Phytochemicals, Vitamins and Mineral Contents of Two Nigerian Medicinal Plants

D.E. Okwu

Department of Chemistry, Michael Okpara University of Agriculture, Umudike P. M. B. 7267 Umuahia, Abia State, Nigeria

Abstract: Samples of two Nigerian medicinal plants (*Garcinia Kola* Heckel) and (*Aframomum melegueta*) were analyzed for their phytochemical composition, vitamins and minerals constituents. The result revealed the presence of bioactive constituents comprising: flavonoids (5.76–1.98 mg 100⁻¹g), phenols (0.09–0.11 mg 100⁻¹g), saponins (1.24–11.48 mg 100⁻¹g), tannins, (0.26–0.38 mg/100g). The medicinal plants contained ascorbic acid (12.32-23.10 mg 100⁻¹g), niacin (0.05–1.60 mg 100⁻¹g), riboflavin (0.22–0.26 mg 100⁻¹g) and thiamin (0.24-0.45 mg 100⁻¹g). The plants are good sources of minerals such as: Ca, P, K, Mg, Na. Fe, Zn, Mn and Cu. These substances may be responsible for the health related properties of the plants, which are based on their antioxidant, anticancer, anti-tumor antiviral, anti-inflammatory and anti-allergic activities. These supports the popular use of *G. Kola* and *A. melegueta* in herbal medicine in South Eastern Nigeria.

Key words: Aframomum melegueta, Garcinia Kola, bioactive compounds, antioxidants, anticancer, anti-inflammatory, anti-allergic, anti-tumor, herbal medicine

INTRODUCTION

In Nigeria, many indigenous plants are used as spices, food or medicine. A great number of these plants are traditionally noted for their medicinal and pesticide properties^[1]. It is widely accepted that fruits and vegetables have many healthful properties. There is considerable amount of epidemiological evidence revealing an association between those who have a diet rich in fresh fruits and vegetables and a decrease risk of cardiovascular diseases and certain forms of cancer^[2-4].

These plants often exhibit a wide range of biological and pharmacological activities, such as anti-inflammatory, anti-bacterial and anti-fungal properties^[5-9]. Extracts from the roots, barks seeds and fruits of these plants are used in the preparation of syrups and infusions in traditional medicine as cough suppressant and in the treatment of liver cirrhosis and hepatitis^[10-11].

It is generally assumed that the active constituents contributing to these protective effects are the phytochemicals, vitamins and minerals^[9]. Phytochemicals are present in a variety of plants utilized as important components of both human and animal diets. These include fruits, seeds, herbs and vegetables. Diets containing an abundance of fruits and vegetables are protective against a variety of diseases, particularly cardiovascular diseases^[12]. Herbs, and spices are harmless sources for obtaining natural antioxidants^[4,13,14].

Phytochemical which posses many ecological and physiological roles are widely distributed as plant constituents. Woody plants can synthesize and accumulate in their cells a great variety of phytochemicals including alkaloids, flavonoids, tannins, cyanogenic glycosides, phenolic compounds, saponins, lignins and lignans^[4].

Phytochemicals exhibit a wide range of biological effects as a consequence of their antioxidant properties. Several types of polyphenols (phenolic acid, hydrolysable tannins and flavonoids) show anti-carcinogenic anti-mutagenic effects^[12]. and polyphenols might interfere in several of the steps that lead to the development of malignant inactivating carcinogens, inhibiting the expression of mutagens^[4,12]. However, some polyphenols have been reported to be mutagenic in microbial assays and cocarcinogens or promoters in inducing carcinogenesis^[4,12]

Several studies have shown that in addition to their antioxidant protective effect, polyphenols, particularly flavonoids inhibit the initiation, promotion and progression of tumors^[4,12].

In recent times, plant flavonoids have attracted attention as potentially important dietary cancer chemo-protective agents^[15,16]. In addition, the possible anti-tumor action of certain flavonoids has also generated interest^[17,19]. Moreover, naturally occurring phytochemicals are potential anti-allergic, anti-carcinogenic, antiviral and antioxidant agents^[4,20].

Isoflavones, which are phytoestrogens effectively, modulate estrogens levels in humans. They are therefore often of clinical value in low estrogen states like menopause or imbalanced and toxic estrogen sensitive conditions such as breast, uterine and prostate tumor growth^[21]. It is now well recognized that people who consume these fruits experience less breast, uterine and prostate cancers.

Aframomum melegueta (Alligator pepper) Zingiberaceae is a plant with both medicinal and nutritive values, found commonly in the rain forest^[22]. The seeds are commonly used in flavouring food in Eastern Nigeria. It is used in Liquors and alcoholic, beverages; medicine for intermittent fever, dysentery and gastrointestinal trouble^[22]. The taste is spicy, hot and a little bitter due to a volatile oil. It contains hydroxyarylalkanone on acetone extract.

Garcinia Kola Heckel (Guttiferare, Bitter kola) is popular in Southern Nigeria. The plant is extensively used in herbal medicine and as food. It is usually found in the tropical rain forest region of West Africa. It prevails as a multipurpose tree crop in the home gardens of Southern Nigeria^[23]. The tree is usually cultivated within villages in Southern Nigeria. It grows to a height of about 14 m and produces reddish, yellowish or orange coloured fruits. Each fruit contains 2 to 4 yellow seeds and a sour tasting pulp. The seeds when chewed have a bitter astringent taste. The flowering of the plant occurs between December and January while the fruits mature between June and August.

Garcinia Kola is highly valued because of its medicinal use^[15,24,25]. The seeds are chewed as an aphrodisiac or use to cure cough, dysentery or chest colds in herbal medicine^[5]. Garcinia Kola could serve as raw material for pharmaceutical industries^[26]. The raw stem bark of G. Kola is a purgative. The powdered bark is applied to malignant tumors. The sap is used for curing parasitic skin diseases. The latex or gum is used internally against gonorrhea^[26] and applied externally on fresh wounds. The seeds prevent or relive colic disorders; cure head or chest colds, suppressed cough and is often use in the treatment of cirrhosis and hepatitis (inflammation of the liver)^[11,26].

The stem of *Garcinia Kola* is used to produce favorite bitter chewing sticks sold in small bundles. The seeds as well as chewing sticks are important products often seen in West Africa markets, particularly Nigeria^[5]. Chewing sticks when used without toothpaste is very effective, efficient and reliable in cleaning the teeth of many people in Southern Nigeria^[9,27].

In Nigeria, indigenous people traditionally use A. melegueta and G. Kola to maintain their health. A. melegueta and G. Kola plants have enormous reservoirs of many secondary metabolites and nutritive compounds which exhibit some medicinal and nutritive properties.

Considering the importance of these plants, which are commonly used in herbal medicine, it is necessary to investigate the properties of *G. Kola* and *A. melegueta* seeds.

This study investigates the fundamental scientific basis for the use of the seeds of these plants by determining the chemical constituents as well as quantifying the percentage of crude phytochemicals, vitamins and mineral constituents present in the seed of *Garcinia Kola* and *Aframonum melegueta*.

MATERIALS AND METHOD

Sample collection: The seeds of *Garcinia Kola* and *Aframomum melegueta* were purchased from Ariam market, Ikwuano Local Government Area of Abia State, Nigeria on 15th July 2002. The plants materials (leaves, flowers and seeds) were identified and authenticated by Dr. A. Nmeregini of Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture Umudike, Nigeria. The voucher specimens were deposited in the Forestry Department, Herbarium of Michael Okpara University of Agriculture Umudike, Nigeria.

Sample preparation: Ripe seeds of *Garcinia Kola* and *Aframomum melegueta* were each weighed (1kg). The plant samples were sun dried and ground into uniform powder using Thomas-Wiley machine and stored in airtight bottles till required for analysis.

Chemical analysis: The major elements comprising calcium, phosphorus, sodium, potassium, magnesium and trace elements (Iron, zinc, copper, manganese, chromium, cobalt and cadmium) were determined according to the method of Shahidi *et al*^[28].

The ground plant samples were sieved with a 2mm rubber sieve and 2 g of each of the plant samples were weighed and subjected to dry ashing in a well-cleaned porcelain crucible at 550°C in a muffle furnace. The resultant ash was dissolved in 5 mL of HNO₃/HCl/H₂O (1:2:3) and heated gently on a hot plate until brown fumes disappeared. To the remaining material in each crucible, 5 mL deionized water was added and heated until a colourless solution was obtained. The mineral solution in

each crucible was transferred into a 100 mL volumetric flask by filtration through a Whatman No 42 filter paper and the volume was made to the mark with deionized water. This solution was used for elemental analysis by atomic absorption spectrophotometer. A 10cm-long cell was used and concentration of each element in the sample was calculated according to the percentage of dry matter.

Phosphorus content of the digest was determined colorimetrically according to the method described by Nahapetian and Bassiri^[29]. To 0.5 mL of the diluted digest, 4 mLs of demineralized water, 3 mLs of 0.75m H₂SO₄, 0.4 mLs of 10% (NH₄)₆Mo₇O₂₄4H₂O and 0.4 mLs of 2% w/v ascorbic acid were added and mixed. The solution was allowed to stand for 20-mins and absorbance readings were recorded at 660 nm. The content of phosphorus in the extract was determined.

PHYTOCHEMICAL DETERMINATION

Preparation of fat free sample: 2 g of the sample were defatted with 100 mL of diethyl ether using a Soxhlet apparatus for 2hrs.

Alkaloid determination: 5 g of the sample were weighed into a 250 mL beaker and 200 mL of 20% acetic acid in ethanol was added and covered to stand for 4hr. This was filtered and the extract was concentrated using 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 by filtration and weighed^[30,31].

Tannin determination: 500 mg of the sample was weighed into 100 mL plastic bottle. 50 mL of distilled water was added and shaken for 1hr in a mechanical shaker. This was filtered into a 50 mL volumetric flask and made up to the mark. Then 5 mL of the filtrate was pipette out into a tube and mixed with 3 mL of 0.1M FeCl₃ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 120 nm wavelengths, within 10 minutes. A blank sample was prepared and the colour also developed and read at the same wavelength. A standard was prepared using tannin acid to get 100 ppm and measured^[32].

Determination of total phenols by spectrophotometric method: For the extraction of the phenolic component, the fat free sample was boiled with 50 mL of ether for 15 minutes. 5 mL of the extract was pipette into a 50 mL flask, and then 10 mL of distilled water was added, 2 mL of ammonium hydroxide solution and 5 mL

of concentration amyl alcohol were also added. The samples were left to react for 30 minutes for colour development. The absorbance of the solution was read using a spectrophotometer at 505 nm wavelengths^[30,31].

Saponin determination: The samples were ground. 20g of each plant samples were dispersed in 200 mL of 20% ethanol. The suspension was heated over a hot water bath for 4hrs with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 mL of 20% ethanol. The combined extracts were reduced to 40 mL over water bath at about 90°C. The concentrate was transferred into a 250 mL separator funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated.

60 mL of n-butanol was added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponin content was calculated in percentage^[31].

Flavonoid determination: 100 g of the plant sample were extracted repeatedly with 100 mL of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed^[33].

Determination of riboflavin: 5 g of the sample was extracted with 100 mL of 50% ethanol solution and shaken for 1hour. This was filtered into a 100 mL flask. 10 mL of the extract was pipette into 50 mL volumetric flask. 10 mL of 5% potassium permanganate and 10 mL of 30% $\rm H_2O_2$ were added and allowed to stand over a hot water bath for about 30 minutes. 2 mL of 40% sodium sulphate was added. This was made up to 50 mL mark and the absorbance measured at 510 nm in a spectrophotometer.

Determination of thiamin: 5 g of the sample were homogenized with ethanolic sodium hydroxide (50 mL). It was filtered into a 100 mL flask. 10 mL of the filtrate was pipette and the colour developed by addition of 10 mL of potassium dichromate and read at 360 nm. A blank sample was prepared and the colour also developed and read at the same wavelength.

Determination of niacin: 5 g of the sample was treated with 50 mL of I N sulphuric acid and shaken for 30 minutes. 3 drops of ammonia solution were added to the

sample and filtered. 10 mL of the filtrate was pipette into a 50 mL volumetric flask and 5 mL of potassium cyanide was added. This was acidified with 5 mL of 0.02 N $\rm H_2SO_4$ and absorbance measured in the spectrophotometer at 470 nm wavelengths. This was used to plot the calibration curve.

Determination of ascorbic acid (vitamin C):5 g of the sample was weighed into an extraction tube and 100 mL of EDTA/TCA (2:1) extracting solution were mixed and the mixture shaken for 30 minutes. This was transferred into a centrifuge tube and centrifuged at 3000 rpm for about 20 minutes. It was transferred into a 100 mL volumetric flask and made up to 100 mL mark with the extracting solution. 20 mL of the extract was pipette into a volumetric flask and 1% starch indicator was added. This was added and titrated with 20% CuSO4 solution to get a dark end point^[34].

RESULTS

The phytochemical constituents of *Garcinia kola* and *Aframomum melegneta* are shown in Table 1. The concentration of saponin was higher in *G. kola* (11. 48 mg 100⁻¹g) than in *A. melegueta*, which contains 5.76mg 100⁻¹g of saponin. *A. melegueta* contained more flavonoids (5.76mg 100⁻¹g) and tamnin (0.38mg 100⁻¹g) while *G. kola* contained 1.98mg 100⁻¹g of flavonoids and 0.26mg 100⁻¹g of tannins respectively.

Quantitative estimates of other phytochemicals showed that G. kola contained $0.36 \text{mg} \ 100^{-1} \text{g}$ of alkaloids while A. melegueta contained $0.28 \text{mg} \ 100^{-1} \text{g}$ of alkaloids. More phenols were detected in G. kola than A. melegueta.

G. Kola seed contained the highest amount of ascorbic acid (23.10mg 100⁻¹g) than A. melegueta, which contained 12.32 mg 100⁻¹g of ascorbic acid. Other vitamins; riboflavin, thiamin and niacin were also detected in both seeds (Table 2).

The results of the mineral composition clearly show that *G. kola* and *A. melegueta* seeds constitute a rich source of mineral elements. Calcium was the most abundant macro element available in *G. kola* seed (1.80 mg 100⁻¹g), followed by potassium (2.50 mg 100⁻¹g) while *A. melegueta* contained 1.52 mg 100⁻¹g of calcium and 0.65 mg 100⁻¹g of potassium, phosphorus, magnesium and sodium were detected in both seeds. Enormous quantity of iron (17.75 mg 100⁻¹g) and zinc (2.30 mg 100⁻¹g) were detected in *G. kola*, seed while *A. melegueta* contained 1.80 mg 100⁻¹g and 0.02 mg 100⁻¹g of iron and zinc respectively. Copper, manganese and cobalt were available in *G. kola* seed while chromium was not detected in *G. kola* and *A. melegueta* seeds (Table 3).

Table 1: Phytochemical constituents of the seeds of *Garcinia kola* and *Aframomum melegueta* on dry weight basis expressed as mg 100⁻¹g

Constituents	G. kola	A. melegueta
Phenols	0.11±0.20	0.09±0.10
Alkaloids	0.36 ± 0.10	0.28 ± 0.20
Tannins	0.26 ± 0.20	0.38 ± 0.11
Sponins	11.48 ± 0.10	1.24 ± 0.30
Flavonoids	1.98 ± 0.20	5.76 ± 0.10

Results are mean of five determinations on dry weight basis \pm standard deviation

Table 2: Vitamin composition of the seeds of *Garcnia kola* and *Aframomum melegueta* on a dry weight basis expressed as $mg\ 100^{-1}g$

Vitamin	G. kola	A.melegueta
Thiamin (Vitamin B ₁)	0.54 ± 0.30	0.24 ± 0.22
Riboflavin (Vitamin B ₂)	0.22 ± 0.01	0.26 ± 0.20
Noacin (Nicotinic acid)	1.60 ± 0.01	0.05 ± 0.11
Ascorbic acid (Vitamin C)	23.10 ± 0.02	12.32±0.10

Results are mean of five determinations on a dry weight basis \pm standard derivation

Table 3: Mineral composition of the seeds of *Garcnia kola* and *Aframomum melegueta* on a dry weight basis expressed as mg 100^{-1} a

Mineral	G. kola	A.melegueta
Macro Elements		
Magnesium	0.42 ± 0.30	0.33 ± 0.11
Calcium	1.80 ± 0.40	0.26±0.20
Potassium	2.50 ± 0.10	0.65 ± 0.01
Phosphorus	0.33 ± 0.10	1.60±0.22
Sodium	0.72 ± 0.10	0.45±0.02
Micro elements		
Iron	17.75±0.30	1.80±0.02
Zinc	2.30 ± 0.01	0.02 ± 0.11
Copper	0.78 ± 0.20	0.63±0.22
Manganese	2.01±0.50	0.17±0.10
Chromium	0.00	0.00
Cobalt	0.55 ± 0.20	0.00
Cadmium	0.29 ± 0.10	0.00

Results are mean of five determinations on a dry weight basis \pm standard deviation

DISCUSSION

G. Kola and A. melegueta seeds are rich in phytonutrients such as flavonoids, phenolic compounds, saponins and alkaloids. (Table1). The tannins, biological functions of flavonoids include protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumors^[4,21,35]. Flavonoids represent the most common and widely distributed groups of plant phenolics. Flavonoids potent water-soluble antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anti-cancer activity and protects against all stages of carcinogensis^[2,4]. Flavonoids in intestinal tract lower the risk of heart disease. As antioxidants, flavonoids from G. kola and A. meleguata seeds provide anti-inflammatory action^[36,37] This may be the reason behind the use of *G. kola* extracts in the treatment of intestinal troubles in herbal medicine^[4]

The presence of phenolic compounds in the seeds of G. kola and A. melegueta indicates that these plants might be anti-microbial agents. This is because phenols and phenolic compounds have been extensively used in disinfections and remain the standard with which other bactericides are compared^[37]. Phenolic compounds as electron donors are readily oxidized to form phenolate ion or quinone, an electron acceptor. This gives rise to practical uses. Protonated phenol is used as cleaning agent^[12]. Extracts from the seeds of G. kola and A. melegueta therefore have potent antiseptic or bactericidal properties[4]. These findings supported the use of extracts from G. kola seeds and A. meleguta in treating wounds that not only heals fast but also prevents the formation of infections^[4].

The presence of phenol further indicated that *G. kola* and *A. melegueta* seeds could act as anti-inflammatory, anti-clotting, antioxidant, immune enhancers and hormone modulators. Phenols have been the subjects of extensive research as disease preventives^[21,38]. Phenols have been responsible in having the ability to block specific enzymes that causes inflammation. They also modify the prostaglandin pathways and thereby protect platelets from clumping.

G. Kola and A. melegueta possessed these constituents, which are known to have anti microbial activity. The observed inhibiting roles on microorganisms explain their utilization in traditional medicine as a cough suppressant, an anti-tumor agent and an aphrodisiac^[1,11,39,40]. G. kola and A. melegueta seeds have high quantity of saponin content. Some of the general characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness^[4,41].

Apart from saponin, other secondary metabolite constituents of G. kola and A. melegueta detected include the alkaloids and tannins. Alkaloids ranked the most efficient therapeutically significant plant substance. Pure isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects^[42]. They exhibit marked physiological activity when administered to animals. The high tannin content could be partly responsible for the bitter principle associated with the raw seed of G. kola and hot, bitter and pungency of A. melegueta seed. Tannins have astringent properties, hasten the healing of wounds and inflamed mucous membrane^[16]. The presence of tannins in A. melegueta and G. kola strongly supports their use in treating wounds, varicose ulcers, hemorrhoids, frostbite and burns in herbal medicine[39,40].

The vitamins are important in the body as their deficiencies adversely affect the metabolism of the body. Lack of ascorbic acid impairs the normal formation of intercellular substances through out the body, including collagen, bone matrix and tooth dentine. A striking pathological change resulting from this defect is the weakening of the endothelial wall of the capillaries due to a reduction in the amount of intercellular substances^[43]. Therefore, the clinical manifestation of scurvy hemorrhage from mucous membrane of the mouth and gastrointestinal tract, anemia, pains in the joints and defects in skeletal calcification can be related to the association of ascorbic acid and normal connective tissue metabolism^[4,43]. This function of ascorbic acid also accounts for its requirement for normal wound healing^[4].

Another beneficial role of ascorbic acid apart from its anti-scurvy property is that it facilitates in the transformation of cholesterol into bile acid in the liver. Ascorbic acid is required for hydroxylation of proline to hydroxyproline and of lysine to hydroxylysine. The hydroxylated forms of these amino acids are found in collagen, and so accounts for the role of the vitamin in maintaining normal connective tissue. The reducing property is beneficial in certain physiological process, such as in the absorption of dietary iron. Iron is absorbed in the ferrous (Fe⁺²) state rather than the ferric (Fe⁺³) form and the reducing capability of ascorbate accounts for the enhanced absorption of the metal in the presence of the vitamin^[4,43].

There has also been interest in the ascorbate ability, as an antioxidant, to prevent or at least minimize the formation of carcinogenic substances from dietary material^[43]. Nitroso compounds, which are known to be carcinogenic, can be formed from the reaction of nitrites with certain amino compounds *in vivo*. The nitrite is formed from the oxidation of nitrate, which are commonly incorporated into foodstuffs used in human nutrition. In this sequence ascorbate can therefore prevent the oxidation of nitrate. As a result of the availability of ascorbic acid in *G. kola* and *A. melegueta* seeds, these plants are used in herbal medicine for the treatment of common cold and other diseases like prostrate cancer^[1,4,6].

The vitamins, though in trace amount are very essential for the body metabolism. Niacin is active in preventing the disease pellagra while a deficiency of thiamin in the diet is the cause of the disease beriberi^[43]. A deficiency of riboflavin does not result to any specific and identifiable disease and one is apt therefore to under estimate its importance. The symptoms are inflammation of the tongue, lesions at the eyes and lips, congestion of conjunctiva blood vessels and desquamation of the skin^[1,44].

The results of the mineral composition clearly indicate that *G. kola* and *A. melegueta* seeds contains rich source of mineral elements. This becomes important when the usefulness of such minerals like phosphorus, calcium, magnesium, potassium, iron and zinc in the body is considered. The zinc content could mean that the plants can play a valuable role in the management of diabetes, which results from insulin malfunctioning. Zinc is essential for the production of insulin, a hormone and carbonic anhydrase, an enzyme in the body^[45]. Iron is a component of hemoglobin. It helps oxygen transport. Iron together with hemoglobin and ferrodixin plays important role in man's metabolism^[45].

However, the lower sodium content of *G. kola* and *A. melegueta* might be an added advantage due to the direct relationship of sodium intake with hypertension in human^[46].

The present study has verified the usefulness of *G. kola* and *A. melegueta* seeds for nutritional and medicinal purposes. This partly explained the use of these plant materials in herbal medicine. As rich source of phytochemicals, coupled with the presence of the essential vitamins and minerals, *G. kola* and *A. melegueta* seeds can be seen as a potential source of useful food and drugs. Further studies are going on with these plants in order to isolate, characterize and elucidate the structure of the bioactive compounds from these plants for industrial drug formulation. The uses of these plants for the treatment of the disease as claimed by traditional healers are also being investigated.

REFERENCES

- Okwu, D.E, 2003. Investigation into the medicinal and nutritional potential of *Garcinia kola* (Bitter kola) Heckel and *Dennettia tripetala* G. Baker, Ph.D Dissertation of Michael Okpara University of Agriculture Umudike, Nigeria, pp. 5-10.
- Salah, W., N.J. Miller, G. Pagauga, Tijburg, G.P. Bolwell, E. Rice and C. Evans, 1995. Prlyphenolic flavonls as seavenger of aqueous phase radicals as ch einbreaking antioxidants. Arch. Biochem. Biorh., 2: 339-346.
- Del-Rio, A., B.G. Obdulio, J. Castillo, R.R. Marin, and A. Ortuno, 1977. Uses and properties of citrus flavonoids. J. Agric. Food Chem., 45: 4505-4515.
- Okwu, D.E., 2004. Phytochemicals and vitamin content of indigenous spices of South Eastern Nigeria. J. sustain Agric. Environ., 6: 30-34.
- Irvine, F.R., 1961. Woody Pants of Ghana, with special Reference to their uses. Oxford University Press London, 9: 20-695.

- Okogun, J.I., 1985. Drug production efforts in Nigeria. Medicinal Chemistry Research and missing link. Being the text of a lecture given to the Nigeria. Acad. Sci., pp. 29-52.
- Oliver-Bever, B., 1986. Medicinal plants in Tropical West Africa Cambridge University press Cambridge, pp: 123-125.
- Okwute, S.K. and M.O. Yakubu 1998 Anti-microbial and Phytochemical screening of *Dallbergia saxatilis* Hook F. fabaccae). ZUMA J. Pure and Applied Sci., 1:2124.
- Okwu, D.E. and O. Ekeke, 2003. Phytochemical screening and mineral composition of chewing sticks in south Eastern Nigeria. Global J. Pure and Applied Sci., 9: 235-238.
- Iwu, M.M., 1986. Research finding on the possible Applications of Nigeria's Raw materials on the pharmaceutical industries in industrial potentials of Nigeria raw materials. Proceedings Ed J.K, Onoh, pp: 251-264.
- Ogu, E.O. and R.C. Agu 1995. A comparison of some chemical properties of *Garcina kola* and Hops for assessment of *Garcinia* brewing value. Bioresource Technology, 54:1-4.
- Uruquiaga, I. and F. Leighton, 2000. Plant polyphenol antioxidants and oxidative stress. Biological Research, 33: 159-165.
- Kim, S.Y, J.H. Kim, S.K. Kim, M.J. Oh and M.Y. Jung, 1994. Antioxidant activities of selected oriental herb extracts. J. American oil chem. Soc., 71: 633-640.
- Hertog, M.G.I., E.J.M. Feskeen, C.H. Hokman and A. Katan, 1993. Dietary antioxidant flavonomids and risk of coronary heart disease, de zutphen elderly study Lancet, 342:2007-1011.
- Okwu, D.E. and M.E. Okwu, 2004. Chemical composition of *Spondias mombia* linn plant parts. J. sustain. Agric. Environ., 6: 140-147.
- Kandaswami, C., E. Perkins, D.S. Soloniuk, G. Arzewiecki and E. Middle, 1991. Anti-preventative effects of citrus flavonoids on a human squamous cell carcinoma *in vitro*. Cancer Lett., 56:147-152.
- Hirano, T., M. Gotch and K. Oke, 1994. Natural flavonoids and ligans as potent cytodtatic agents against human lenkemia HL-60 cells. Life Sci., 55:1061-1069.
- Elangovan, V., N. Sekar and S. Gorindasamy, 1994.
 Chemopreventive potential of dietary bioflavonoids against 20-methy/cho/anthrene-induced tumoringenesis. Cancer Lett., 87: 107-113.
- 20. Close, D.C. and McArthur, 2002. Rethinking the role of many plant phenolics protection from photodamage. Oikos 99: 166-172.

- Okwu, D.E. and O.D. Omodamiro, 2005. Effects of hexane extract and phytochemical content of xylopia acthiopica and ocimum gratissimum on the uterus of Guinea pig.. Bio-Res., 3: (in press).
- Agoha, R.C., 1974. Medicinal Plant of Nigeria. Offsetdikker Facuiteet Waskunden Natnurwenten Schoppen Netherlands, pp. 22-158.
- 23. Nzegbule, E. and R. Mbakwe, 2001. Effect of pre-sowing and incubation treatment on Germination of *Garcina kola* Heckel seed. Fruits, 56:437-442.
- Manimi H., M. Kinoshita, Y. Fukuyama, M. Kodama, T. Yoshizawa, M. Sugiura, T. Nakagawa, M. Sugiura, K. Nakagawa and H. Tago, 1994. Antioxidant Xanthones from Garcinia subelliptica. Phytochemistry, 41:533-629.
- Chairungsrilerd, N., K. Takenchi, Y. Ohizum, S. Noezoe and T. Ohta, 1996 Mangostanol, A prenyl xanthon from Garcinia mangostana. Phytochemistry, 43: 1099-1102.
- Iwu, M.M. 1989. Food for medicine Ed M Iwu in Dietary plants and masticatories as sources of biologically active substances. University of Ife, Nigeria Ife Press, pp: 303-310.
- Olabanji, S.O., O.V. Makanju, D.C.M. Haque, M.C. Buoso, D. Ceccato, R. Cherubini and G. Moschini, 1996. PIGE-PIXE Analysis of chewing sticks of pharmacological importance, Nuclear instruments and methods in physics Research, 113:368-372.
- Shahidi, F., U.D. Chavan, A.K. Bal and D.B. Mckenzie, 1999. Chemical composition of beach pea (*Lathyrus maritimus L.*) plant parts. Food Chemistry, 64:39-44.
- Nahapetian, A. and A. Bassiri, 1995 Changes in concentration and interrelationships of phytate, P. Mg, Cu, Zn in wheat during maturation. J. Agric. Food Chemistry, 23: 1179-1182.
- 30. Harborne, J.B., 1973. Phytochemical methods. Chapman and Hall London, pp:113.
- Obadoni B.O., and P.O. Ochuko, 2001. Pytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. Global J. Pure and Applied Sciences, 8:203-208.
- Van-Burden, T.P. and W.C. Robinson, 1981.
 Formation of complexes between protein and tannin acid. J. Agric Food Chem., 1: 77-82.

- Boham A. B. and Kocipai A. C. 1994 Flavonoids and condensed tannins from leaves of *Hawaiian* vaccinium vaticulum and V. calycinium. Pacific Science, 48: 458-463.
- Barakat, M.Z., S. K. Shehab, N. Darwish and E.I. Zahermy, 1993. Determination of ascorbic acid from plants Analyst Biochemistry, 53: 225-245.
- 35. Farquar, J.N., 1996. Plant sterols, their biological effects in humans, Handbook of Lipids in Human Nutrition. BOCA Rotan HL CRC Press, pp. 101-105.
- 36. Okwu, D.E., 2001. A Improving the Nutritive value of Cassava tapioca meal with local spices. J. Nutraceutical, functional and medical Food, 3: 43-51.
- 37. Okwu, D.E., 2001b. Evaluation of the chemical composition of indigenous spices and flavouring Agents. Global J. Pure and Applied Sci., 8: 455-459.
- 38. Duke, J., 1992. Handbook of biological active phytochemicals and their activities. BOCA Raton (FL) CRC Press, pp. 99-131.
- Igboko, D.O., 1983. Phytochemical studies on Garcinia kola Heckel. MSc. Thesis. University of Nigeria Nsukka Nigeria, pp. 202.
- Maduinyi, I., 1983. Biochemical and pharmacological studies of Active principles of the seeds of Garcinia kola Heckel. MSc. Thesis university of Nigeria Nusukka, Nigeria, pp. 108.
- 41. Sodipo, O.A. and J.A. Akiniyi, 2000. Studies on certain characteristics of extracts from bark of *pansinystalia macruceras* (K. schum) pierre Exbeille. Global J. Pure and Applied Sciences, 6: 83-87.
- 42. Stray, F., 1998. The Natural Guide to medicinal Herbs and plants. Tiger Books International, London, pp. 12-16.
- Hunt, S., J.L. Groff and J. Holbrook 1980. Nutrition principles and chemical practices. John Wiley and Sons, New York, pp: 49-52, 459-462.
- 44. Taylor, O.J., 1972. Micronutrients A Uniliver educational Booklet, Unilever International Company, London, 9: 10-25.
- 45. Okwu, D.E. and F.N. Morah, 2004. Mineral and Nutritive value of *Dennrttia tripetala* fruits. Fruits, 59: 437-442.
- 46. Dahl, L.K., 1972. Salt and Hypertension. American J. Clin. Nutr., 25: 231-238.