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 Cola. The tannin, saponin and oxalate content were 6.98 ± 0.01, 15.79 ± 0.28 and 1.707 ± 0.13mg/100g of dry sample respectively. The cyanogenic and cardiac glycosides were 59.56 ± 0.05 and 67.10 ± 0.03mg/100g dry matter respectively. The levels of oxalate, tannin and cyanogenic glycosides of the cola 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. (Russells, 1955).

The seed of G. Kola is chewed as masticatory, stimulant and for it’s 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 (Aniche 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 haemolytic in nature (Ommco 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; Chandelier 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 distill 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. (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 AgNO₃ to a faint and permanent turbidity. The hydrocyanate was calculated taking 1ml of 0.02 AgNO₃ as equivalent to 1.08mg HCN.

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

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

<table>
<thead>
<tr>
<th>Plant Metabolites</th>
<th>Concentration (mg/100g)</th>
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<tbody>
<tr>
<td>Tannin</td>
<td>0.69 ± 0.01</td>
</tr>
<tr>
<td>Saponin</td>
<td>15.79 ± 0.28</td>
</tr>
<tr>
<td>Oxalate</td>
<td>1.707 ± 0.02</td>
</tr>
<tr>
<td>Cyanogenic glycoside</td>
<td>59.56 ± 0.05</td>
</tr>
<tr>
<td>Cardiac glycoside</td>
<td>67.10 ± 0.03</td>
</tr>
</tbody>
</table>

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 ± 0.03mg/100g 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 ± 0.02mg/100g). 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 anthocyanides, 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 ± 0.28g/100g 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 (Oakonful and Siddhu, 1990, Mahanato et al. 1982). A concentration of 0.15 – 5mg/kg body weight decreased the frequency of cardiac contration while 5 – 500μg/100g body weight in rats prevented pregnancy (Mahanato, et al. 1982). The abortifacent 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 (Omung, 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 (Laifita, 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 ± 0.8mg/kg of sample in Garri while Tinchy (1977) observed, that a dose of 50mg/100g sample in foods. In the present work, 59.56 ± 0.05mg/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 ± 0.03mg/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


