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PHYTOCHEMICAL SCREENING AND PROXIMATE COMPOSITION OF BITTER KOLA (*GARCINIA KOLA*) FRUIT MESOCARP

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Abstract - The study evaluated the proximate composition and phytochemical content of *Garcinia kola*. Colour, shape, texture, odour, flavour and colour of the pod were the physical characteristics studied. The proximate analysis revealed that carbohydrate (62.23%) was observed to be the most abundant biological component. The ash content (12.01%) was significantly high followed by the fat/oil (11.20%), while crude fibre (6.80%), moisture content (4.95%) and crude protein (2.81%) were present in appreciable amount. The phytochemical screening showed that the pod contains tannins, saponins, phenolics, reducing sugar, flavonoids and cardiac glycosides. These results shows that *Garcinia kola* pod, which is ordinarily considered as waste, possess medicinal and nutritional benefits.

Key words: Phytochemical, proximate, bitter, kola, fruit

INTRODUCTION

Plants continue to be a major source of medicine as they have been through human history. Medicinal plants or healing herbs are used in treating and preventing specific ailments and diseases and as such are considered to play a very vital role in health care. As a product of nature, they exert all their curative and preventing powers when used in combination with other health improving natural elements like sun, water, fresh air and foods. One of their important virtues is precisely their ability to regulate life processes and prevent diseases (Ajiwe, *et al.*, 2007).

Strivast-Ava and Viet (1991) stated that hundreds of species of plants are recognized as having medicinal value and four out of every five of those plants are collected from the forest while most of them are from the floras of developing countries. They also aserted that medicinal properties maybe in one or all the plants parts like the root, stem, bark, leaf, fruit or seed. Medicinal plants are particularly important for rural residents who are not well served by formal health care system. Studies on the medicinal value of some Nigeria plants have attracted so much attention from various professionals all over the world. This was one of the compelling reasons that made the federal government of Nigeria to adopt the national policy on traditional medicine in 1997 (Olunkun, 2000).

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World Health Organization (WHO, 1999) estimates 80% of the world's population presently use herbal medicine for some aspect of primary health care or the other. In many developing countries, the use of plant drugs is increasing because modern life saving drugs are beyond the reach of three – quarters of the third world's population although many of such countries spend 40 – 50% of their total wealth on drugs and health care. As a part of strategy to reduce the financial burden on developing countries, it is obvious that an increased use of plant drugs will be observed in future (Joy *et al.*, 1998).

Medicinal plants in addition to providing the basis for between 30 - 40% for today's conventional drugs, the medicinal and curative properties of various plants are also employed in herbal supplements and teas (Balick and Allan 1996). Medicinal plants are considered as rich resources of ingredients which can be used in drug development and synthesis (Rassol, 2012).

Garcinia Kola, belong to the family *Guttiferae*. It is commonly known as bitter kola (English), Orogbo (Yoruba), Namijingoro (Hausa) and Mvule (Swahili). It is a widespread tree of evergreen forest and it is found in Benin republic, Cameroon, Democratic Republic of Congo, Ivory Coast, Gabon, Ghana, Liberia, Nigeria, Senegal and Sierra Leone. Its natural habitat is subtropical and tropical moist lowland forest. *Garcinia Kola* is a well branched evergreen and grows as a medium size tree. The specie is one of the most important trees valued in Nigeria for its medicinal values and its exploitation in the natural forest has been very heavy (Adegoke *et al.*, 1998; Adebayo *et al.*, 2013).

Traditionally, the nuts were chewed to stimulate the flow of saliva but today they are consumed as a snack. Bitter kola is thought to clean the digestive system, without side effects such as abdominal problems, even when a lot of nuts are eaten. Bitter kola is culturally very important for the Yoruba and for many other people living in the sub – Saharan region. They are used in traditional ceremonies, marking special events like births, marriages and the conference of chieftaincy.

Garcinia Kola has been proven to exhibit pharmacological uses in treating coughs, throats infections, bronchitis, hepatitis and liver disorders (Adebayo *et al.*, 2013). It serves as bitter stimulant and snake repellent when they are placed round the compound (Nair, 1990). Other medicinal value of this plan include purgative, anti-parasitic and antimicrobial. This plant has shown bronchodilator effect (Orie and Ekon 1993), anti-inflammatory, anti-bacterial and anti-viral properties (Ebana *et al.*, 1991,

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Akoachere *et al.*, 2002), anti-hepatotoxic effect (Wegwu and Didia 2007) and anti-oxidant activity (Adaramoye, 2005) in south west Nigeria. *Garcinia Kola* is one of the constituent of traditional recipe that is used in the management of sickle cell disease (Egunyomi, *et al.*, 2009).

The plant has been referred to as "wonder plant" because every part of it has been found to be of medicinal importance (Dalziel, 1937). From its roots to its leaves, the plant is known to contain several phytochemicals noted for their medicinal value (Akintonwa and Essien 1990). *Garcinia Kola* is used in folklore remedies for the treatment of ailments such as liver disorder, hepatitis, diarrhoea, laryngitis, bronchitis and gonorrhoea (Iwu *et al.*, 1999; Adesina *et al.*, 1995). The seed is masticatory and also used to prevent and relieve cough and can as well be used to treat headache (Ayensu, 1978). The plant also found usefulness in the treatment of stomach ache, bacterial infection, tuberculosis and fever (Ajebesone and Aina, 2004). Traditionally the plant is used as natural antimicrobial. Other medicinal properties of the plants include its usage in the treatment of skin infections in Liberia and Congo. The plant latex is taken internally for gonorrhoea and externally to seal new wounds and prevent sepsis. The fruit pulp is used for the treatment of jaundice or high fever. In Nigeria cold water extract of the roots and bark with salt are administered in cases of cough and vomiting (Akintonwa and Essien 1990). *Garcinia Kola* has anti-oxidant properties and it is used as tonic for the liver and the gall bladder, which helps to detoxify the body system. It helps to reduce eye pressure and relieves arthritis by reducing swelling, pain and increase joints movement. *Garcinia Kola* improves lungs function by expanding the alveolar duct and sac in the lungs thereby improving and strengthening the fibres in the lung tissues (Ogunmoyole *et al.*, 2012).

The mesocarp of *Garcina kola* has received little or no attention from researchers because it is generally considered as waste and unimportant part of the plant. The study was therefore conducted to analyze the constituents, as well as establish the nutritional and medicinal potentials, of the dry powder of *Garcinia kola* pods.

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MATERIALS AND METHODS

Plant sample collection and preparation.

The sample (bitter kola pod) used in the research work was obtained from Uwalor Village in Esan North-East Local Government Area, Edo state, Nigeria. The plant was identified and in the Department of Plant Biology and Biotechnology, University of Benin, Nigeria.

The pods were thoroughly washed to remove sand and other debris after which they were cut into bits to aid fast drying. The sample was placed in a drying oven (Jenway Model DHG-9023a) set at 60^0C. The sample was dried for two weeks after which it was ground to powder using an electrical grinding machine (domestic machine).

Preliminary extraction

A hot aqueous extraction was carried out on the sample by weighing 10 g of the powdered sample into a 250 mL beaker and adding 50 mL of distilled water. The beaker was placed in a water bath and allowed to boil for 5minutes after which the mixture was filtered hot and allowed to cool. The cool filtrate was then used for the phytochemical screening (AOAC, 2012) .

Phytochemical screening

The phytochemicals present in *Garcinia kola* pods were qualitatively analyzed using the methods described by Soforowa, 1993 as well as Trease and Evans, 2002.

Proximate analysis

Proximate analysis is also known as Weende analysis. It is a chemical method of assessing and expressing the nutritional value of feed. It gives the nutritional composition of the sample in question (Whitney and Rolfes, 2005). The proximate composition was carried out in accordance with the methods described in AOAC, 2012.

Determination of Moisture Content

The crucible was weighed empty and the sample material was added and weighed again with the sample. The crucible containing the sample was dried in oven at 105^0 C for 24 hrs and allowed to cool in a desiccator. This drying and weighing cycle was continued until a constant weight was obtained. The moisture content of the sample was then calculated using the relationship below.

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$$\%\ moisture = \frac{weight\ of\ sample+dish\ before\ drying - weight\ of\ sample+dish\ after\ drying}{weight\ of\ sample}\ x\ 100$$

Determination of Ash Content

The crucible was weighed empty before the sample was added. Ashing of the sample was carried out for 4hrs in a muffle furnace at 500^0C. After ashing, the sample was kept in desiccator for 3 hours after which the crucible containing the ashed sample was weighed again. To calculate the ash content, the formula below was used.

$$ash\ content\ (\%) = \frac{(wt\ of\ crucible+ash) - wt\ of\ crucible}{Wt\ of\ sample} x\ 100$$

Determination of Crude Fibre

50g sample were weighed and transferred to an extraction flask. Extraction was carried out with petroleum ether by stirring, settling and decanting three times. The extracted sample was transferred to a dry 100ml conical flask and allowed to evaporate at room temperature. 30 mL of 1.25% sulphuric acid was added to disperse the sample and boiled for a minute. Thereafter, an additional 150mL was added and the mixture was boiled for exactly 30minutes. During the period of boiling, the flask was rotated intermittently in order to mix the content and remove particles from the sides. The mixture was then allowed to stand for one minute and then poured immediately into a shallow layer of hot water under gentle suction in the prepared Buchner funnel. The insoluble matter was washed with boiling water until the washing were free from acid.

It was then washed twice with alcohol and three times with ether. The insoluble matter was transferred to a dried and weighed ash-less filter paper in a porcelain crucible and placed in an oven at 106^0C until a constant weight was obtained. The crucible and content were ignited in a muffle furnace at a dull red heat (600^0C) until the organic matter had been destroyed in about 20minutes. The loss in weight represents the weight of the crude fibre in the sample.

$$\%\ crude\ fibre = \frac{dry\ wt\ of\ residue\ before\ ashing - wt\ of\ residue\ after\ ashing}{Wt\ of\ sample}\ x\ 100$$

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Determination of Ether Extract

5g of the sample was extracted with 150ml of petroleum ether using a soxhlet extractor for 4 hours. At the end of the extraction process, the flask was disconnected and placed in an oven at 65^0C for 4hrs for the solvent to evaporate, after which it was cooled in desiccator and weighed.

$$\%\ ether\ extract = \frac{wt\ of\ flask + extract - tare\ weight\ of\ the\ flask}{wt\ of\ sample\ x\ 100}$$

Determination of Crude Protein

Crude protein is calculated from the nitrogen content of the sample. The method involves, digestion, distillation and titration.

Digestion

2g of the sample was weighed into a Kjeldahl flask and 25mL of concentrated H_2SO_4 was added, 0.5g $CuSO_4$, 5g of Na_2SO_4 and a speck of selenium tablet was also added, after which it was heated in the fume cupboard to prevent undue frothing. The digestion was carried out for 45minutes and continued until a clear pale green solution was obtained. It was allowed to cool and 100mL of distilled water was added gently.

Distillation

10mL of the digest was taken and transferred into the distillation flask and allowed to boil. 10 mL of NaOH was added to the solution in other to prevent loss of ammonia. It was distilled into 50mL of 2% boric acid containing methyl red indicator.

Titration

Alkaline ammonium borate formed during distillation was titrated directly with 0.1HCl, the titre value which is the volume of acid used was recorded. The volume of acid used was fitted into the formula:

$$\%\ Nitrogen = \frac{14\ x\ VA\ x\ 0.1\ x\ w}{1000\ x\ 100}\ x\ 100$$

VA = volume of acid used, W = weight of sample

% crude protein = % N x 6.25

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Determination of Nitrogen Free Extract (NFE)/Carbohydrate

NFE is determined by mathematical calculation. It is obtained by subtracting the sum of percentage of all the nutrients already determined from 100

$$\% NFE = 100 - (\% moisture + \% crude\ protein + \% crude\ fibre + \% ether\ extract + \% ash)$$

NFE represents soluble carbohydrates and other digestible and easily utilizable non - nitrogenous substances in feed.

RESULTS AND DISCUSSION

The results for the phytochemical screening of *Garcinia kola* (bitter kola) pod are presented in Table 1 below. The extract was found to contain alkaloids, terpenoids and eugenols in abundance. There were moderate amounts of saponins, flavonoids, phenolics, reducing sugars and glycosides. However, steroids was absent.

Table 1. Result of phytochemical screening of *Garcinia kola* pod.

Phytochemicals	Results
Phenolics	+
Saponins	+
Terpenoids	++
Alkaloids	++
Steroids	-
Reducing sugar	+
Flavonoids	+
Eugenols	++
Glycosides	+

Key: ++ = largely present + = present - = absent

The presence of these secondary plant metabolites shows a great potential for the application of *Garcinia kola* as a useful source of plant medicines. The phytochemical screening of the pod showed that it is rich in alkaloids, terpenoids, flavonoids, eugenols, glycoside and reducing sugar. Plants containing alkaloids extract have been reported to inhibit the growth of *staphylococcus aureus*, used as antimycotic and in the treatment of stomach ache (Akinpelu, *et al.*, 2008). Flavonoids are known for diuretics, antibacterial and antioxidants activities (Odezue and Ugwu, 2010). They also help in the healing of wounds and in the treatment of skin disorders due to its ability to neutralize the acidity of

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wounds and inflammation. They also find use in the treatment of diarrhoea. Tannin extract are anti – inflammatory, controls gastritis and irritating bowel disorder, they also contribute to anti – microbial power which heals wounds and stops bleeding (Gills, 1992). Saponin detected in the plant has been found to be an anti – bacterial and antimycotic substance on the cell walls of many organisms. The presence of reducing sugar in the plant indicates that it has high nutritive value. Cardiac glycosides in the plant are also of importance pharmaceutical. All these phytochemicals are well known for their medicinal as well as physiological activities. They are starting materials in the synthesis or production of new drugs. (Alinnor, 2007).

The results for the proximate analysis of *Garcinia kola* (bitter kola) pod are presented in Table 2 below.

Table 2. Proximate composition of Bitter Kola Pod (Dry Powder)

Parameters	Values %
Moisture content	4.95
Crude protein	2.81
Crude Fat	11.20
Crude fibre	6.80
Crude Ash	12.01
Carbohydrate	62.23

The results obtained from proximate analysis of the *Garcinia Kola* pod (dry powder) show that it can be ranked as a carbohydrate rich plant. Also the low moisture content of the powdered sample would hinder the growth of micro–organisms and the shelf life would be high (Adeyeye and Ayejuyo, 1994).

CONCLUSION

From the results of this study, it can be concluded that *Garcinia Kola* can be used as a good source of carbohydrate especially in the compounding of animal feed. The phytochemical composition also shows that *Garcinia Kola* can be useful in the pharmaceutical industry and medical science for the production of drugs and supplements that can prevent or cure diseases. It will also contribute to the development of the plant-based products towards standardization and drug development. The result of the proximate composition shows that it can be used as a raw material for compounding animal feeds.

REFERENCES

Adaramoye A.O., Farombi F.O., and Emerole G.O. (2005). Antioxidant properties of flavonoids of *Garcinia Kola* seeds. *Pakistani Journal of Medical Science*, 21: 331 – 339.

Adebayo O. R, Forombi A. G and Oyekanmi A. M. (2013). Proximate, Mineral and Anti-nutritonal evaluation of pumpkin pulp (Cucurbita pepo). *Journal* of applied chemistry. 4(5): 25-28

Adegoke G.O., Kumar M.V., Sambaiah K, and Lokesh B.R. (1998). Inhibitory effect of *Garcinia Kola* on lipid peroxidation in rat liver homogenate. *Indian Journal of Experimental Biology*, 36: 907 - 910.

Adesina S.K., Gbile Z.O., Odukoya O.A., Akinwusi D.D., and Yeola A. A. (1995). Survey of indigenous useful plants of West Africa with special emphasis on medicinal plants and issues associated with management. The United Nations Programme on Natural Resources in Africa; 2nd Edition. 84 - 85.

Adeyeye E. and Ayejuyo O.O. (1994). Chemical composition of *Cola accuminata* and *Garcinia kola* seeds grown in Nigeria. *Inter. J. Food Sci and Nut*. 45(4):223 - 230.

Ajebesone P.E., and Aina J.O. (2004). Potential African substances for Hops in Tropical Beer Brewing. *Journal of Food Technology*, 9 (1): 13 - 16.

Ajiwe V.I.E., Ofojekwu A.M., Onuegbu T.U. and Okonkwo S.I. (2007). The preliminary study on the pharmaceutical constituents of *Desmodium velutinum* leaf (Wild D.C.*). J. Chem. Soc. Nig. Anachem*, 1: 192-197.

Akinpelu D. A., Adegboye M. F., Adeloye O. A., and Okoh A. I. (2008). Biocidal activity of partially purified fractions from methanolic extract of *Garcinia kola* (Heckel) seeds on bacterial isolates. *Biological Research* 41(3):277–287.

Akintonwa A. and Essien A.R. (1990). Protective effects of *Garcinia Kola* seed extract against paracetamol - induced hepatotoxicity in rats. *J. Ethnopharmacol* 29(2):207-211.

Akoachere J. F., Ndip R.N, Chenwi E.B., Ndip L.M., and Njock T.E. (2002). Antibacterial effect on Zingiber officinale and *Garcinia kola* on respiratory tract pathogens. *East African Medical Journal*, 79: 588 - 592.

Alinnor I.J. (2007). Preliminary phytochemical and antibacterial activity screening of seed of *Garcinia kola, J. Chem. Soc. Nigeria* 32(2); 41 - 47.

AOAC (2012) Official Method of Analysis: Association of Analytical Chemists. 19th Edition, Washington DC, 121-130.

Ayensu E.S (1978). Medicinal plants of West Africa, Reference Publ. Inc. Algonac, Michigan 162

Balick M.J. and Alan P. C. (1996). Plants that heal in their plants, people and culture, the science of ethnobotany, New York, scientific American Library, 25 - 61.

Braid V.B. (1971). Inhibition of drug metabolism by flavonoids extract of *Garcinia Kola* seeds in rat Phytother. Res. 5:38 - 40

Dalziel J.M. (1937). The useful plants of West Tropical Africa. Crown Agents for the colonies. London.

doi: 10.3109/09637489409166162.

Ebana R.U., Madunagu B.E, Ekpe E.D., and Otung I.N. (1991). Microbiological exploitation of Cardiac glycosides and alkaloid from *Garcinia kola*, *Borreria ocymoldes*, *kola nitida* and *citrus aurantifolia*. *Journal of AppliedBacteriology*, 71: 398 – 401.

Egunyomi A., Moody J.O., and Eletu O.M., (2009). Antisikling activities of two ethnomedicinal plant recipes used for the management of sickle cell anaemia in Ibadan, Nigeria. *African Journal of Biotechnology*, 8: 20 - 25

Gills (1992). Ethnomedical uses of plants in Nigeria. University of Benin Press, Nigeria. 276

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Herbalism - http://en.wikipedia.org/wiki/herbalism accessed on the 14th of July, 2011.

http://www.bioline.org.br/bk

Iwu M.M., Duncan A.R., and Okunyi C.O. (1999). New antimicrobials of plant origin. In: J. Janick (Ed.), Perspectives on New crops and new uses. ASHS Press, Alexandria, VA. pp 457- 462.

Joy I.M., Okoye P.A., and Ajiwe V.I.E. (1998). Phytochemical and antimicrobial screening of root, stem and leaf extracts of *acanthus montanus. Anachem Journal*, 4 (2) 261 - 364

Nair P.K.R. (1990). The prospects for Agro forestry in the tropics. Technical paper 131. Nairobi, ICRAF

Odezue J.C.L and Ugwu N.M. (2010). Phytochemical studies of the root of *Erythreva mildbreadii* as a cure for baterial and fungal infections . *Anachem J.* 4(2): 890 -894.

Ogunmoyole T., Olalekan O.O., Fatai O., Makun J. O. and Kade I.J. (2012) Antioxidant and phytochemical profile of aqueous and ethanolic extract of *Garcinia kola. J. Pharmacog Phytoth* 4(5): 66-74. http://www.academicjournals.org/JPP DOI: 10.5897/JPP12.020

Olunkun, A.A. (2000). Chemistry in National Health Development of Food and Medicines from Natural Sources for Primary Health Care in Nigeria CSN Abstract, pp: 62.

Orie N.N., Ekon E.U. (1993). The Bronchiodilator effect of Garcinia kola. East Africa Med. *J*. 70: 143 - 145

Rasool Hassan B.A. (2012). Medicinal Plants (Importance and Uses). Pharmaceutica Analytica Acta 3: e139. http://dx.doi.org/10.4172/2153-2435.1000e139

Sofowora A. (1993) Medicinal plants and Traditional Medicine in Africa. Spectrum books ltd., Ibadan 95 - 238

Strivast – Ava., L.L. and Viet M. N. (1991). Medicinal plant, an expanding role in development. World Bank Technical paper No. 320 Washington D.C., 20433, USA, 1 - 2

Wegwu M. O. and Didia B. C. (2007). Hepatoprotective effects of *Garcinia kola* seed against hepatotoxicity induced by carbon tetrachloride in rats. BIOKEMISTRI 19(1):17-21.

Whitney, E.N. and Rolfes (2005). Understanding Nutrition. 10th Edition, Thompson/Wadsworth Publishing Company, Belmont, CA, 132-137.

WHO. Energy and protein requirement (1999). Report of a joint FAO|WHO|UNU expert consultation. WHO technical report series, No. 724, 163 – 166.