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Phenolic Content, Antioxidant Capacity and Toxicity of 3 Varieties of Living Stone Potato (Rizga)

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ABSTRACT

The phenolic content, antioxidant activity and toxicity of three varieties of extracts of rizga flour on white albino male rats was evaluated. The antioxidant activities of the extracts as determined by the quantities of phenols present and scavenging activities on 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical indicated that all three varieties of rizga flour contained significant quantities of antioxidants and phenols. However, the total antioxidant capacity and polyphenolic contents of langaat was lower than that of beebot and riyom ($p<0.05$) with an IC_{50} of 90.16 μg mL^{-1} for langaat, 30.28 μg mL^{-1} for beebot and 60.27 μg mL^{-1} for riyom as compared with the standard (Quercetin) that had an IC_{50} of 23.17 μg mL^{-1}. The phytochemical screening of the flours was 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 3 varieties of rizga had a low toxic effect on the rats studied in addition with an LD_{50} of 630.1 mg kg^{-1} for Riym, 398.1 mg kg^{-1} for Langaat and 446.7 mg kg^{-1} for Beebot. Correlation between the total antioxidant capacity and polyphenolic composition of the 3 varieties of rizga was found to be significant ($R^2 = 0.917$). 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 is safe for consumption.

Key words: Phenolic composition, antioxidant activity, rizga, varieties, antioxidant activity

INTRODUCTION

Living stone potato (*Plectranthus esculentus* N.E.Br) which is known locally 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 (Schippers, 2000; Olojede *et al*., 2004). It is grown in Nigeria as it is rich in carbohydrates like most other tuber crops. In terms of protein content, when compared with yam, cassava, sweet potato and cocoyam, living stone potato ranks highest relative to major food crops grown in Nigeria. Despite its nutritive potential, it is classified among the lesser known and under exploited species of food crops in Africa (Schippers, 2000).

The medicinal value of plants have assumed a more important dimension in the past decades owing largely to the discovery that their extracts contain not only minerals but also a diverse array of secondary metabolites with antioxidant potentials (Akinmoladun *et al*., 2007). These antioxidants have been implicated in the therapeutic effects of several plants and vegetables that are used in traditional medicine (Ames *et al*., 1993; Kumar *et al*., 2005). As, these compounds are predominantly found in fruit tissues, it would be worthwhile investigating the nature of

phenols that are present in them. In addition, phytochemicals also act as potent antioxidants in both fat soluble and water soluble body fluids and cellular components (Marthur and Marthur, 2001) and also possess biological characteristics like anti-carcinogenicity, anti-mutagencity, anti-aging 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 either anti-oxidative 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 to be ascertained. This leads to the basis of this research which is designed to understudy the total antioxidant capacity, phenolic composition and toxicity of three varieties of living stone potato (rizga) to white albino male rats.

MATERIALS AND METHODS

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

Plant materials: Living stone potato used was freshly harvested from the experimental farm of National Root Crops Research Institute, Potato Programme, Jos. It was thoroughly washed, peeled and dried in an oven for 24 h at a temperature of 50°C.

Preparation of plant materials for analysis: The peeled portion of the Living stone potato (rizga) was ground into flour using a food processor 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 was carried out using standard qualitative procedures (Trease and Evans, 1984; Sofowara, 1984).

Assay of DPPH radical scavenging activity: The free radical scavenging activity of the extracts of the rizga varieties was determined using the modified method of Blois (1985). One milliliter of different concentrations (500, 250, 125, 62.5 and 31.25 μg mL^{-1}) of the methanolic extracts and standard quercetin was added to 1 mL of 0.3 mM DPPH in a test tube methanol to bring the final concentration to 250, 125, 62.5, 31.25 and 15.62 μg mL^{-1}. The mixture was vortexed and incubated in a dark chamber for 30 min and the absorbance was read at 517 nm using a uv spectrophotometer against a DPPH control which contained 1 mL of methanol. The percentage inhibition was calculated using the equation:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of control-absorbance of sample}}{\text{Absorbance of control}} \times 100$$

IC_{50} values denote the concentration of the samples which is required to scavenge 50% of DPPH radical. Higher IC_{50} values denote lower antioxidant activities.

Total phenol assay: Ten grams each of the fresh samples was soaked in 100mls of ethanol overnight. The mixture was filtered and centrifuged at 3000 x g for 10mins to obtain a clear supernatant. The total phenol assay was measured using the modified Folin-Ciocalteau method (Singleton *et al.*, 1999). The hydrophilic extract (0.5 mL) was diluted with distilled water to 5 and 0.5 mL of Folin-Ciocalteau reagent was added and allowed to react at room temperature for 3 min.

One milliliter of 1N sodium carbonate was added and allowed to react at room temperature for 1 h. The absorbance was measured at 725 nm using a UV spectrophotometer with distilled water as blank. Chlorogenic acid and gallic acid were used as standards. Total phenol content was reported as mg of chlorogenic acid equivalents per gram fresh weigh sample (mg CAE g^{-1} fw) which could be converted to mg of gallic acid by multiplying by a factor of 0.445.

Toxicity assay

Selection of animals: Fourty two matured male albino rats weighing between 119.86±48.2 g were used for the toxicity tests. Animals were acclimatized for a period of 7 days to the laboratory conditions prior to the experiment. Rats were housed in colony cages with 2 rats per cage at room temperature with 12 h light and dark cycle and they had access to drinking water and their food.

Experimental procedure: The rats were divided into 3 groups with 14 animals in each group. The animals received varying doses of ethanolic extracts of langaat, beebot and riyom flour corresponding to 330, 300, 280, 250, 220 and 120 mg kg^{-1} body weight. The dosage that killed 50% of the experimental animals after 24 h 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 methods of Karber (1931) and Litchfield and Wilcoxon (1949) and results were expressed in mg kg^{-1} body weight. The percentage dead for 0 and 100% mortality were corrected before the determination of probits as follows: For 0% mortality, corrected = 100 (0.25/n) and for 100% mortality, corrected = 100 (n-0.25/n). The probit values were plotted against log-doses and the dose corresponding to probit 5 was noted and its antilog taken to be the LD_{50}.

Statistical analysis: Statistical analysis was conducted using the Mean±SD 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 screening 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 1).

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

Table 1: Phytochemical screening of 3 varieties of rizga (langaat, beebot and riyom)

	Langaat	Beebot	Riyom
Alkaloid	-ve	+ve	Slightly+ve
Flavonoid	+ve	+ve	+ve
Tannin	+ve	+ve	+ve
CG	+ve	+ve	+ve

+ve: Present, -ve: Absent, CG: Cyanogenic glucoside

in blood pressure and death in severe cases. However, we could not determine the actual concentration of these glucosides in the 3 varieties of rizga studied.

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.

DPPH radical scavenging activity: The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability (Liu *et al*., 2008). The DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts (Devasagayam *et al*., 2004). The inhibitory activities of the extracts and quercetin decreased in the following order: Langaat<Riyom<Beebot<quercetin: The inhibitory activities of the extracts on DPPH free radical indicates that the extracts have a proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. The inhibitory actions of the three varieties of rizga is attributed to the phenolic content and presence of other phytochemicals in them. This suggests that rizga might be a useful therapeutic agent for treating radical-related pathological damage. The lower antioxidant activity of langaat when compared with beebot and riyom is thought to have arisen from the absence of alkaloids in it which are polyphenols with high antioxidant capacity (Table 2). Kirakosyan *et al*. (2003) have reported that polyphenols present in plants are responsible for their antioxidant activities. The generation of reactive oxygen species beyond what the body's ability can cope with leads to oxidative stress and can lead to tissue damages and necrosis in many instances. Furthermore, oxidative stress plays an important role in malaria (Kremser *et al*., 2000; Kulkarni *et al*., 2003). The immune system of the body is stimulated by infection including malaria thus causing the release of reactive

Table 2: DPPH radical scavenging activity of three rizga varieties

Sample	Concentration (μg mL^{-1})	Log concentration	Scavenging activity (%)	IC_{50} (μg mL^{-1})
a. Langaat	1000.00	3.00	87.92	90.16
	500.00	2.70	77.50	
	250.00	2.40	60.25	
	125.00	2.10	52.00	
	62.50	1.80	43.00	
	31.25	1.50	39.25	
b. Beebot	1000.00	3.00	88.00	30.28
	500.00	2.70	84.00	
	250.00	2.40	72.10	
	125.00	2.10	63.25	
	62.50	1.80	55.22	
	31.25	1.50	54.00	
c. Riyom	1000.00	3.00	82.25	60.27
	500.00	2.70	78.25	
	250.00	2.40	75.22	
	125.00	2.10	58.00	
	62.50	1.80	47.34	
	31.25	1.50	42.25	
d. Quercetin	1000.00	3.00	98.00	23.17
	500.00	2.70	96.55	
	250.00	2.40	94.28	
	125.00	2.10	85.09	
	62.50	1.80	55.88	
	31.25	1.50	52.25	

Linear equation: (a) y = 33.81x-16.10 (b) y = 25.25x +12.60 (c) y = 29.51x-2.532 (d) y = 34.28x+3.209

Table 3: Total phenolic contents of 3 varieties of rizga (langaat, beebot and riyom)

Variety	Total phenolic composition (mg CAE g^{-1} FW)
Langaat	0.1500±0.008
Beebot	0.4000±0.006
Riyom	0.3361±0.030

Each value in the table is the average of triplicate Experiments±SD at ($p<0.05$). CAE: Chlorogenic Acid Equivalent, FW: Fresh weight

Table 4: Lethal dose (LD_{50}) (oral) of extracts of 3 varieties of rizga on white albino rats

Group	Dose (mg kg^{-1})	Log dose	Dead (%)	Corrected (%)	Probit	LD_{50} (mg kg^{-1})
Riyom	330	2.52	52	52	5.05	630.1
	300	2.48	48	48	4.95	
	280	2.45	45	45	4.87	
	250	2.40	38	38	4.69	
	220	2.34	32	32	4.53	
	120	2.08	23	23	4.26	
Langaat	330	2.52	57	57	5.18	398.1
	300	2.48	51	51	5.03	
	280	2.45	42	42	4.80	
	250	2.40	37	37	4.67	
	220	2.34	31	31	4.50	
	120	2.08	25	25	4.33	
Beebot	330	2.52	54	54	5.10	446.7
	300	2.48	45	45	4.87	
	280	2.45	37	37	4.67	
	250	2.40	30	30	4.48	
	220	2.34	25	25	4.33	
	120	2.08	19	19	4.12	

LD_{50}: Lethal dose

oxygen species. In addition, the malaria parasite also stimulates certain cells to produce reactive oxygen species resulting in hemoglobin degradation (Das and Nanada, 1999). Results indicate that these rizga varieties could play significant roles in malaria therapy.

Total phenolic contents of 3 varieties of rizga flour: In recent years, phenolics have attracted the interest of researchers because they show promise of being powerful antioxidants that can protect the human body from free radicals, the formation of which is associated with the normal metabolism of aerobic cells (Mohammadpour and Rocha, 2007; Eleazu *et al*., 2011a,b). That the phenolic compounds present in plants possess antioxidant activity and may help protect cells against the oxidative damage caused by free radicals has been reported by Kirakosyan *et al*. (2003). All three varieties investigated contained significant quantities of phenols (Table 3). However, the phenolic composition of langaat was lower than that of the other 2 varieties analyzed and this is also attributed to the absence of alkaloids in it. Results obtained indicate that rizga contains significant quantities of phenolics.

Toxicity assay: The extracts of the 3 varieties of rizga flour had low toxic effects on the rats studied with an LD_{50} of 630.1 mg kg^{-1} for riyom, 398.1 mg kg^{-1} for langaat and 446.7 mg kg^{-1} for beebot (Table 4). LD_{50} denotes the dosage of the extracts that can kill 50% of the experimental

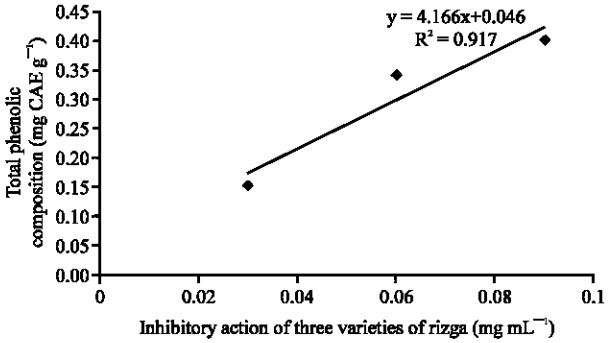

Fig. 1: Correlation of total phenolic composition versus antioxidant activity of 3 rizga varieties

animals in 24 h. High LD_{50} values indicate low toxic levels of the extracts and vice versa (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 antioxidant activities of the rizga varieties correlated positively with their total phenolic contents (Fig. 1) suggesting that the phenols present in all the varieties rizga evaluated could be responsible for their antioxidant activities. This is consistent with the findings of many researchers who reported such correlation between total phenolic content and IC_{50} antioxidant activity (Llobera and Canellas, 2007; Lecumberri *et al.*, 2007).

CONCLUSION

The results of the research analysis carried out reveals that Living stone 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 was found to be high, its use could be recommended especially in 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 Living stone potato and thus confirm the safety of its consumption.

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