



Estimation of Tannin, Saponin, Oxalate, Cyanogenic and Cardiac Glycosides in *Garsinia Kola*

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ABSTRACT: The presence of some secondary plant metabolites – tannin, saponin, oxalate, cyanogenic and cardiac glycosides were done in *Garsinia Kola*. The tannin, saponin and oxalate content were 0.69 ± 0.01 , 15.79 ± 0.28 and 1.707 ± 0.13 mg/100g of dry sample respectively. The cyanogenic and cardiac glycosides were 59.56 ± 0.05 and 67.10 ± 0.03 mg/100g dry matter respectively. The levels of oxalate, tannin and cyanogenic glycosides of the kola were observed to be very low while the levels of saponin and cardiac glycoside were observed to be very high when compared to some internationally accepted standards. @JASEM

The genus, *Cola* is found growing widely in the gardens and forests of West Africa and mostly in Nigeria. In Eastern part of West Africa we have over Fifty (50) species of *Cola*. In Nigeria, we have around twenty three (23) species, out of which five are edible. (Russels, 1955).

The seed of *G. Kola* is chewed as masticatory, stimulant and for its bitter taste. Medicinally, *G.Kola* has been used as antihepatotoxic drug extract. Many works have been done to this effect and many liver diseases were reported treated by chewing this cola. (Braide, 1991, Iwu, et al. 1990; Akintonwa and Essien, 1990). Antiulcerogenic and gastric lowering effects of *G. Kola* was also reported by Okunji and Iwu, (1991). The aqueous extract decreased the mycelial weight of *Aspergillus parasiticus* in yeast extract. Also Aflatoxin production was most effectively decreased by *G. Kola* extract showing some antimicrobial activity (Olojede, et. Al. 1993).

Industrially, *G. Kola* is being investigated for possible hop substitute in beer production (Aliche and Uwakwe, 1990; Aina and Uko, 1991). The bitterness, hop substitution and microbial action were suspected to be as a result of the presence of some phenolic compounds (Aina and Uko, 1990). Phenolic compounds likely to be present in *Cola* are mostly secondary plant metabolites like tannin, saponin, oxalates etc. High consumption of these compounds is therefore dangerous to health. Saponin for example is hemolytic in nature (Onning and ASP, 1995). Major adverse effects of these secondary plant metabolites are known – divalent metal chelators, protein binders, antinutrients, respiratory system and oxidative phosphorylation poisons. (Mahato, et. al. 1982; Chandel and Rastogi, 1980; Duncan et. al. 2000; Gondwe, et. al. 1978).

In respect to high consumption of *G. Kola* in Nigeria and West Africa as a whole, we deem it necessary to estimate the levels of these secondary plant metabolites in order to advice on the average quantity to be consumed at a given time.

MATERIALS AND METHODS

Sample Preparation: Fresh seeds of *G. Kola* were collected from the pods. Dry sample of the seed were ground into fine powder and dried in the oven at 40°C. This was stored in the refrigerator and used throughout this experiment.

Determination of tannins was based on the method of A.O.A.C (1975). The tannins were extracted into boiling distil water for one hour. Colour development was done with Folin-dennis reagent and sodium carbonate solution. Absorbance was measured spectrophotometrically at 750nm. The tannic acid concentration was calculated from the tannic acid standard curve.

Oxalate was determined by the method of Munro and Basir (1969). The oxalate was extracted with dilute HCL at 50°C and treated with ammonium hydroxide and glacial acetic acid. Further treatment with CaCl₂ solution, precipitated calcium oxalate, which was solubilized with hot dilute H₂SO₄ and titrated against KMnO₄ as equivalent to 2.2mg of oxalate.

Saponin content was determined by the modified method of Fenwick and Oakenfull (1981). Saponin was extracted for 2 hours in a reflux condenser containing pure acetone. Exhaustive re-extraction over heating mantle with methanol in the soxlet apparatus was done for 2 hours. The extract was weighed after allowing the methanol to evaporate. The saponin content was calculated as a percentage of the sample.

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This was done according to the method A.O.A.C (1975). A gram of the sample was soaked for 4 hours in distilled water. The suspension was steam-distilled into a dilute NaOH solution. The distillate was then treated with dilute KI and titrated against AgNO_3 to a faint and permanent turbidity. The hydrocyanate was calculated taking 1ml of 0.02 AgNO_3 as equivalent to 1.08mg HCN.

The modified method of Siddique *et. al.* (1987) was employed. The sample was refluxed in methanol for 2hrs, the methanol extract was treated with HCL and re-extracted twice with water and dried under anhydrous Na_2SO_4 . The weight of the extract was calculated as the percentage of the sample.

RESULTS AND DISCUSSION

The tannin, saponin, oxalate, cyanogenic and cardiac glycosides content of *G. Kola* is shown on Table 1.

Table 1: Tannin, Saponi, Oxalate, Cyanogenic and Cardiac Glycosides content of *G. Kola*

Plant Metabolites	Concentration (mg/100g)
Tannin	0.69 ± 0.01
Saponin	15.79 ± 0.28
Oxalate	1.707 ± 0.02
Cyanogenic glycoside	59.56 ± 0.05
Cardiac glycoside	67.10 ± 0.03

Remark: result represents mean ± S.D. of the triplicate analysis.

The tannin, saponin, oxalate, cyanogenic and cardiac glycosides of *G. Kola* are 0.69 ± 0.0 , 15.7 ± 0.28 , 1.707 ± 0.02 , 59.56 ± 0.05 and $67.10 \pm 0.03\text{mg}/100\text{g}$ of dry matter respectively. When these values were compared with other works, the cardiac glycoside and saponin were observed to be very high; cyanogenic glycoside was moderately high; while tannin and oxalate levels were considered low. The essence of estimating the concentrations of these secondary plant metabolites is to establish and advice on the quantity one can chew at a time. The higher the concentration of these metabolites the more dangerous they become to health.

Oxalate, for example, will form a strong chelate with dietary calcium and other divalent metals at certain concentration (Aremu, 1989; Abara *et. al.* 2000). The strong-complexed calcium is limited, unavailable for absorption and sometimes becomes precipitated as insoluble salts that accumulate in the renal calculi (Hui, 1992). The level of calcium found in *G. Kola* is low ($1.707 \pm 0.02\text{mg}/100\text{g}$). Hui, (1992) stated that intake of 5g or more of oxalic acid could be fatal to humans while Munro and Basir (1969) estimated the threshold of oxalate toxicity in man to be 2 – 5g/100g of the sample. The high content of oxalate in some plants deter even herbivores from feeding on such plants (Duncan, *et. al.* 2000; Frutos, *et. al.* 1998).

Tannins also complexes proteins, divalent metals, cellulose, hemicellulose, pectin and other carbohydrates. (Mahanato, *et. al.*, 1982). High consumption of tannin is dangerous to health, being a phenolic secondary plant metabolite with one or more

hydroxyl substitutes bonded to aromatic ring, it produces anthrocyanides, another toxic product on acid degradation (Gatachew, *et. al.* 2000; Waterman and Cole, 1994). Another danger of consumption of high concentration of tannins is that it is not normally extracted either with solvents or detergents thus tannin-protein complexes cannot easily be broken down or digested (Perez Maldonado, *et. al.* 1996). The tannin content of *G. Kola* is low and thus can be assumed to be non-toxic. Mole and Waterman, (1987) showed that addition of 1mg/ml of tannic acid to a standard trypsin solution led to the formation of an insoluble complex.

There is remarkable high concentration of saponin in *G. Kola* i.e. $15.79 \pm 0.28\text{g}/100\text{g}$ dry matter. This concentration may be deleterious when high concentration is consumed. Saponins of 1mg/100g in diet of rats was shown to decrease the plasma cholesterol and increase bile acid production (Oakenful and Sidhu, 1990, Mahanato *et. al.* 1982). A concentration of 0.15 – 5mg/kg body weight decreased the frequency of cardiac contraction while 5 – 500ug/100g body weight in rats prevented pregnancy (Mahanato, *et. al.* 1982). The abortifacient effect was also seen in pregnant goats, and cows.

Price *et. al.* (1987) observed an irreversible combination of saponins with membranes in animals and cells, thus rendering the membrane non semi-permeable. At a concentration of 1mg/ml, the saponins increased the membrane permeability of ovalbumin significantly in the proximal tubules of the intestine. Hemolysis of erythrocytes after

consumption of Oat saponin of 2mg/ml was observed in rats after 5 minutes. When the concentration was halved to 1mg/ml about 50% of the erythrocyte were lysed (Onning *et al.* 1996). Given orally in high doses, 300mg/kg body weight to rats, saponin cause diarrhoea, restlessness and histopathological changes in liver and kidney, ultimately leading to death (Lalitha, *et al.* 1990).

Cyanogenic glycosides yield hydrogen cyanide upon hydrolysis and thus toxic at certain concentrations. It's also a known inhibition of the respiratory chain, inhibiting metalloenzymes such as cytochrome oxidases (Montgomery, 1980). This makes oxygen unavailable to tissues and might result to death. The lethal dose of hydrocyanate is believed to be about 60mg per day in adult man (Oyenuga and Amazigo, 1957). Maduagwu, (1979), observed a concentration of 32.0 ± 0.8 mg/kg of sample in Garri while Tinchy (1977) observed, that a dose of 50mg/100g sample in foods. In the present work, 59.56 ± 0.05 mg/100g was observed in dry matter of *G. Kola*. This may be slightly toxic in those people that consume high quantity of this kola.

Cardiac glycoside in *G. Kola* is relatively very high in that cardiac glycosides influences the sodium potassium ion movement of cardiac membrane. It also inhibits the ATPase activity which regulates the sodium-potassium ion pump (Schilol, 1980). This stimulates contractility of the heart muscle, vasoconstriction that 0.5 – 4mg/100 of *Cola acuminata* can decrease blood pressure and heart rate while higher concentration of 10 – 100mg/100g gave a higher decrease. A concentration of 67.10 ± 0.03 mg/100g in *G. Kola* is very high and should be considered as a high dose.

In conclusion, high quantity *G. Kola* should not be consumed at a time considering the adverse effects of high saponin, cyanogenic glycoside and cardiac glycosides.

REFERENCES

- Abara, A.E., Udosen, E.O.J. and Eka, O.U. (2000). Estimation of calcium, zinc, hydrocyanate, oxalate and phytate in *Dioscorea bulbifera* tuber. *Global J. of pure and App. Sci.* 10: 79 – 84.
- Aina, J.O. and Uko, E.E. (1991). Preliminary studies on tropical hop substitutes. *Tropical Science*; 31(4) 379 – 385.
- Akintonwa, A. and Essien, A.R. (1990). Protective effects of *Garcinia Kola* Seed extract against Paracetamol induced hepatotoxicity in rats. *J. of Ethnopharmacology* 29(2); 207 – 211.
- Aniche, G. N. and Uwakwe, G. U. (1990). Potential use of *Garcinia Kola* as hop substitute in beer brewing. *World J. of Micro. And Biotech.* 6(3): 323 – 327.
- Aremu, C.Y., (1989). Quantitative estimation of dietary contribution of phytate, oxalate and hydrocyanate by six popular Nigerian foodstuffs. *Nig. Nutri. Sci.*, 10:79-84.
- Association of Official Analytical Chemists (1975). *Official methods of analysis of the Association of Official Analytical Chemists, Washington, D.C. (A.O.A.C.)*.
- Braide, V.B. (1991). Antihepatotoxic biochemical effects of Kolaviron, a biflavonoid of *Garcinia Kola* seeds. *Phytochemical Research*, 5(1): 35 – 37.
- Chakraborty, R. and Eka, O.U. (1978). Studies on hydrocyanic, oxalic and phytic acid content of foodstuffs. *West Afri. J. Biol. Appl. Chem.*, 21:50-55.
- Chandel, R.S. and Rastogi, R.P. (1980): Review – triterpenoid, saponins and saponogenins: 1973 – 1978. *Phytochemistry*, 19:1889 – 1908.
- Duncan, A.J.; Frutos, P. and Young, S. A. (2000). The effect of rumen adaptation to oxalic acid on selection of oxalic acid rich plants by goats. *British Journal of Nutrition*, 83:59-65.
- Essien, E. U. Esenowo, G.J. and Akpanabiatu, M. I. (1995). Lipid Composition of lesser known tropical seeds. *Plant foods for Human Nutrition*, 48(2): 135 – 140.
- Fenwick D. E. and Oakenfull, D. (1981). Saponins content of soyabeans and some commercial soyabean products. *J. Sci. Food Agric.* 32:273 – 278.
- Frutos, P; Duncan, A. Kynazakis, I. And Gordon, I. (1998). Learned aversion towards oxalic acid – containing foods by goats. *J. of Chem. Eco:* 24: 383:397.
- Gatachew, G; Makkar, H. and Becker, K. (2000). Effect of polyethylene glycol on in vitro degradability of nitrogen from tannin rich browse and herbaceous legumes. *Brit. J. of Nutri.*, 84:73-83.
- Gatachew, A. T. David, S.S. and Dunn, J. E. (1978). Two cyanogenic glycosides, tetraphyllin B and Epi-tetraphyllin B, from *Adenia volkensii*. *Phytochemistry*, 17: 271 – 274.

- Hui, Y. H. (1992). Oxalates in Encyclopedia of Food Science and Technology. John Wiley and sons, inc. vol. 3 p.58.
- Ibironke, G. F., Olaleye, S. B.; Balogun O. and Aremu, A. (1997). Effects of diets containing seeds of *Garcinia Kola* on gastric acidity in experimental ulceration in rats. *Phytotherapy – Research*, 11(4): 312 – 313.
- Iwu, M. M., Igboko, O. A.; Elekwa, O.K. and Tempesta, M. S. (1990). Prevention of thioacetamide – induced hepatotoxicity by biflavones of *Garcinia Kola* *Phytototherapy – Research*, 4(4): 157 – 159.
- Korubo, O.; Dapper, D. and Siminialayi, I. (2000). XXVIII Annual Regional Conference of the W. Afri. Society for Pharmacology suppl. 10:15.
- Lalitha, T.; Vishwanatha, S. and Venkataraman, L. (1990). Oral toxicity of *Modhuca butyracea* mach. Saponins to albino rats. *Indian J. of Exp. Biol.*, 28:642 – 647.
- Maduagwu, E. N. (1979): Cyanide content of Garri. *Toxi col. Lett.* 3(1): 21 - 24.
- Mahanato, S.B., Ganguly A. N., Sadhu, N. P. (1982). Review-Steroid saponins. *Phytochemistry* 21(5): 959 – 978.
- Mole, S. and Waterman, P. G. (1982). Tannic acid and proteolytic enzymes: Enzyme inhibition or substrate deprivation. *Phytochemistry*, 216(1): 99 – 102.
- Montgomery R. D. (1980). Cyanogens. In toxic constituents of plant foodstuffs Liener S. E. (ed) 2nd Edu. Acad. – press New York.
- Munro, and Basir, O. (1969). Oxalate in Nigeria vegetables. *W. Afri. J. Biol. Appl. Chem.*, 12:14-18.
- Oakenful, D. G. and Sidhu, G. S. (1990). Could Saponins be a useful treatment for hypercholesterolaemia? *European J. of Clin. Nutri.* 44:79-88.
- Okunji, C. O. and Iwu, M. M. (1991). Molluscidal activity of *Garcinia Kola* biflavonones. *Fitoterapia* 62: (1) 74 – 76.
- Olojede, F.; Engelhardt, G. Wallnofer, P. R. and Adegoke, G. O. (1993) Decrease of growth and Aflatoxin production in *Aspergillus parasiticus* caused by spices. *World J. of Micro. and Biotech.* 5(9) 505 – 606.
- Omode, A. A. Fatola, O. S. and Oloagun, K. A. (1995). Physicochemical properties of some under-exploited and non-conventional oilseeds. *J. of Agric. And Food chem.* 43(11): 2850 – 2853.
- Onning, G., and ASP, N. G. (1995). Effect of oat saponins on plasma and liver lipids in gerbils and rats. *British J. of Nutri.* 73:275 – 286.
- Onning, G. Wang Q, Westrom B. ASP, N. G. and Karlsson B. W. (1996). Influence of oat saponins on intestinal permeability in vitro and in vivo in the rat. *British J. of Nutri.* 74:141 – 151.
- Oyenuga, V. A., and Amazigo, E. O. (1957). A note on hydrocyanic acid content of cassava. *W. Afri. J. Biol. App. Chem.*, 1:39 – 43.
- Perez Maldonado, R. A. and Norton, B. W. (1996). Digestion of ¹⁴C-labelled condensed tannins from *Desmodium intortum* in sheep and goats. *British J. of Nutri.*, 76: 501 – 513.
- Price, K. R.; Johnson I. T. and Fenwick G. R. (1987). The Chemistry and biological significance of saponins in foods and feeding stuffs. *CRC Critical Reviews in Food Science and Nutrition* 26:27 – 135.
- Russels, T. A. (1995). The Colas of Nigeria and Cameroon. *Cameroon Tropical Agric. Innilda*, 32:210 – 240.
- Schild, H. O. (1983). The heart. *Applied Pharmacology*. 12th edition Churchill Livingstone London, 134 – 145.
- Siddique, S. Hafeez, F. and Begum (1987). Isolation and Structure of two cardiac glycosides from the leaves of *Nerium oleander*. *Phytochemistry* 26(1): 237 – 241.
- Somarin, O. (1973). Physiological actions of Kola nuts. *Journal of Food Science* 38:911.
- Tichy, W. (1977). *Poisons, Antidotes*; Sterling publishing Co. Inc. New York p. 80.
- Waterman, PG and Cole, S. (1994) *Analysis of phenolic plant metabolites*, London: Blackwell Scientific Publications.