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Isolation and Characterization of Flavanone Glycoside 4[I],5, 7-Trihydroxy Flavanone Rhamnoglucose from *Garcinia kola* Seed

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Abstract: The ethanolic extract of *Garcinia kola*, Heckel (Guttiferae), which had previously been shown to have biological activity were studied. Preliminary phytochemical screening of the plants showed the presence of flavonoids, phenolic compounds, tannins and saponins. The ethanolic extract of *Garcinia kola* seeds resulted in the isolation and characterization of flavanone glycoside 4[I], 5, 7-trihydroxyflavonone rhamnoglucose (that is naringin-7-rharmnoglucoseside) from its spectral data. [1]HNMR spin system analysis and acid hydrolysis were performed to characterize the higher order rhamnoglucosyl moiety comprising glucose and rhamnose linked to carbon 7 of the flavanone ring system of the isolate. It is concluded that 4[I], 5, 7-trihydroxyflavanone rhamnoglucose may be a contributor to the antioxidants, anti-inflammatory, anti-microbial, anti-tumor and anti-hepatotoxic properties exhibited by *Garcinia kola* seed.

Key words: *Garcinia kola*, guttiferae, flavanone, rhamnoglucose, phenolic compounds, antioxidant, phytomedicine

INTRODUCTION

Garcinia kola Heckel (Bitter kola), belongs to the family Guttiferae found mainly in the tropical rain forest region of Central and West Africa (Keay, 1989; Uko *et al*., 2001). In addition to its use as alternative to hops in the brewing industry (Ogu and Agu, 1995; Aniche and Uwakwe, 1990) and in the treatment of cirrhosis and hepatitis in phytomedicine (Okwu, 2005, 2003; Nwankwo *et al*., 2000), *Garcinia kola* seed have been investigated for its ability to suppressed colic disorders, cure head or chest colds (Okwu, 2005; Iwu, 1986). The anticancer and chemo-preventive properties of the plant have been studied (Farombi *et al*., 2005, Akintonwa and Essien, 1990).

The main flavonoids content isolated from *G. kola* seed is kolaviron, a defatted ethanol extract from the seeds. Kolaviron is a mixture compounds comprising garcinia biflavonoids and kolaflavanone (Nwakwo *et al*., 2000; Iwu *et al*., 1990). It have been reported (Adaramoye *et al*., 2005; Farombi *et al*., 2005; Olaleye *et al*., 2000; Ibironke *et al*., 1997) that the flavonoids and phenolic compounds present in the plant are responsible for antioxidants, anti-inflammatory, anti-tumor, anti-hepatotoxic, anti-ulcer and anti-microbial properties exhibited by the plant.

This research describes the isolation and identification of new phytoconstituents from the seed of *Garcinia kola*.

MATERIALS AND METHODS

Sample collection: Mature seeds of *Garcinia kola* were purchased from Ariam market, Ikwuano Local Government Area of Abia State, Nigeria. On 15th July 1997. The plant materials (leaves, flowers, fruits 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 Forestry Department Herbarium of Michael Okpara University of Agriculture Umudike, Nigeria.

Treatment of plant materials: Plant materials were treated and analyzed at the Chemistry laboratory, Michael Okpara University of Agriculture Umudike. Mature seeds of *Garcinia kola* (1 kg) were dried on the laboratory bench for 10 days. The dry sample was milled and ground into powder (450 kg) using Thomas Wiley Machine. The powdered plant materials is dried and stored in airtight bottles for chemical analysis. The powdered plant sample (100 kg) was packed into a Soxhlet apparatus (2 L) and

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extracted exhaustively with 1000 mL ethanol for 24 h. The ethanolic extract was concentrated using a rotary evaporator at 45°C and in a hot air circulatory oven afforded a dark brown oil (16 kg).

Thin layer chromatography (chloroform: methanol 7:3) iodine vapour gave one band R_f 0.98 (blue). The resultant EtOH extract was applied to a silica gel column and eluted with acetone: ethanol (7:3). The eluted sample was re-chromatographed through the column with 50% chloroform in diethyl ether afforded a brown crystal (7 mg). This was re-crystallized from hexane to afford compound 1, yellow solid (6.1 mg), melting point 262-264°C, UV (MeOH) max 290 nm, IR Vmax 1641 (C = C) aromatic, 1726 (C = O) ether, 2859 (OH-phenol). ^{1}HNMR $CDCl_3$, TMS δ-scale ppm) 7.39 2 Hd (H6^1 and H2^1) 6.0 bs (OH), 6.85 2 H b poorly resolved (H_6 and H_8), 5.20 1Hm (H_2), 3.0-4.2 unresolved (Sugar OH and CH_2 protons), 5.91 (1H brs H-Rha-1), 5.31 (1H brd H-6), 4.96 (1Hd H-GLC-1), 3.92 (1Hm) 1.05 (3 Hs-H-Rha).

Acid hydrolysis of 1: Compound 1 (5.0 mg) was refluxed with 1M HCl/dioxane 1:1 V/V 2 mL) on a water bath for 6 h. The reaction mixture was evaporated to dryness. The dry reaction mixture was partitioned four times between $CHCl_3$ and H_2O. The $CHCl_3$ layer was concentrated and subjected to silica gel column chromatography CHCl/EtOAC to provide compound 2 (3 mg), white amorphous powder, 1 R (KBr) V max 3416, 2933, 1456, 1068, 1050, 190 cm^{-1}, ^{1}HNMR (pyridine-d_2) 5.19 (^{1}Hbrs H-Rha-1), 5.31 (1Hbr d H-6), 4.96 (1Hd H-GLC-1, 4.77 (1Hd-H-GLC-1), 3.92 (1Hm H-3) 1.72 (3Hd H-Rha-6) 1.08 (3Hd) 1.05 (3Hs-H rha).

Acid hydrolysis of 2: Compound 2 was subjected to acid hydrolysis as described for 1 to give D. glucose acid L. rhamnose as sugar moieties shown by sugar analysis with Benedicts reagent (Moore *et al.*, 1982).

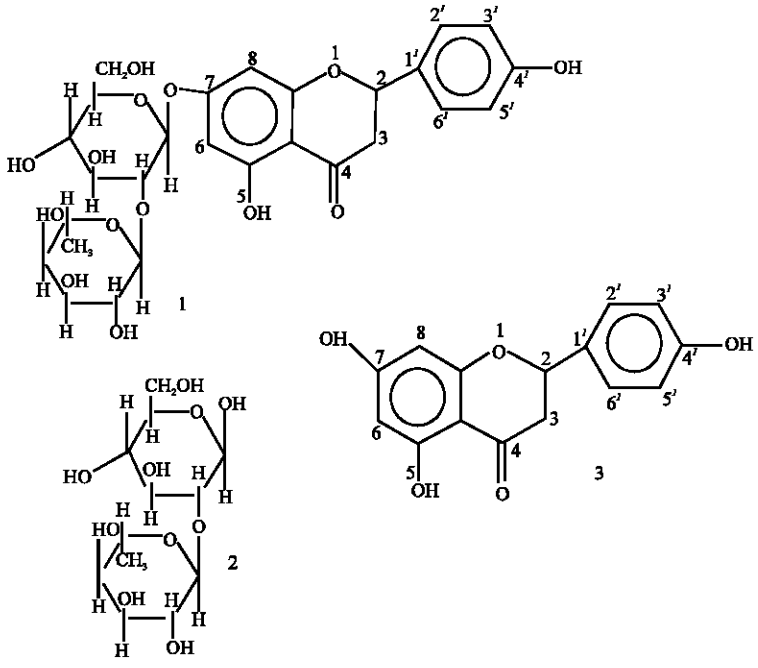
RESULTS AND DISCUSSION

Garcinia kola seed afforded a compound Mp 262-264°C, the compound was identified as flavonone rhamnose glycoside (naringin-7-rhamnoglucoside (i.e., 4^1, 57-trihydroxyflavonone rhamnoglucoside) 1.

This identification is based on the physical data, Mp, UV, IR and ^{1}HNMR spectroscopy, which is in accord with literature data (Harborne *et al.*, 1975; Alias and Linden, 1999).

The ^{1}HNMR spectrum of 1 showed a methyl signal at δ1.085. Acid hydrolysis of 1 produced the rahmnoglucosyl moiety 2 comprising glucose and rhamnose linked to Carbon 7 of the flavonone ring system and 4^1, 5, 7-trihydroxyflavanone compound 3. Compound 3 is non bitter yellow compound known as naringenin (Alias and Linden, 1999). Acid hydrolysis of 2 with 1 M HCl gave L-rhamnose and D-glucose as sugar residues.

The conjugation of the electrons of the heterocyclic ring within the aromatic system may be responsible for the



yellow colour of the flavonoid compound isolated from the *Garcinia kola* seed. The presence of this phenolic compound in *Garcinia kola* indicates that this plant may be anti-microbial agent since phenols and phenolic compounds are extensively used in disinfections and remain the standard with which other bactericides are compared (Okwu, 2005, 2003). Phenolic form a large group of naturally occurring diverse and widespread compounds. They are characterized by the presence of an aromatic ring with one or more hydroxyl groups. These phenolic compounds in *Garcinia* seed may be responsible for the therapeutic, antiseptic, antifungal or bactericides properties of the plants (Okwu, 2005).

This agreed with the findings of Akoachere *et al*., (2002) who reported that *G. kola* seed extracts exhibited inhibition on *Streptococcus pyogenes*, *Streptococcus pneumoniae* and *Hemophilis influenza.* The observed inhibiting role on these microorganism explains the reason behind the utilization of *G. kola* extract in traditional medicine as cough suppressant, anti-tumor agent, wound healing activity and an aphrodisiac (Akoachere *et al*., 2002; Okwu, 2003, 2005). Flavonoids are phenolic potent, water-soluble super anti-oxidant and free radical scavengers which prevent oxidative cell damage, have strong anti-cancer activity and anti-inflammatory properties (Olaleye *et al*., 2000). This plant, has been used in phytomedicine to protect and regenerate liver cells and in the treatment of cirrhosis and hepatitis (Okwu, 2005; Nwankwo *et al*., 2000). These phytochemicals isolated are responsible for the marked medicinal properties of the plant. Compound 1 have earlier been isolated from grape fruits, lemons, hops and oranges and is responsible for the bitter taste of these fruits (Alias and Linden, 1999). Flavanone is partly responsible for the bitter principle or astringent flavour of the *Garcinia* seed. Acid hydrolysis with 1 M HCl removes the rhamnoglucosyl moiety of compound 1 and also its bitter properties.

With naringin-7-rhamnoglucoside and the anti-microbial activity of *Garcinia kola* seeds, it will serve as a good substitute for the brewing of larger beer. The isolation and characterization of this bitter principle lend credence to the common use of *Garcinia kola* as an antibacterial and antifungal drug. This supports its utilization in phytomedicine in Nigeria.

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REFERENCES

Adaramoye, O.A., E.O. Farombi, E.O. Adeyemi and G.O. Emerole, 2005. Comparative study of the antioxidant properties of the flavonoids of *G. kola* seeds. Pak. J. Med. Sci., 21: 331-339.

Akintonwa, A. and A.R. Essien, 1990. Protective effects of *Garcinia kola* seed extracts against paracetamol-induced hepatotoxicity in rats. J. Ethnopharmacol., 29: 207-211.

Akoachere, J.E., R.N. Nidip, E.B. Chenwi, L.M. Nidip, T.E. Njock and O.N. Anong, 2002. Antibacterial effect of *Z. officinale* and *Garcinia kola* on respiratory pathogens. East African Med. J., 79: 588-592.

Alias, C. and G. Linden, 1999. Food Biochemistry. An Aspen Publication. Gaithersburg, Maryland. pp: 113-115, 145-147.

Aniche, G.N. and G.U. Uwakwe, 1990. Potential use of *Garcinia kola* as hop substitute in large beer brewing. World J. Microb. Biotechnol., 6: 323-327.

Farombi, E.O., B.F. Adepoju, O.E. Ola-Davies and G.O. Emerole, 2005. Chemoprevention of aflatoxin Bi-induced genetoxicity and hepatotoxic oxidative damage in rats by kolaviron, a natural bioflavnoid of *Garcinia kola* seeds. Eur. J. Cancer Preventions, 14: 207-214.

Harborne, J.B., T.J. Mabry and H. Mabry, 1975. Natural Distribution of Flavonoid. Chapman and Hall Ltd. London, pp: 216-217, 756-758, 1106-1113.

Ibironke, G.F., S.B. Olaleye, O. Balagun and D.A. Aremu, 1997. Antiulcerogenic effects of diets containing seeds of *Garcinia kola* (Heckel) Phytotherapy Res., 11: 312-313.

Iwu, M.M., 1986. In Plant Flavonoids in Biology and Medicine. Cody, V., E. Middleton and J.B. Harborne (Eds.), Ala R, Liss, New York, pp: 485.

Iwu, M.M., O.A. Igboko, O.K. Elekwa and M.S. Tempesta, 1990. Prevention of thioacetamide induced hepatoxicity by biflavanones of *Garcinia kola*. Phytotherapy Res., 4: 157-159.

Keay, R.W.J., 1989. Trees of Nigeria. Clarendon Press Oxford, pp: 19-30.

Moore, J.A., D.L. Dalrymple and O.B. Rodig, 1982. Experimental Methods in Organic Chemistry. Sanders College Publishing New York, pp: 98-134.

Nwankwo, J.O., D.O. Tahatengy and G.O. Emerole, 2000. Inhibition of aflatoxin Bi-genotoxicity in human liver derived Hep G2 cells by kolaviron biflavonoids and molecular mechanism of action. Eur. J. Cancer Prevention, 9: 351-361.

Ogu, E.O. and R.C. Agu, 1995. A comparism of some chemical properties of *Garcinia kola* and Hops for assessment of garcinia brewing value. Bioresour. Technol., 54: 1-4.

Okwu, D.E., 2003. Investigation into the medicinal and nutritive potential of *Garcinia kola* (Bitter kola) heckel and *Dennettia tripetala*. G. Baker, Ph.D Thesis of Michael Okpara Univ. Agric. Umudike, Nig., pp: 5-10; 130-140.

Okwu, D.E., 2005. Phytochemicals. Vitamins and mineral contents of two Nigeria medicinal plants. Int. J. Mole Med. Adv. Sci., 1: 375-381.

Olaleye, S.B., E.O. Farombi, E.A. Adewoye, B.V. Owoyele, S.A. Onasanwo and R.A. Elegbe, 2000. Analgesic and anti-inflammatory effects of kolaviron (A *Garcinia kola* seed extract). Afr. J. Biomed. Res., 3: 171-174.

Uko, O.J., A. Usman and A.M. Ataja, 2001. Some biological activities of *Garcinia kola* in growing rats. Veterinarski Arh., 71: 287-297.