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TANNIN, OXALATE, SAPONIN, CYANOGENIC AND CARDIAC GLYCOSIDES CONTENTS OF *COLA NITIDA AND COLA ACUMINATA*

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ABSTRACT

Two species of cola, *Cola nitida* and *Cola acuminata*, were investigated for their possible relative contents of the secondary plant products:- tannin, oxalate, saponin, cyanogenic and cardiac glycosides. The two cola species gave low levels of tannin and oxalate but very high levels of cyanogenic and cardiac glycosides as well as high levels of saponin. Tannin concentrations of 0.69 ± 0.01 and 0.71 ±0.02mg/100g were obtained for *C. nitida* and *C. acuminata* respectively. *C. nitida* and *C. acuminata* also produced saponin levels of 10.49 ± 0.24 and 13.33 ± 0.16mg/100g respectively. The cyanogenic and cardiac glycoside contents were 60.00 ± 0.08 and 42.60 ± 0.09 mg/100g respectively for *C. nitida* and 80.10 ± 0.05 and 68.51 ± 0.08 mg/100g respectively for *C. acuminata* . The two cola species showed no significant ($P < 0.001$) difference in their tannin and oxalate contents. However, their saponin, cyanogenic and cardiac glycosides levels differed significantly ($P < 0.001$) with *C. acuminata* showing the higher levels.

KEY WORDS: Tannins, saponins, cyanogenic glycoside, cardiac glycoside, *Cola nitida, Cola acuminata*

INTRODUCTION

The genus, cola is found within the family sterculiaceae. It is widely distributed in the gardens and forests of wet tropics. In West Africa, mostly at the Eastern part, there is over 50 species of cola. In Nigeria we have around 23 species of cola, out of which five are edible (Russel, 1955).

The seeds of these nuts are chewed as masteratories, stimulants and for their bitter taste. The chewing is mostly seen among the Moslem communities and hence was recently found to be economically important in Labanese countires (Laan-HI-Vander and Abott, 1993).

The stimulatory effect was found to be as a result of some content of alkaloids like caffeine, theophylline and theobromine (Somarin, 1973). The anti-hypertensive effect of C. nitida has been studied (Osim and Udia, 1993). Blood pressure and heart rate reduction have been found in anaesthesized cats with C. acuminata (Korubo *et al.*, 2000). Antimicrobial properties were seen to be associated with the phenolic (Tannin) content and bitter taste. The bitterness was suspected to be as a result of tannin or phenolic content of the seed.

Tannin, saponin, oxalate, cardiac and cyanogenic glycosides are toxic to humans and have been regarded as antinutrients. Their presence in the food prevents the digestion, availability and assimilation of other food substances depending on the concentration (Mahato, *et al.*, 1982, Onning *et al.*, 1996, Duncan *et al.*, 2000;). Consumption of these colas is on the increase as they serve as stimulants but their adverse effects have not been reported. It is hoped that by determining the concentrations of these anutrients in the colas, one can predict the possible damage these colas may have on their habitual consumers.

MATERIALS AND METHODS

Sample Preparation

Fresh seeds of *Cola nitida* and *Cola acuminata* were collected from the pods plucked from their tress in Anambra State, Nigeria. Dry samples of the seeds were prepared by blending the seeds into fine powders and oven-drying at 40° C for 48 hrs. The samples were stored in the refrigerator and used throughout the experiment.

Determination of Oxalate

Oxalate was determined by the method of Munro and Basir (1969). The oxalate was first extracted with dilute 0.1M HCl at 50°C on a magnetic stirrer for 1 hour. The extract was diluted with distilled water and treated with ammonium hydroxide and glacial acetic acid. Calcium oxalate was precipitated from the solution, by treatment with 0.5M $CaCl_2$ solution centrifuged and washed repeatedly with hot water at 90°C. The precipitate was solubilized with hot dilute H_2SO_4 and titrated against a dilute 0.01 M $KMnO_4$ solution. The oxalate content was calculated taking 1ml of 0.01M $KmnO_4$ as equivalent to 2.2mg of oxalate.

Determination of Tannins

Determination of tannins was based on A. O. A. C. (1975) method. The tannins were extracted into boiling distilled water for 1 hour. The mixture was filtered and diluted . Colour development was done with Folin-Dennis reagent and 17% v/w sodium carbonate solution. It was allowed to stand in boiling water for 20 minutes. The absorbance was measured spectrophotometrically at 750nm. The tannic acid concentration was calculated from a tannic acid standard curve.

Determination of Cyanogenic Glycosides

Determination of cyanogenic glycosides was done according to the A.O.A.C (1975) method. A gram of the sample was soaked for 4 hours in distilled water. The suspension was steam-distilled into a dilute NaOH solution (0.5g in 20ml H_2O). The distillate was then treated with 2ml of 5% solution of KI and titrated against 0.02N $AgNO_3$ to a faint permanent turbidity. The hydrocyanate was calculated taking 1ml of 0.02N $AgNO_3$ as equivalent to 1.08mg HCN.

Determination of Saponin

Saponin content was determined by the modified method of fenwick and Oakenful (1981). Saponin was extracted for 2 hours in a reflux condenser containing pure acetone to remove fat pigments. Exhaustive re-extraction over a heating mantle with 300ml of 95% methanol in soxlet apparatus for 2 hours was done. The methanol in the

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extract after extraction was allowed to evaporate. The saponin content was calculated as a percentage of the sample.

Determination of Cardiac Glycosides

The modified method of Saddique *et. al.* (1987) was employed, to determine the concentration of cardiac glycosides. The sample was refluxed in 95% methanol for 2 hours, condensed to a smaller volume and neutralized. The neutralized sample was adjusted to pH 3.0 with 0.1% HCl, re-extracted twice with pure petroleum ether (bp. 60°C) and washed with distilled water. The washed ether extract was treated with 10ml portions of 0.5M $NaHCO_3$ and then 0.5M NaOH. The ether layer was dried with anhydrous Na_2SO_4 filtered and evaporated. The weight of the extract was taken and calculated as the percentage of the sample.

Statistical Analysis

All data obtained were statistically analysed using students t-tests. The data were expressed as mean ± standard error of mean.

RESULTS

The tannin, oxalate, saponin, cyanogenic and cardiac glyosides content of Cola nitida and Cola acuminata are shown in table 1.

Table 1: CONCENTRATION OF TANNIN, OXALATE, SAPONIN, CYANOGENIC AND CARDIAC GLYCOSIDES IN *COLA NITIDA* AND *COLA ACUMINATA*

	Cola nitida (mg/100g) mean ± SEM	*Cola acuminata* (mg/100g) mean ± SEM	Remarks
Tannin	0.69 ± 0.01	0.71±0.02	NS
Oxalate	1.04 ± 0.08	1.03±0.07	NS
Saponin	10.49 ± 0.24	13.33±0.16	***
Cyanogenic Glycoside	60.06 ±0.08	80.10±0.05	***
Cardiac Glycoside	42.60 ± 0.09	68.51±0.08	***

Values represent mean of triplicate analysis with standard error of the mean.
N.S = not significant at $P < 0.001$
*** = highly significant at $P < 0.001$

Table 1 shows that the concentration of Tannin and oxalate in both colas are relatively the same; there is no significant difference between the oxalate and tannins of both colas at $P < 0.001$. The saponin content of 10.49+0.24 and 13.33±0.16 mg/100g for *C.nitida* and *C. acuminata* respectively showed a highly significant difference at $P < 0.001$. Cyanogenic and cardiac glycosides of both colas also showed a highly significant difference at $P<0.001$. The cyanogenic glycosides content of *C. nitida* was found to be 60.06± 0.08 while that of *C. acuminata* was found to be 80.00±0.05 mg/100g. Cardiac glycoside is also present in both colas, at a concentration of 42.60±0.09 and 68.51±0.08 mg/100g for *C. nitida* and *C. acuminata* respectively.

DISCUSSION

Tannins, oxalates, saponins cyanogenic and cardiac glycosides are among several secondary metabolites found in plants (Mahato *et al.*, 1982; Onning *et al.*, 1996; Duncan *et al.*, 2000). The toxicity of these nutrients have been documented (Muhato *et al.*, 1982; Duncan *et al.*, 2000). The present study has revealed the presence, in *C. nitida* and *C. acuminata*, of low levels of tannin and oxalate but high levels of saponin, cyanogenic glycosides and cardiac glycosides (Table 1).

Oxalate at high concentrations is known to strongly chelate with dietary calcium and other divalent metals (Abara *et al.*, 2000). The complexed calcium is consequently unavailable for absorption. It is also reported that oxalate causes assimilated calcium to be precipitated as insoluble salts which accumulate in the renal calculi (Hui, 1992). Hui (1992) stated that intake of 5g or more of oxalic acid could be fatal to humans. Munro and Basir (1969) on the other hand, estimated the threshold of oxalate toxicity in man to fall between 2 to 5g daily. Oxalate toxicity is such that its presence in high concentrations in some plants even deter herbivores from feeding on such plants (Frutos *et al.*, 1998; Duncan *et al.*, 2000). This study showed low oxalate levels of 1.04 ± 0.08 and 1.03 ± 0.07mg/100g of *C. nitida* and *C. acuminata* respectively in comparism with the reported toxic levels of 2-5g/100g in plant materials (Munro and Basir, 1969). It therefore means that consumers of these cola species (*C. nitida* and *C. acuminata*) stand little or no risk of oxalate toxicity.

Like oxalates, tannins also form complexes with proteins, divalent metals, cellulose, hemicellulose, pectin and other carbohydrates. Tannins, therefore, reduce the availability of these nutrients. Being phenolic secondary plant metabolites with one or more hydroxyl substitutes bonded to aromatic ring, tannins produce anthocyanins (toxic metabolites) on acid hydrolysis (Waterman and Mole, 1994; Gatachew *et al.*, 2000). Moreover, tannins are normally extracted either with solvents or detergents and consequently tannin-protein complexes are not easily broken down or digested (Mole and Waterman, 1987; Perezmaldonado *et al.*, 1996).

The tannin levels of 0.69 ±0.01 and 0.71 ±0.02 mg/100g found, in this work, for *C. nitida* and *C. acuminata* respectively may not be regarded as high concentrations and thus can be assumed non-toxic. Consequently consumers of these cola species may stand no risk of tannin toxicity.

Our study has revealed remarkably high concentration of saponin in the two cola species investigated. Saponin values of 10.49 ± 0.24 and 13.33 ± 0.16mg/100g were obtained for *C. nitida* and *C. acuminata* respectively. Saponin concentration of 1mg/100g in diet of rats was shown to decrease their plasma cholesterol and increase bile acid production (Mahato *et al.*, 1982). Saponins have been noted to inhibit cholesterol absorption through complexation and also have minor effect on lipid metabolism (Oakenful and Sidhu, 1990). Saponin concentrations of 0.15 – 5mg/kg body weight of rat were observed to decrease the frequency of cardiac contraction (Mahato *et al.*, 1982). It has also been reported that rats fed with saponin-containing diets (at a concentration of 5-500ug/100g body weight) for 15 days were unable to get pregnant. The abortificient effect was also shown by saponin of *C. speciosus* when given to pregnant goats, rats and cows (Mahato, *et al.*, 1982). The abortificient effect was interpreted as resulting from an irreversible combination of saponins with membranes in animals and in effect limiting their permeability (Price *et al.*, 1987). Saponins are also haemolytic in nature, and have detergent effects which can disrupt and disintegrate membranes and hence their ability to cause haemolysis of erythrocytes. Oat saponin has a haemolytic activity at a concentration of 2mg/ml; complete haemolysis of rat

erythrocytes was observed in 5 minutes. When the concentration was halved to 1mg/ml about 50% of the erythrocytes were lysed (Onning *et al.*, 1996). Given orally in high doses, 300mg/kg body weight, to rats, saponins cause diarrhoea, restlessness and histopathological changes in liver and kidney and ultimately death (Lacitha *et al.*, 1990). From the foregoing therefore, the high level of saponin obtained in this work for *C. nitida* and *C. acuminata* could well mean that habitual consumers of these colas stand a high risk of saponin toxicity. This effect could either be short term or long term depending on ones nutritional status or general metabolic state.

Cyanogenic glycosides are compounds that yield hydrogen cyanide (HCN) on hydrolysis. Hydrogen cyanide is a known inhibitor of the respiratory chain; inhibiting metallo-enzymes such as cytochrome oxidases (Montgomery, 1980), an action that may eventually result in the death of the organism. Other enzyme systems inhibited by cyanide include carboxylase of liver (De Metz *et al.*, 1982), mitochondrial catalase (Kremer and Mordechai, 1981), and bovine heart dehydrogenase (Phelps and Hatefi, 1981). The lethal dose of hydrocyanate is believed to be about 60mg per day in adult man (Oyenuga and Amazigo, 1957). Tichy (1977) observed that the fatal dose of cyanogenic glycoside in food is 50mg/100g while Montgomery (1980) suggested a level of 10-20mg/100g sample in foods for safety.

In the present study, cyanogenic glycoside levels of 42.6 ± 0.59 and 68.51 ± 0.08mg/100g obtained for *C. nitida* and *C. acuminata* respectively may be toxic especially in communities where large quantities of these colas are consumed daily.

Cardiac glycosides influence the sodium potassium ion movement of the cardiac membrane, and inhibit the ATPase activity which regulates the sodium / potassium ion pump. These glycosides also increase oxygen consumption, stimulus contractility of the heart muscle and vasoconstriction of the blood vessels (Schild, 1983); Korubo et al (2000) observed that 0.5 – 4 mg/100g of this glycoside in *C. acuminata* decreased blood pressure and heart beat rate of cats while higher concentrations of 10-100mg/100g gave more pronounced decreases. On the contrary however, Osim and Udia (1993) had earlier observed that feeding rats with *C. nitida* at high concentration increased blood pressure. Could the cardiac glycosides from the two cola species be having opposite effects in the two animal species? Nevertheless, the high cardiac glycoside levels of 68.15 ± 0.08mg/100g and 42.60 ± 0.09 obtained in this work for *C. acuminata* and *C. nitida* respectively are of significance in terms of their possible toxicity to the habitual consumers of these colas.

In conclusion therefore, this work has established the presence, in high concentrations of some toxic metabolites in the two cola species *C. acuminata* and *C. nitida*. It is therefore advised that the consumption of these colas be done with moderation and indeed with caution.

REFERENCES

Association of Official Analytical Chemists, 1975. Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC) Washington, D. C.

Abara, A. E., Udosen, E. O. and Eka D. U., 2000. Estimation of calcium, Zinc, hydrocyanate, Oxalate and Phytate in Dioscorea *bulbifera* tuber, Global Journal of Pure and Applied Science 6(3): 449-453.

Chakraborty, R. and Eka, O. U., 1978. Studies on hydrocyanic, oxalic and Phytic acid contents of Foodstuff. West African J. Biol. Appli. Chemistry 21: 50-55.

De-Metz M. S., hauker, B. A. and Vermeer C., 1982. Inhibitors of Vit. K-dependent carboxylase of Liver by Cyanogenic glycosides F.E.B.S. letters 137(2):253-256.

Duncan, A. J., Frutos P. and Young S. A., 2000. The effect of rumen adaptation of Oxalic acid on selection of oxalic acid-rich-plants by goats. British Journal of Nutrition, 83: 59-65.

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; Kyriazakis, I. and Gordon, I., 1998. Learned aversion towards oxalic acid – containing foods by goats: does rumen adaptation to oxalic acid influence diet choice? Journal of Chemical Ecological 24: 383-397.

Gatachew, G. Makkar, A. and Becker, K., 2000. Effect of Polyethylene glycol on invitro degradability of Nitrogen from Tannin rich browse and herbaceous legumes. Briti. J. of nutri. 84:73-83.

Hui, Y. H., 1992. Oxalates, In Encyclopedia of Food Science and Technology 12th ed./New York Vol. 3 p. 58.

Kremer, N. and Mordechai, L., 1981. Non-stationary inhibition – the cyanide inhibition of Catalase J. Physio chem.. 87(7): 835-839.

Korubo O., Dapper, D. and Siminalayi, 2000. 11th Annual Regional Conference of the West Africa Society for Pharmacology suppl. 10:15.

Laan - Hl-vander and Abott, J., 1993. The Labanese traders in Sierra Leone. Agriculture and Food marketing in developing countries – Selected readings 95: 103.

Lacitha, T., Vishwanatha, S. and Venkataraman, L., (1990). Oral toxicity of *Modhuca butyracea* .nacb. Saponins to albino rats. Indian J of Experimental biology 28: 642-647.

Maduagwu, E. N., 1979. Cyanide content of Garri. Toxicol. Letters, 3(1): 21-24.

Mahato, S. B., Ganguly, A. N. and Sahu, N. P., 1982. Review – Steriod Saponins Phytochemistry 21 (5): 959-978.

Mole, S. and Waterman, P. G., 1987. Tannic acid and Proteolytic Enzymes: Enzyme Inhibition or Substrate Deprivation. Phytochemistry, 26(1):99-102.

Montgomery, R. D., 1980. Cyanogens. In toxic constitution of Plant feedstuffs. Liener S.E. (ed) 2nd Ed. Acad - Press, New – York.

Munro, O. and Basir, O., 1969. Oxalate in Nigeria vegetables. West Africa J. biol. Appli. Chem. 12:14-18.

Oakenful, D. G. and Sidhu, A. S. (1990). Could Saponins be a useful Treatment for Hypercholesterolaemia. European Journal of Clinical Nutrition 449-79-88.

Onning, G. Wang, Q. Westrom, B. ASP, and Karlsson, B., 1996. Influence of Oat Saponins on Intestinal Permeability in Vitro and in vivo in the Rat. British J. of Nutrition: 76: 141-151.

Osim, E. E. and Udia – P. M., 1993. Effects of consuming a Cola nut (*Cola nitida*) diet on mean Arterial pressure in rats. International Journal of Pharmacognosy: 31(3): 193-197.

Oyenuga, V. A. and Amazigo , E. O., 1957. A note on the hydrocyanic acid content of Cassava. West African J. Biol. App. Chem. 1: 39-43.

Perez – Maldonado, R. A. and Norton, B. W., 1996. Digestion of "C-Labelled condensed tannins from Desmodium Intortum in Sheep and Goats. British J. of Nutri. 76:501-513.

Phelps, D. C. and Hatef., V., 1981. Inhibition of B-Hydroxybutyrate dehydrogenase by Cyanogenic glycoside. Biochemistry 20(3): 493-498.

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.

Russel, T. A., 1955. The Cola of Nigeria and Cameroon. Cameroon torpical Agric Innilda 32:210-240.

Schild, H. O., 1983. The Heart Applied Pharmacology. 12th Edition Churchill Livingstone, London 134-145.

Saddique, 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 cola nuts. Journal of Food Science 38:911.

Tichy, W., 1977. Poisons, Antidotes; Sterling Publishing co. Inc., New York p. 80

Waterman, P. G. and Mole S., 1994. Analysis of Phenolic Plant Metabolites London: Blackwell Scientific Publications.