TOTAL ANTIOXIDANT CAPACITY, POLYPHENOLIC COMPOSITION AND CYTOTOXICITY OF THREE VARIETIES OF LIVINGSTONEPOTATO (*RIZGA*).

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ABSTRACT

The growing interest in the substitution of synthetic food antioxidants with natural ones in the maintenance of human health has fostered increased research on the screening of plants for the identification of antioxidants. The total antioxidant capacity and polyphenolic content of the extracts of the three varieties of rizga flour as measured by the quantities of quercetin and polyphenols present showed that all three varieties contained significant quantities of antioxidants and polyphenols. However, the total antioxidant capacity and polyphenolic composition of langaat was lower than that of beebot and riyom ($P< 0.05$). The phytochemical screening of the flours as determined using standard qualitative methods indicated the presence of cyanogenic glucosides, tannins and flavonoids in all the three varieties studied but beebot and riyom contained alkaloids in addition. The extracts of the 3varieties of rizga had a low cytotoxic effect on the rats studied with their LD50 higher than that of potassium dichromate used. Correlation between the total antioxidant capacity and polyphenolic composition of the 3varieties of rizga was found to be significant ($R^2=0.725$).These findings suggest rizga to be a natural source of antioxidants and thus could be used in the treatment of ailments implicating free radicals. In addition, it's safe for consumption.

INTRODUCTION

Living Stone Potato (*Plectranthus esculentus* N.E.Br) locally known as rizga is one of the widely cultivated minor root crops in the middle belt regions especially Kaduna and Plateau States of Nigeria for the finger-like edible tubers (Schiffers, 2000 and Olojede et al., 2004). It's grown in Nigeria as it is rich in carbohydrates like most other tuber crops (Schippers, 2000).In terms of protein content, when compared with yam, cassava, sweet potato and cocoyam, livingstone potato ranks highest in protein content relative to major food crops grown in Nigeria.

Despite its nutritive potential, it's classified among the lesser known and under exploited species of food crops in Africa (Schippers, 2000). For a long period of time, plants have been a reliable source of natural products for maintaining human health, especially in the last decade, with more intensive studies devoted to natural therapies (Kumar *et al*., 2005; Pourmorad *et al.,* 2006).The World Health Organization has recommended that this should be encouraged especially in areas where access to conventional treatment is not adequate (WHO, 1980). Fruits and vegetables are good sources of phytochemicals such as carotenoids, flavonoids, and other phenolic compounds. Studies have indicated that these phytochemicals especially polyphenols have high antioxidant and free radical scavenging activity, which helps to reduce the risk of chronic diseases such as cardiovascular disease, cancer, etc (Ames *et al*., 1993). In addition, phytochemicals also act as potent antioxidants in both fat soluble and water soluble body fluids and cellular components (Mathur and Mathur, 2001) and also possess biological characteristics like anticarcinogenecity, antimutagenecity, anti-aging activity and anti-cholesterol activity.
Since free radicals have been associated with some of these disorders and being that the phytochemicals present in plants are known to possess antioxidative or free radical scavenging activity, the antioxidant and phytochemical composition of this plant ought to be investigated. In addition, the biosafety of its consumption need be ascertained. This thus leads to the basis of this research which is designed to understudy the total antioxidant capacity, polyphenolic composition and cytotoxicity of Livingstone potato to rats.

MATERIALS AND METHODS

Chemicals

Quercetin (3, 3' ,4' ,5, 7 -pentahydroxyflavone), Folin-ciocalteau reagent and Chlorogenic acid were products of Sigma Chemical Company (UK).All other chemicals used were purchased from John-J-Scientific Company, Jos, Nigeria and were of analytical grade.

Plant Materials

Livingstone Potato used was freshly harvested from National Root Crops Research Institute, Potato Programme. Kuru Station, Jos. It was thoroughly peeled, and freeze dried in a freezer for 48hrs.

Preparation of Plant Materials for Analysis
The peeled portion of the Livingstone potato (*rizga*) was ground into flour using a mortar and blender and the flour was then used for analysis.
Phytochemical Screening of *Rizga* Flour
The phytochemical screening of the plant flour using organic solvent and hot water extracts were carried out using standard qualitative procedures (Trease and Evan, 1984 and Sofowara, 1986).

Total Antioxidant Composition of *Rizga* Flour
The total antioxidant activity was determined using quercetin as standard as described by Velasquez (1991).The calibration curve was plotted using serial dilutions of quercetin at 517nm and the results were expressed in quercetin equivalence.

Total Phenolic Assay
Total phenolic assay was measured using the modified Folin-Ciocalteau method (Singleton *et al*, 1999).The hydrophilic extract(0.5ml) was diluted with distilled water to 5ml and 0.5ml of Folin-Ciocalteau reagent was added and allowed to react at room temperature for 3mins. One millitre of 1N sodium carbonate was added and allowed to react at room temperature for 1hr.The absorbance was measured at 725nm using a UV spectrophotometer with distilled water as blank. Chlorogenic acid and garlic acid were used as standards. Total phenolic content was reported as mg of chlorogenic acid equivalents per gram fresh weigh sample(mg CAE/g fw) which could be converted to mg of gallic acid by multiplying by a factor of 0.445.

Cytotoxicity Analysis
Selection of Animals
56 matured male albino rats weighing between 71.66g and 168.06g were used for the toxicity tests. Animals were acclimatized for a period of 7days to the laboratory conditions prior to the experiment. Rats were housed in colony cages with 2rats per cage at room temperature with 12hr light and dark cycle and they had access to drinking water and their food.

Experimental Procedure
The rats were divided into 4groups with 14 animals in each group. Groups 1-4 rats received varying doses ranging from 330,300,280,250,220 to 120ml/kg body weight of the flour from each of the extracts of the three varieties of *rizga*
Same procedure was followed using potassium dichromate as the reference standard for group 4 rats which served as the control group. The dosage that killed 50% of the experimental animals after 24hrs of oral administration of the extracts of the different varieties of rizga flour was recorded and the LD_{50} of each of the *rizga* varieties computed using the modified methods of (Karber, 1931) and Litchfield and Wilcoxon, (1949) and results were expressed in ml/kg bogy weight which is equal to mg/kg body weight.

Statistical Analysis
Statistical analysis was conducted using the means ± std of 3
experiments. Results were considered significant at $P<0.05$.
Correlation analysis was carried out using Pearson's model and results were considered significant at $P<0.05$.

RESULTS AND DISCUSSION
Phytochemical Composition of the *Rizga* Flours
The phytochemical screening of the 3 varieties of *rizga* showed that they contained some quantities of flavonoids, tannins and cyanogenic glucosides but *beebot* and *riyom* contained alkaloids in addition (Table 4).
Flavonoids, alkaloids and tannins are polyphenolic compounds with antioxidant properties. Phenolics have been associated with antioxidant properties of food (Robbins, 2003). Kirakosyan *et al*. (2003) reported that phenolic compounds in plants possess antioxidant activity and may help protect cells against the oxidative damage caused by free radicals.

Cyanogenic glucosides are the compounds in cassava that make them toxic. This is due to the production of cyanide which activates the enzyme cytochrome oxidase in the mitochondria of cells by binding to the $Fe^{2+/}Fe^{3+}$ contained in the enzyme. This leads to a decrease in the utilization of oxygen in the tissues, increase in blood glucose, lactic acid levels, decrease in ATP/ADP ratio, drop in blood pressure and death in severe cases (Food Standards

Australia,2005)..However we could not determine the actual concentration of these glucosides in the 3 varieties of *rizga.* The present study shows that *rizga* flour contains considerable amount of phenolics and this implies that they may be useful in relation to diseases involving free radical reactions.

Antioxidant Activity of *Rizga* Flour

The 3 varieties of *rizga* contained significant quantities of quercetin which was used as an index for the measurement of the total antioxidant composition of the *rizga* flours. However the total antioxidant composition of *langaat* was lower than that of *beebot* and *riyom*. (Table 1).The lower antioxidant activity of *langaat* when compared to *beebot* and *riyom* are thought to arise from the absence of alkaloids in it which are polyphenols with high antioxidant capacity. Kirakosyan *et al.* (2003) have reported that polyphenols present in plants are responsible for their antioxidant activity. Quercetin which is a flavonoid commonly found in plants has been reported to have both medicinal and antioxidant properties (Aligianis *et al*., 2001). The antioxidant composition of the varieties of *rizga* is attributable to the phenolic content and presence of other phytochemicals in it. However, we could not determine the actual status of the phytochemicals present.

Total Polyphenolic Composition of 3varieties of *Rizga* Flour

Analysis of the polyphenolic composition of the *rizga* flour showed that all three varieties contained significant quantities of polyphenols (Table 2). However, the polyphenolic composition of *langaat* was lower than that of the other 2 varieties analyzed. These results obtained show that *rizga* flour is a potential source of natural antioxidants and could be of medicinal purposes in treatment of ailments implicating reactive oxygen species and oxidative stress.

Cytotoxicity of 3 Varieties of *Rizga* on Rats

The inhibitory activity of the extracts of the three varieties of *rizga* extracts and their corresponding values are shown in Table 4. The extracts of the 3 varieties of *rizga* flour had very low cytotoxic effects on the rats studied with the LD50 of 315.93ml/kg for *riyom* , 322.5ml/kg for *langaat* and 357.8ml/kg for *beebot* (Table 4). The implication of this is that all three varieties of *rizga* flour have a very low toxic level and are thus safe for consumption.

Correlation analysis carried out revealed that the total quercetin equivalence present in all the varieties of the *rizga* correlated positively with their total phenolic composition suggesting that the concentration of polyphenols present in all the varieties of the sample could be responsible for their quercetin composition confirming earlier reports by Aligiannis *et al*.(2001) that the antioxidant properties of quercetin arise from the polyphenols present in it.

CONCLUSION

The results of the research analysis carried out reveals that Livingstone potato (*Rizga*) is a potential natural source of antioxidants with free radical scavenging activity.

Since the lethal dosage of its consumption in the rats studied were found to be quite low, its use could be recommended especially regarding ailments implicating free radicals and oxidative stress.

However, since it was found to contain cyanogenic glucosides and being that these compounds could be toxic in high concentrations, more efforts need to be geared to determine the actual status of these glucosides in Livingstone potato and thus confirm the safety of its consumption.

Table1: Quercetin Equivalence of 3 varieties of Rizga (Langaat, Beebot and Riyom)

The values above are the means ± std of 3 experiments carried out on three varieties of rizga ($P<0.05$).

Variety	**Quercetin Equivalence(mg/ml)**
Langaat	0.00059 ± 0.0003
Beebot	0.0016 ± 0.0009
Riyom	0.001 ± 0.00006

Table 2: Total Phenolic Composition of 3 varieties of Rizga (Langaat, Beebot and Riyom).

Variety	Total Phenolic Composition(mg CAE/g fw)
Langaat	0.15 ± 0.008
Beebot	0.40 ± 0.006
Riyom	0.3361 ± 0.030

CAE=Chlorogenic Acid Equivalent., fw =Fresh weight.
The values above are the means±std of triplicate experiments carried out on 3 varieties of Rizga ($P<0.05$).

Table 3. Phytochemical Screening of 3 varieties of *Rizga* (*langaat. Beebot* and *Riyom*).

Group	Sample	Dos (ml/kg)	% Lethality	LD50(ml/kg)
Riyom	Crude Methanol Extract	330	52	880.28
		300	48	
		280	45	
		250	38	
		220	32	
		120	23	
Langaat	Crude Methanol Extract	330	57	315.93
		300	51	
		280	42	
		250	37	
		220	31	
		120	25	
Beebot	Crude Methanol Extract	330	54	357.8
		300	45	
		280	37	
		250	30	
		220	25	
		120	19	
		330	100	52.896
		300	98	
		280	90	
Potassium dichromate	Crude Methanol Extract	250	88	
		220	85	
		120	80	

Table 4. Inhibitory activity of crude methanolic extracts of 3 Varieties of *rizga* on rats.

	Langaat	***Beebot***	***Riyom***
Alkaloid	**-ve**	**+ve**	**Slightly +ve**
Flavonoid	**+ve**	**+ve**	**+ve**
Tannin	**+ve**	**+ve**	**+ve**
Cyanogenic Glucoside	**+ve**	**+ve**	**+ve**

+ve = Present -ve = Absent

Figure 1.Correlation of Quercetin Equivalence of all the varieties of *Rizga* against their total Phenolic Composition.

Correlation betweem Quercetin Equivalence and Total Phenolic Content

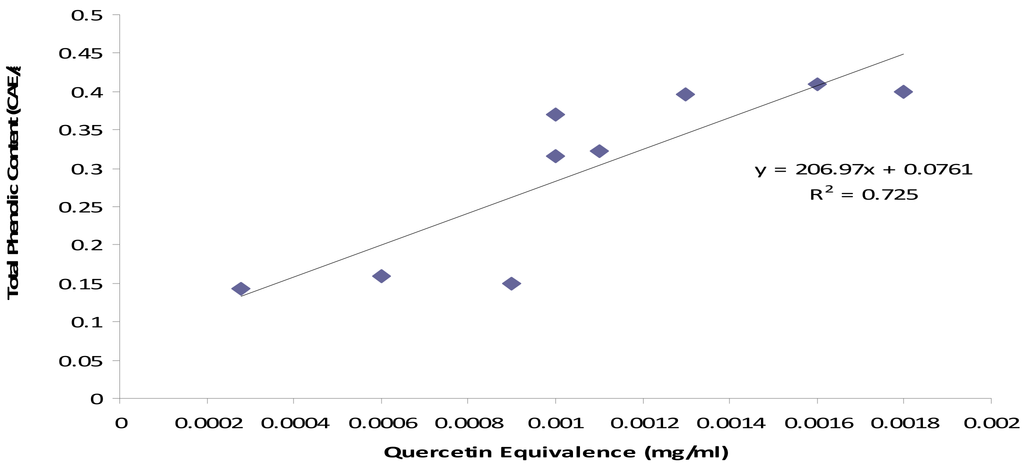


Fig 2. Effect of dosage rate (mg/ml) of Riyom on the percentage mortality of rats.

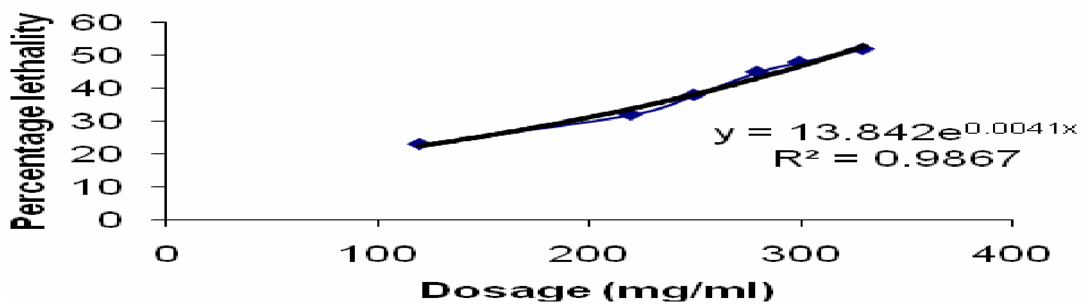
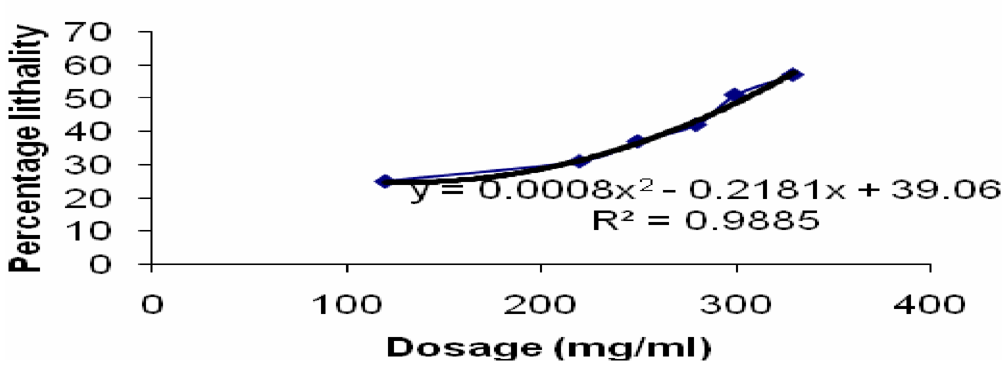
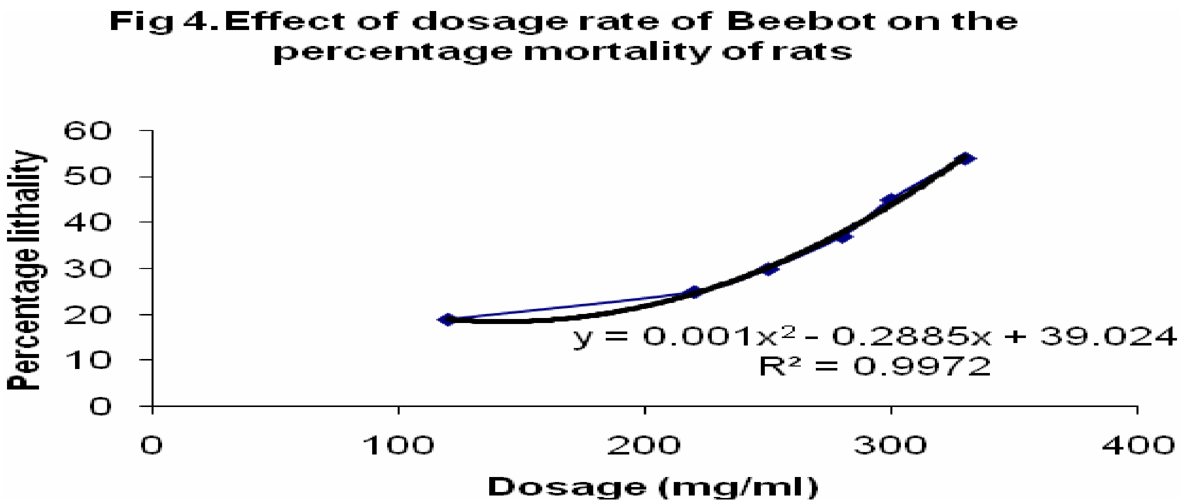
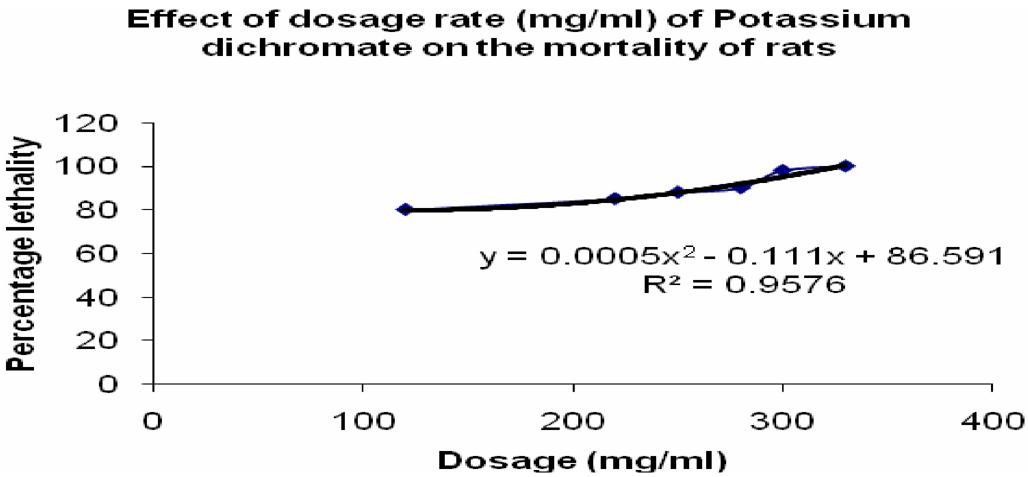


Fig 3. Effect of dosage rate (mg/ml) of long'at on the percentage mortality of rats







REFERENCES

Aligiannis.N.,Mitaku,S.,Mitrocotsa,D.and Lecler,S.(2001).Flavonoids as cycline-dependent kinase inhibitors:inhibition of cdc 25 phosphatase activity by flavonoids belonging to the quercetin and kaemferol series,*Planta Med*.67.468-470.

Ames,B,M.,Shigena,M,K.and Hagen,T,M.(1993).Oxidants,antioxidants and the degenerative diseases of ageing.Proceedings of the National Academy of Science.90,7915-7922.

Association of Official Analytical Chemists.(1990).Official Methods of Analysis. W Horwitz (ed) 13th edn.233-234.

Association of Official Analytical Chemists.(1984).Official Methods of Analysis.14th edn.242-245.

Blois,M,S.(1985).Antioxidant determination by use of stable free radicals.*Nature*,29:1199-1200. Food standards. (2005).Australia.*New Zealand*.4.

Harbone,J,B.(1967).Comparative biochemistry of the flavonoids.New York Academic Press.221-222.

Hsu,C,L.,Chen,W.,Weng,Y,M.and Tseng,C,Y.(2003).Chemical composition, physical properties and antioxidant activities of yam flours as affected by different drying methods. *Food chemistry*.83 (1)85-92.

Karber,G. (1931). Contribution to the collective treatment of pharmacological serial experiments.Arch.Exp.Path.*Pharmacol.*162:480-483.

Ketiku,A,O. (1973). Chemical composition of Unripe Plantain (*Musa Paradisiaca*).*J Science Food and Agriculture*.24, 703-707.

Kirkosyan,A.,Seymour,E.,Kaufman,O,B.,Warber,S,E.and Chang, S. C. (2003). Antioxidant capacity of polyphenolic extracts from leaves of crataegus Laevigata and Crataegus monogyna (Hawthorn) subjected to drought and cold stress. *J Agricultural and Food Chemistry*.51.3973-3976.

Kumar,R,S.,Sivakuma,T.,Sunderem,R,S.,Gupta,M.,Murujesh,K.,Rajeshwa,Y.,Kumar,M,S.and Kumar, K, A. (2005). Antioxidant and antimicrobial activities of Bauhima Recemosa L Stem Bark. *J. Med .Biol. Res* .38. 1015 - 1024.

Litchfield J .and Wilcoxon, F. (1949). A simplified method of evaluating dose-effect experiments .*J pharmacol*. Exp Ther 96.99-113.

Marthur, N. K. and Marthur, V. (2001). Antioxidants: Natural ingredients and additives for food. *Beverage Food World* 5.13-15.

Olojede, A, O., Nwokocha, C, C. ,Eke-Okoro, O. N. and Emehue, J, K, U. (2004). Varietal response of Livingstonepotato to NPK fertilizer application at Umudike. NRCRI. *Annual Report*.86-87.