed is directly proportional to the concentration of terpenoids present in the samples.

Determination of total flavonoid content: Total flavonoid contents were measured by the modified aluminium chloride (AlCl₃) colorimetric method as described by Dewantoet *al.* (2002), Marinovaet *al.* (2005) and Nabaviet *al.* (2008). An aliquot of 1mL extracts or standard solution of catechin (20, 40, 60, 80 and 100 mg/L) was diluted with 4mL distilled H₂O in a 10 mL volumetric flask. Then 0.3 mL of 5% NaNO₂ was added. After 5 min, 0.3 mL of 10% AlCl₃ was added; at 6 min, 2 mL of 1 M NaOH was added and the total volume was made up to 10 mL with distilled H₂O. The solution was thoroughly mixed. Absorbance of the reaction mixture was measured at 510 nm against a reagent blank using a UV-Vis spectrophotometer 1000 series (CECIL Instruments Limited, Milton Technical centre Cambridge CB24 6AZ, England). The total flavonoid contents in VA leaves and GK seeds were expressed as mg catechin equivalents (CE)/100 g of fresh matter.

Determination of alkaloids: One milligram of the plant extract was dissolved in dimethyl sulphoxide (DMSO) and 1 mL of 2 N HCl was added and then filtered. This solution was transferred to a separating funnel where 5 mL of bromocresol green solution and 5 mL of phosphate buffer were added. The mixture was vigorously shaken with 1, 2, 3 and 4 mLs of chloroform and made up to 10 mL with chloroform in a 10-mL volumetric flask. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100 µg/mL) were prepared in the same manner as described earlier. The absorbance for test and standard solutions



was determined against the reagent blank at 470 nm with an UV-Vis spectrophotometer 1000 series (CECIL Instruments Limited, Milton Technical centre Cambridge CB24 6AZ, England). The total alkaloid content was expressed as mg of atropine equivalent (AE)/100 g of extract (Shamsa *et al.*, 2008; Malikarjuna *et al.*, 2012).

Micronutrient and toxic metal contents analyses: The freeze-dried and aqueous extract samples of VA and GK were processed and the resultant ash was dissolved in 5 mL of HNO₃/HCl/H₂O (1:2:3) and heat applied gently on a hot plate until brown fumes disappeared. To the remaining material in each crucible, 5 mL of deionized water was added and heat applied until a colourless solution was obtained. The mineral solution in each crucible was filtered into a 100 mL volumetric flask through a Whatman No. 42 filter paper and the volume was made to the mark with deionized water.

Using the appropriate hollow cathode lamp, the digested samples and the salts of the metals to be analysed used as standards were sprayed and read on Buck Atomic Absorption Spectrophotometer (AAS) model 210/211 VGP. A 10 cm-long cell was used and concentration of each element in the sample was calculated on percentage of dry matter. This was done for Cu, Fe, Mn, Zn, Cd and Pb (Shahidi *et al.*, 1999). Vitamins A, C and E were determined using Kirk and Sawyer (1991) method as described by the Association of Vitamin Chemists using High Performance Liquid Chromatography (HPLC).

Data analysis: Data were presented as means with standard deviations (SD) and analysed using a Statistical Software Package for Social Sciences (SPSS) version 20 (IBM SPSS, Armonk, NY, USA). The Student's 't-test' was used to determine significant differences between the means. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

The proximate results revealed low moisture contents with higher levels in crude protein, crude fat, crude fibre, total ash and carbohydrate of the freeze-dried samples compared with the aqueous extracts of VA leaves and GK seeds ($p < 0.05$). Similarly, significantly higher levels of tannins, polyphenols and flavonoids were found in the freeze-dried samples of VA leaves and GK seeds compared with their corresponding aqueous extract samples. Terpenoids and alkaloids were higher in the freeze-dried samples of VA leaves compared with the aqueous extracts of the leaves; however, terpenoids and alkaloids were not detected in both the freeze-dried samples and the aqueous extracts of GK. Furthermore, the levels of micronutrients: Mn, Fe, Cu, Zn, vitamin A, vitamin C and vitamin E were higher in the freeze-dried samples compared with the aqueous extracts of VA leaves and GK seeds ($p < 0.05$); while Cd and Pb were not detected in both the VA leaves and GK seeds (See Table 1 below).

DISCUSSION

High moisture content in any organic compound or material can cause microbial contamination and growth and this may have negative impact on the long term storage of the aqueous form of medicinal plants (Ruberto and Baratha, 2000; Dah-Nouvlessounon *et al.*, 2015). In this study, the low moisture content of the freeze-dried samples observed for VA and GK implied that these samples could be stored for long period without fear of spoilage or microbial contamination. The aqueous extracts of VA leaves and GK seeds had higher moisture contents than those reported by Okeke *et al.* (2015) and Odebunmi *et al.* (2009) respectively. The differences observed from the various studies could be attributed to the differences in the nature of soil and environmental conditions where the VA and GK plants were grown and harvested.

Protein is one of the major macronutrients vital for survival of man and animal (Voet *et al.*, 2008). This study revealed higher protein content in the freeze-dried samples of VA leaves and GK seeds compared with their corresponding aqueous samples. This may be due to protein retention in the plant residues with little quantity extracted into the aqueous solution. However, the freeze-dried sample of VA leaves contain more protein than the freeze-dried sample of GK seeds. The high protein contents of the freeze-dried samples may complement the body's need of this important macronutrient for growth, development and repair of worn-out tissues in man and other animals and thus enhance species survival. Similarly, enzymes



Table 1: The proximate, phytochemical, micronutrient and toxic metal components of freeze-dried and aqueous extracts of *Vernonia amygdalina* leaves and *Garcinia kola* seeds

Components	Freeze-dried VA Mean±SD	Aqueous extract VA Mean±SD	Freeze-dried GK Mean±SD	Aqueous extract GK Mean±SD	p'- values	p''- values	p#- values	p## values
Proximate								
Moisture (%)	7.70±0.07	99.10±0.03	7.10±0.03	98.30±0.07	0.000	0.000	0.074	0.056
Crude protein (%)	28.52±0.04	3.17±0.01	5.47±0.07	2.53±0.04	0.000	0.004	0.001	0.020
Crude fat (%)	5.30±0.07	0.04±0.00	5.80±0.28	0.95±0.04	0.006	0.022	0.186	0.021
Crude fibre (%)	9.09±0.01	0.02±0.00	3.32±0.04	0.56±0.07	0.001	0.005	0.002	0.059
Total Ash (%)	4.45±0.07	0.18±0.03	0.82±0.03	0.00±0.00	0.010	0.016	0.005	0.070
Carbohydrate (%)	44.97±0.09	5.98±0.03	77.49±0.05	0.00±0.00	0.001	0.000	0.001	0.002
pH	5.90±0.28	6.05±0.21	5.60±0.01	5.80±0.03	0.205	0.032	0.359	0.305
Phytochemical								
Tannins (g/100g TAE)	3.54±0.10	2.23±0.04	2.24±0.04	1.07±0.05	0.019	0.033	0.046	0.003
Polyphenols(g/100g GAE)	17320.00±7.07	313.33±0.04	10372.55±0.04	286.84±0.06	0.000	0.000	0.000	0.000
Terpenoids (mg/kg)	1.60±0.03	1.23±0.04	ND	ND	0.008	-	-	-
Flavonoids (mg/100g CE)	8.32±0.06	6.76±0.06	6.03±0.04	37.71±0.07	0.002	0.002	0.021	0.000
Alkaloid (mg/g AE)	1.05±0.03	0.08±0.01	ND	ND	0.016	-	-	-
Micronutrients								
Mn (mg/kg)	245.33±0.04	1.17±0.01	25.11±0.01	1.45±0.03	0.000	0.001	0.000	0.023
Fe (mg/kg)	378.43±0.01	7.75±0.07	25.86±0.03	6.39±0.01	0.000	0.001	0.000	0.028
Cu (mg/kg)	35.30±0.02	2.96±0.08	5.98±0.11	2.54±0.06	0.001	0.006	0.001	0.149
Zn (mg/kg)	59.59±0.01	2.12±0.03	12.68±0.07	1.33±0.04	0.000	0.004	0.001	0.040
Vitamin A	^a 4.85±0.07	^b 0.73±0.04	^a 4.37±0.03	^b 0.91±0.01	0.012	0.006	0.092	0.119
Vitamin C	^a 22.49±0.01	^b 0.13±0.03	^a 9.48±0.04	^b 0.60±0.04	0.001	0.004	0.001	0.014
Vitamin E	^a 16.45±0.04	^b 4.62±0.03	^a 10.33±0.03	^b 0.46±0.01	0.003	0.002	0.001	0.002
Toxic Metals								
Cd (mg/kg)	ND	ND	ND	ND	-	-	-	-
Pb (mg/kg)	ND	ND	ND	ND	-	-	-	-

SD = Standard deviation; ND = Not detected; ^aUnit = mg/kg; ^bUnit = mg/L; Mn = Manganese; Fe = Iron; Cu = Copper; Zn = Zinc; Cd = Cadmium; Pb = lead

p' – value = Freeze-dried VA compared with aqueous extracts of VA

p# – value = Freeze-dried VA compared with Freeze-dried GK

p'' – value = Freeze-dried GK compared with aqueous extracts of GK

p## – value = Aqueous extracts of VA compared with aqueous extracts of GK



(antioxidant and non-antioxidant), hormones and a lot of body components are made of protein (Kelley *et al.*, 2007; Cuesta *et al.*, 2015). The high protein in the freeze-dried samples may suggest also the involvement in free radical scavenging since protein is a major components of these enzymes. In this study, the obtained values of protein in VA leaves were much lower than the reported values by Anyasor *et al.* (2010) and Okeke *et al.* (2015). Similarly, values obtained for GK seeds were higher than those reported by Eleyinmi *et al.* (2006), Odebunmi *et al.* (2009) and Adesuyi *et al.* (2012) but lower than the values reported by Ajaiet *et al.* (2012) and Maziet *et al.* (2013). This difference in the reported protein may be due to the different geographical locations of collected samples which may vary in soil contents, since the soil contents determine the nutrient contents of the plants.

Similarly, higher contents of crude fat were obtained for freeze-dried VA leaves and freeze-dried GK seeds while aqueous extracts of VA revealed a slightly lower value than the aqueous extracts of GK. Fat is insoluble in water, therefore, the low contents found in aqueous extract samples of VA and GK may results from this insolubility whereas, the freeze-dried samples (whole plant) was able to retain the fat contents. The obtained fat contents in VA leaves were slightly higher than the values reported by Okeke *et al.* (2015) but lower than those reported by Anyasor *et al.* (2010) and Asaolu *et al.* (2012). However for GK seeds, the fat contents obtained were slightly lower than the values reported by Odebunmi *et al.* (2009) and Eleyinmi *et al.* (2006) but higher than the reported values by Adesuyi *et al.* (2012).

The crude fibre contents of both VA and GK suggested the presence of cellulose, hemi-cellulose and lignin in the herbs (Adesuyi *et al.*, 2012). The freeze-dried samples of VA leaves and GK seeds revealed higher contents of the crude fibre than the aqueous extract samples. However, the freeze-dried VA leaves revealed higher content of crude fibre than that of freeze-dried GK seeds while the crude fibre contents of aqueous extracts of GK seeds were slightly more than that of the aqueous extracts of VA leaves. The obtained values of crude fibre in VA leaves were lower than that reported by Anyasor *et al.* (2010), Asaolu *et al.* (2012) and Okeke *et al.* (2015). Similarly, the fibre contents of GK seeds were lower than that reported by Odebunmi *et al.* (2009) and Eleyinmi *et al.* (2006) but higher than that reported by Adesuyi *et al.* (2012). This study might suggest that intake of freeze-dried VA and freeze-dried GK could increase the body's fibre requirement. Fibres help to increase intestinal bulk and motility as well as improve the transitory time.

The total ash contents in the freeze-dried samples of both plants were slightly higher than the aqueous extract samples. Aqueous extract of VA leaves revealed a small content of total ash while it was not detected in aqueous extracts of GK seeds. Also, the freeze-dried VA leaves were higher in total ash contents than the freeze-dried GK seeds. In this study, the total ash values obtained for VA leaves were much lower than the reported values by Anyasor *et al.* (2010), Asaolu *et al.* (2012) and Okeke *et al.* (2015). However for GK seeds, the obtained values were comparable to the values reported by Ajai *et al.* (2012) and Odebunmi *et al.* (2009), but lower than the values reported by Eleyinmi *et al.* (2006) and Adesuyi *et al.* (2012).

Carbohydrates, another major macronutrient, which the body requires for growth, development and survival, are the most abundant biological molecules. They play very important functions in the body as the main sources of energy as well as structural materials (Voet *et al.*, 2008). They also provide accessible fuel for physical performance and regulate nerve tissues (Whitney and Rolfes, 2005). In this study, the freeze-dried sample of VA leaves had lower carbohydrate contents than the freeze-dried samples of GK seeds. However, higher levels were observed in the aqueous extracts of VA leaves than the GK seeds suggesting that most of the carbohydrate was retained in the samples' residue after extraction. Higher carbohydrate contents in VA leaves were much lower than those reported by Okeke *et al.* (2015) but higher than the values reported by Anyasor *et al.* (2010) and Asaolu *et al.* (2012). However, the carbohydrate contents observed for GK seeds were in agreement with other findings by Adesuyi *et al.* (2012), Mazi *et al.* (2013) and Dah-Nouvlessounon *et al.* (2015).



Tannins are responsible for the astringent taste of foods and beverages (Amarowicz, 2007; Chikezie *et al.*, 2008). They are reported to be beneficial because of their possession of anticarcinogenic, antimutagenic, antimicrobial and antioxidant properties (Zhang *et al.*, 2004; Amarowicz, 2007). In this study, the tannin contents of the freeze-dried samples of VA leaves and GK seeds were higher than their corresponding aqueous extracts. Premised on their beneficial effects, this result suggested that the freeze-dried samples may provide better medicinal and therapeutic activities than the aqueous extracts. The obtained values for tannins in VA and GK were respectively twice as low as those reported by Udochukwu *et al.* (2015) and Omehet *et al.* (2014), but the tannin contents in VA and GK were much higher than those reported by Okeke *et al.* (2015) and Eleazu *et al.* (2012). This difference may be due to the different sources where the herbs were obtained.

Polyphenols are natural occurring compounds found largely in fruits, vegetables, cereals and beverages (Scalbert *et al.*, 2005; Spencer *et al.*, 2008). Their long term consumption have been shown to provide protection against the development of cancers, cardiovascular diseases, diabetes mellitus, osteoporosis and neurodegenerative diseases (Pandey and Rizvi, 2009). In this study, the freeze-dried samples of VA leaves and GK seeds showed higher values of polyphenols than the aqueous extracts. This suggested that better protection, against oxidative stress related diseases, might be achieved with freeze-dried samples than the aqueous extracts of VA leaves and GK seeds. Further studies are required to elucidate this.

Terpenoids represent the most diverse and largest group of chemicals among the broad variety of different substances produced by plants. They have been traditionally used by man in the food, pharmaceutical and chemical industries (Tholl, 2015). Also, terpenoids have been demonstrated to possess antioxidant properties and are used as health promoters (Grassmann, 2005). In this study, the terpenoids were only detected in the freeze-dried and aqueous extracts of VA leaves but not detected in the freeze-dried and the aqueous extracts of GK seeds. This could suggest that terpenoids might not be one of the essential components of GK seeds.

Flavonoids are basic component of diet such as vegetables, fruits, nuts, seeds, stem, flower, tea and wine (Sandhar *et al.*, 2011). They are a group of plant secondary metabolites with vast range of biological and medicinal activities, which increases their potential for therapeutic drug development (Banjarnahor and Artanti, 2014). In this study, the flavonoid contents in the freeze-dried samples of VA leaves were higher than the aqueous extract sample, whereas for GK seeds, the aqueous extract contents of flavonoids were substantially higher than the freeze-dried sample. This implied that the freeze-dried sample of VA leaves may possess a better free-radical scavenging property than the freeze-dried GK seeds. The values for VA were comparable with those reported by Okeke *et al.* (2015), while the aqueous extract values were higher than the reported value by Udochukwu *et al.* (2015). However, the obtained flavonoid content in aqueous extract of GK is higher than the reported value by Eleazu *et al.* (2012) and Omeh *et al.* (2014).

Several studies have demonstrated the antioxidant properties of alkaloids (Maiza-Benabdesselam *et al.*, 2007; Moura *et al.*, 2007). In this study, the freeze-dried samples of VA leaves revealed slightly higher alkaloidal contents than the aqueous extracts of the leaves. However, the alkaloid contents of the freeze-dried and aqueous extracts of GK seeds were not detected. This result, in terms of alkaloids contents, suggested that VA leaves may possess a better alkaloid related bioactivity than GK seeds. The observed alkaloid contents in the aqueous extract of VA leaves were lower than the reported values by Udochukwu *et al.* (2015), but the values for GK seeds were contrary to the reports of Eleazu *et al.* (2012) and Omeh *et al.* (2014).

Micronutrients are vitamins (A, B, C, D, E, K) and minerals (Zn, Cu, Mn, Se, Fe, I, and Cr among other) that the body requires in small quantities for specific functions (Shils *et al.*, 1999). These functions range from prevention of deficiency diseases such as scurvy, pellagra and rickets, to regulation of metabolism and gene expression as well as deterrence of the development and progression of many chronic diseases (Shils *et al.*, 1999). In this study, the freeze-dried samples of VA leaves and GK seeds revealed greater micronutrient contents than the aqueous samples.



However, the freeze-dried sample of VA leaves revealed higher micronutrient contents than GK seeds. Iron, Cu, Zn and vitamin E contents were higher in aqueous extracts of VA leaves compared with the aqueous extracts of GK seeds while Mn, vitamins A and C were lower in aqueous extract of VA leaves than that of GK seeds. Interestingly in this study, toxic metals such as Cd and Pb were not detected in both the freeze-dried and aqueous extract samples of VA leaves and GK seeds. These findings suggested that VA leaves, especially the freeze-dried form, may possess better protective and scavenging abilities to prevent diseases associated with oxidative stress and maintain good health than GK seeds. The obtained mineral contents in VA leaves were much higher than those reported by Asaolu *et al.* (2012), Adewole *et al.* (2015) and Offor (2014). However, the mineral contents in GK seeds were higher than the reported values by Odebunmi *et al.* (2009) and Adesuyi *et al.* (2012) while vitamins A and E contents were higher and vitamin C lower than the values reported by Mazi *et al.* (2013).

CONCLUSION/RECOMMENDATION

In this study, the freeze-dried (whole plant) samples of both *Vernonia amygdalina* leaves and *Garcinia kola* seeds revealed higher phytochemical and micronutrient contents than their corresponding aqueous extracts. However, the freeze-dried samples of *Vernonia amygdalina* leaves revealed higher phytochemical and micronutrient contents than *Garcinia kola* seeds. Thus suggesting better medicinal and therapeutic properties for preventing oxidative stress related diseases and maintaining good health. Therefore, in order to achieve better health benefits from the use of plants in health maintenance, disease prevention and treatment, especially for chronic diseases, the freeze-dried samples should be preferred to the aqueous extracts.

CONFLICT OF INTEREST: No conflicts of interest

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AUTHORS' CONTRIBUTIONS:

The contributions of the authors are stated as follows:

Bolajoko, E.B., Onyeaghala, A.A., Akinosun, O.M., Attah, A.F., Moody, J.O., and Khine, A.A. – Conceived and designed the study.

Bolajoko, E.B. – Did Data acquisition, analysis, and manuscript preparation

Onyeaghala, A.A., Attah, A.F., Akinosun, O.M., Moody, J.O., and Khine, A.A. – Contributed to the drafting of the manuscript, performed manuscript editing and review. All Authors approved the final version.

