Global Science Research Journals

ISSN: 0274-3248 Vol. 2 (3), pp. 139-146, March, 2016.
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Global Journal of Medicinal Plants Research

Full Length Research Paper

Contrast between African seeds and vegetables: Identification of their high antioxidant capacity

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Accepted 10 February, 2016

The antioxidant capacity of 2 African seeds and 8 vegetables were analyzed using ferric reducing antioxidant power assay (FRAP assay) after water and acetone extractions. The contents of ascorbic acid, phenolics and flavonoids were determined and their correlations with FRAP value were investigated. The results showed that *Vernonia amygdalina* was stronger (4.84 mM/100 g dry weight (DW)) than other vegetables analyzed in antioxidant capacity based on total FRAP values. *Baphia nitida* was the lowest in total FRAP value (1.26 mM/100 g DW). *Treculia africana* seed was higher than *Telfairia occidentalis* seed in total FRAP value. *T. occidentalis* leaf had antioxidant capacity than its corresponding seed. All water extracts were higher in FRAP value than the acetone extracts. FRAP value was significantly correlated with the contents of ascorbic acid, phenolics, or flavonoids in water extracts and with flavonoids in acetone extract, in which ascorbic acid and flavonoids contributed most in the water extracts based on multivariate regression analysis. In conclusion, the different African seeds and vegetables were remarkably different in antioxidant capacity.

Key words: Antioxidant capacity, ascorbic acid, flavonoids, phenolics, African seeds and vegetables.

INTRODUCTION

Natural antioxidants, particularly in fruits and vegetables have gained increasing interest among consumers and the scientific community because epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer (Temple, 2000). These antioxidants are a class of compounds thought to prevent certain types of chemical damages caused by an excess of free radicals-charged molecules that are generated by a variety of sources including pesticides, smoking, radiation and exhaust fumes. The defensive effects of natural antioxidants in fruits and vegetables against these free radicals are related to three major groups: vitamins, phenolics and carotenoids. Ascorbic acid and phenolics are known as hydrophilic antioxidants, while carotenoids are known as lipophilic antioxidants (Halliwell, 1996). These antioxidants are effective in scavenging various free radicals, inhibiting initiation of chained reactions by binding to metal ions (Peschel et al., 2006).

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Previously, a group of Spanish researchers discovered a novel and powerful natural antioxidant (Feruloylnoradenaline) which is 4.5 times more potent than vitamin E and 10 times more potent than vitamin C in tomato plants when under stressful condition (Lopez et al., 2011). The free radical theory of aging researched by Dr. Siegfried Hekimi and his student Dr. Wen Yong was tested by creating mutant worms that had increased production of free radicals, predicting they would be short lived even longer than regular worms! Moreover, their enhanced longevity was abolished when they were treated antioxidants such as vitamin C (Wen and Siegfried, 2010). Although non-antioxidant mechanisms are still undefined, flavonoids and other polyphenols may reduce the risk of cardiovascular disease and cancer (Arts and Hollman, 2005). Many epidemiological studies showed a significant inverse correlation between the intake of fruits and vegetables and the incidence of some chronic diseases (Dauchet et al., 2006). Therefore, increase in the consumption of fruits and vegetables have been frequently recommended to be one of the strategies in the prevention against oxidative stress related diseases (Kaliora et al., 2006; Garrido et al., 2010, 2014).

Several assays have been frequently used to estimate antioxidant capacities in fresh fruits and vegetables and their products and foods for clinical studies including 2,2-azinobis (3-ethyl-benzothiazoline-6-sulforic acid) (ABTS) (Leong and Shui, 2002), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Gil et al., 2002), ferric reducing antioxidant power (FRAP) (Benzie and Strian, 1999), and the oxygen radical absorption capacity (ORAC) (Prior et al., 2003).

The vegetables under review in this research studies include: *Heinsia crinita*- a local shrub commonly known as "atama", "*Vernonia amygdalina*" locally known as "bitter leaf" because of its bitter taste, "*Lasianthera africana*" called "editan" by the Efiks and Ibibios, "*Gongronema latifolium*" a tropical rainforest plant commonly known as "utasi" by the Efiks and Ibibios in the South-South states of Nigeria, "*Telfairia occidentalis*", a tropical vine grown in West Africa, commonly known as "fluted pumpkin", " *Acalypha torta*", commonly known as "nettle", *Alchornea cordifolia",* a perennial shrub with a height of 4 m, commonly called "Christmas bush", "*Baphia nitida*" a shrubby hard- wooded African tree commonly known as "camwood". *T. africana* seed" known locally as "ukwa seed" by the Igbos (South-Eastern Part of Nigeria), "*T. occidentalis* seed", a dark-red seed which bears fluted pumpkin leaves.

In the present study, the ferric reducing antioxidant power assay (FRAP assay) was used to determine the antioxidant capacity of different African seeds and vegetables. The correlations between the FRAP value and content of ascorbic acid, phenolics, or flavonoids were also analysed. The main objective of this study is to make a comprehensive comparison among different African seeds and vegetables and to identify the ones with high antioxidant capacity.

MATERIALS AND METHODS

Collection of plant material

A total of 8 different vegetables and 2 seeds were obtained from different plants at the Botanical Garden, which were identified by Botanists at the Department of Biological Sciences, University of Calabar.

The 2 seeds were (*T. occidentalis*, that is, fluted pumpkin seed and *T. africana* seed, that is, african breadfruit seed), while the 8 were vegetables were (*T. occidentalis*) (fluted pumpkin), *H. crinita* (atama), *A. torta* (nettle), *A. cordifolia* (Christmas bush), *B. nitida* (camwood), *G. latifolium* (utazi), *Lasianthera africana* (editan), *V. amygdalina* (bitter leaf). The vegetables and seeds were properly washed and dried in the laboratory under room temperature for 2 weeks to make them moisture free. Subsequently, they were ground into powdery form with a blender before extraction using water and acetone (Fatope et al., 1999).

Extraction with water and acetone

The extracts where prepared using standard procedures described by Fatope et al. (1999). This involves soaking 50 g of the powdered plant extract in both 95% water and 95% acetone for 48 h at room temperature to allow for maximum extraction. This was subsequently filtered to obtain the water fraction of the plant extracts. The filtrate obtained from the mixture of the powdered extract and acetone was evaporated using a rotary evaporator. The residues were retained as a crude extract for each of the test vegetables and stored in reagent bottle s. Both water and acetone extracts were used directly for Ferric Reducing Antioxidant Power (FRAP) assay on frozen at 20°C before the analysis of ascorbic acid, phenolics and flavonoids within two weeks (Fatope et al., 1999).

Ferric reducing antioxidant power assay (FRAP)

The procedure described by Benzie and Strain (1996) was followed. The principle of this method is based on the reduction of the ferric-tripyridyltriazine complex to its ferrous coloured form in the presence of antioxidants. The FRAP reagent contained 2.5 ml of 10 mM 2,4,6-tripyridy-s-triazine solution in 40 mM Hcl and 2.5 ml of 20 mM $Fecl_3$ and 25 ml of 0.3M acetate buffer, pH 3.6, and was prepared freshly and prewarmed at 37°C. Aliquots of 40 µl of extracts were mixed with 0.2 ml of distilled water and 1.8 ml of FRAP reagent. The absorbance of reaction mixture at 593 nm was measured spectrophotometrically after incubation at 37°C. The final result was expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1 mM $FeSO_4$. Adequate dilution was performed if the FRAP value measured was over the linear range of the standard curve.

Determination of ascorbic acid, phenolics and flavonoids

Ascorbic acid

A colorimetric procedure for the measurement of ascorbic acid, including dehydroascorbic acid in fruits, vegetables and derived products was followed in the determination of ascorbic acid in water extracts. Briefly, ascorbic acid was oxidized by activated charcoals to yield dehydroascorbic acid, which was further reacted with 2,4-dinitrophenylhydrazine to form osazone, a light-absorbing substance. The absorbance was read at 490 nm spectrophotometrically and the content of ascorbic acid was calculated based on a standard curve.

Phenolics

The content of phenolics in both water and acetone was determined according to the method reported by Singleton et al. (1999). Aliquots of 0.5 ml of water extracts were mixed with 2.5 ml of 10-fold-diluted Folin Ciocalteu reagent and 2 ml of 7.5% sodium carbonate (Na_2CO_3). The mixture was allowed to stand for 90 min at room temperature before the absorbance was measured at 760 nm spectrophotometrically. The contribution by ascorbic acid contained in water extract was calculated. The measurements were conducted in triplicate and the results reported as mean ± standard deviation (SD) values. The final results were calibrated to deduct the contribution from ascorbic acid and expressed as gallic acid equivalent.

Flavonoids

The content of flavonoids in both water and acetone extracts was measured using a modified colorimetric method described by Jia et al. (1999). A volume of 2.5 ml of water and acetone extract was transferred to a test tube, mixed with 0.15 ml of 5% sodium nitrite for 5 min. Then, 0.15 ml of 10% aluminium nitrate were added. After 6 min, the reaction was stopped by adding 1 ml of 1M sodium hydroxide. The mixture was further diluted with distilled water up to 5 ml. The absorbance of the mixture was immediately measured at 510 nm. The flavonoid content was calculated and expressed as rutin equivalent.

Statistical analysis

All statistical analyses were performed using Statistical Package for Social Science (SPSS) version 20. Measurements were carried out in triplicate. Data were presented as mean ± standard error of mean (SEM). The correlation between the FRAP value and the content of ascorbic acid, phenolics or flavonoids were analyzed. Multivarate regression analysis was also applied to investigate the relative importance to the contribution of the FRAP value by ascorbic acid, phenolics or flavonoids. Significant levels were tested and accepted at value of $P<0.05$.

RESULTS

Antioxidant capacity of different African seeds and vegetables

The FRAP values of 2 seeds and 8 vegetables of African origin (Table 1) shows that out of the 8 vegetables analyzed, *V. amygdalina* ranked the highest in total FRAP value (4.84 mM/100 g dry weight (DW)). This value is consistent with that obtained by Kelly et al. (2013). *G. latifolium* ranked second in total FRAP value (4.26 mM/100 DW), while the remaining vegetables had FRAP value ranging from 1.26 to 3.49 mM/100 DW. *B. nitida* was the lowest (1.26 mM/100 g DW). Two seed fractions (*T. africana* and *T. occidentalis*) were also analyzed. *T. africana* seed was higher in total FRAP value than *T. occidentalis* seed.

All water extracts were higher in FRAP value than the acetone extracts among all the vegetables analyzed in this study. Similar report was given by Ji et al. (2011), though with their own local vegetables. It was also shown that the leaf fraction of *T. occidentalis* was stronger in antioxidant capacity as measured by FRAP assay than the corresponding seed fraction. This is in agreement with the report by Mohammed et al. (2012) whose work showed that *T. occidentalis* leaf had antioxidant activity.

It is time-consuming and also difficult technically to isolate the antioxidants one by one from the vegetables, because there are too many compounds displaying antioxidant activity in the vegetables, such as ascorbic acid, β-carotene, phenolics, flavonoids, and others (Hein et al., 2002; Seifried, 2007). In the current study, the contents of ascorbic acid, phenolics and flavonoids of different African seeds and vegetables were measured in an attempt to investigate the relative contribution to the antioxidant capacity by different antioxidants.

DISCUSSION

Ascorbic acid is a well-known antioxidant and plays an important role in collagen synthesis and iron absorption (Mandl et al., 2009). It was found that *V. amygdalina* contained more than 300mg of ascorbic acid per 100g DW of the leaf fraction analyzed (Table 2) indicating that this vegetable is a good source of ascorbic acid.

This value is in contrast with that obtained by Olajire et al. (2011). Odukoya et al. (2007) reported a lower value for *V. amygdalina*. *T. occidentalis* leaf contained more than 200 mg of ascorbic per 100 g DW. However, the ascorbic acid content of the remaining vegetables (*G. latifolium*, *A. torta*, *L. africana*, *B. nitida* and *A. cordifolia*) was below 194.0 mg/100 g DW. Between the two (2) seeds analyzed, it was shown that *T. occidentalis* seed had higher ascorbic acid content (193.33 mg/100 g DW) than *T. africana* seed (178.33 mg/100 g DW). Comparatively, it was found that the leaf fraction of *T. occidentalis* was higher (218.00 mg/100 g DW) in ascorbic acid content than the corresponding seed fraction (193.33 mg/100 g DW) in the water extract.

Phenolics are small molecules containing one antioxidant activity *in vitro*. They play an important role in the protection of plants against ultraviolet radiation, or pathogens and predators (Strack, 1997). Most phenolics present in vegetables are water soluble in nature. For example, Toor and Savage (2005) reported that the hydrophilic phenolics contributed 78 to 87% of the total phenolics presents in the tomato pulps. In this study, the phenolics content of different vegetable fractions were determined in water extracts. The result showed that *G. latifolium* had the highest phenolic content (327.67±1.15 mg/100 g DW), followed by *T. occidentalis* leaf and *H. crinita*, in which more than 100 mg of phenolics per 100 g DW was detected. The other vegetables contained less than 100 mg of phenolics per 100 g DW. The seed (*T. africana* seed and *T. occidentalis* seed) contained less hydrophilic phenolics than the vegetable leaf fraction. The leaf fraction of *T. occidentalis* had high phenolic content (137.67 mM/100 g) than the corresponding seed.

Table 1. FRAP values of 2 different seeds and 8 vegetables of African origin (mg/100 g DW).

Vegetable	Fraction			
	Water	Acetone	Total	Rank
Vernonia amygydalina	4.31±0.07	0.54±0.01	4.84	1
Gongronema latifolium	3.85±0.04	0.41±0.01	4.25	2
Acalypha torta	1.84±0.04	0.19±0.00	2.04	9
Lasianthera africana	2.03±0.05	0.20±0.01	2.23	8
Baphia nitida	1.09±0.07	0.17±0.01	1.26	10
Alchornea cordifolia	2.08±0.06	0.20±0.01	2.28	6
Heinsia crinita	3.33±0.11	0.35±0.02	3.68	3
Teculia africana seed	3.18±0.03	0.31±0.00	3.49	4
Telfairia occidentalis leaf	2.93±0.03	0.27±0.03	3.20	5
Telfairia occidentalis seed	2.04±0.04	0.22±0.01	2.26	7

Data are expressed as mean±SEM. Each seed and vegetable was analyzed 3 times.

Table 2. The contents of ascorbic acid, phenolics and flavonoids of 2 different African seeds and 8 vegetables.

Vegetable	Ascorbic acid (mg/100 g DW)	Phenolics in water extract (mg/100 g DW)	Flavonoids (mg/100 g DW)		
			Water	Acetone	Total
Vernonia amygydalina	311.00±6.08	97.33±0.67	10.40±0.46	0.50±0.03	10.90
Gongronema latifolium	193.33±2.40	327.67±1.45	18.00±0.58	0.52±0.04	18.52
Acalypha torta	57.33±0.88	88.00±0.58	5.00±0.15	0.35±0.01	5.35
Lasianthera africana	122.00±1.15	96.33±0.88	4.57±0.15	0.32±0.01	4.88
Baphia nitida	70.00±2.52	79.33±2.60	3.50±0.06	0.09±0.01	3.59
Alchornea cordifolia	91.67±2.03	67.00±2.08	1.71±0.03	0.20±0.01	1.91
Heinsia crinita	46.33±1.0.88	115.33±2.60	9.17±0.20	0.48±0.02	9.64
Teculia africana seed	178.33±0.88	33.67±2.40	0.70±0.02	-[b]	0.70
Telfairia occidentalis leaf	218.00±1.53	137.67±1.45	11.00±0.58	0.52±0.03	11.52
Telfairia occidentalis seed	193.33±2.40	46.33±0.88	0.47±0.03	-[b]	0.47

Data are expressed as mean±SEM. Each seed and vegetable was analyzed 3 times. -[b]Means not detected.

Odukoya et al. (2007) reported that the phenolic content of twelve Nigerian vegetables including *T. occidentalis*, *V. amygdalina*, *L. africana*, and *G. latifolium* ranged from 21.83 mg/100 g DW to 546.97 mg/100 g DW, while a range between 33.67 mg/100 g DW to 327 mg/100 g DW was obtained in this work.

Flavonoids are a class of plant secondary metabolites and possess many biological actions, such as anti-allergic, anti-inflammatory, anti-microbial, and anti-cancer activities (Middleton et al., 2000). *G. latifolium* had the highest (18.00 mg/100 g DW) content of flavonoids among all the vegetable in water extract. The seeds in the water extract were poor in flavonoids. The vegetables in acetone extracts were also poor in content of flavonoids (Values <0.60 mg/100 g DW). No detectable flavonoids were found in the seed fractions (*T. africana* seed and *T. occidentalis* seed) in acetone extract. The leaf fractions contained more flavonoids than the seeds. More flavonoids were distributed in the water extract than the acetone extracts. Ji et al. (2011) reported that the water extracts of vegetable fractions was higher in FRAP value than the acetone extracts.

Correlations between the FRAP value and content of ascorbic acid, phenolics, or flavonoids in different African seeds and vegetables

The individual correlation graph is indicated (Figures 1 to 4). For the water extracts, the FRAP value of different African seeds and vegetables was significantly correlated with the content of ascorbic acid, phenolics or flavonoids (R^2=0.4285, 0.2132, 0.4331). For the acetone extracts, the FRAP value was also significantly correlated with the content of flavonoids (R^2=0.28). Based on multivariate regression analysis (Table 3), it was indicated that among ascorbic acid, phenolics, and flavonoids, ascorbic acid and flavonoids are the most important factor in

Table 3. Multivariate regression analysis among the FRAP value and contents of ascorbic acid, phenolics, and flavonoids in water extracts.

Variable	Unstandardized coefficient		Standard coefficient		
	B	S.E	Beta	T	Sig (p)
Constant	1.234	0.240	-	5.130	0.000
Asorbic acid	0.006	0.001	0.488	3.008	0.000
Phenolics	-0.005	0.003	-0.407	-1.501	0.145
Flavonoids	0.89	0.22	0.494	4.052	0.000

Significant difference at p>0.05.

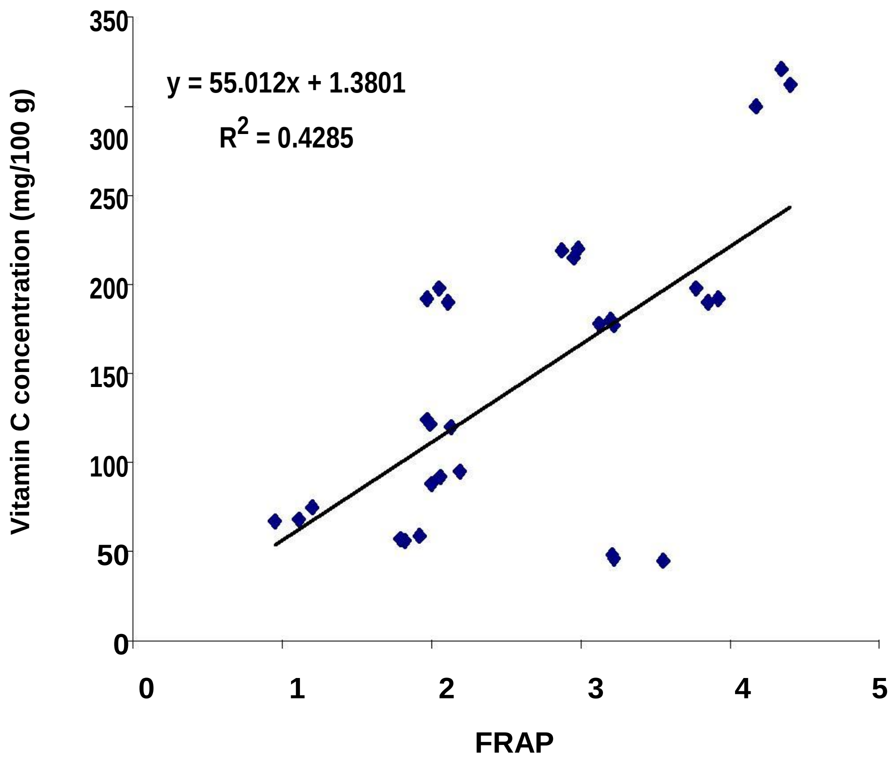

Figure 1. Relationship between FRAP and vitamin C content of water extract.

contributing to the FRAP value of the different African seeds and vegetables in water extracts (Table 3). This is in contrast with the result obtained by Oboh et al. (2004). They reported that phenol contributed more to the antioxidant properties of vegetable than ascorbic acid. This is because the content of ascorbic acid reduces when the vegetables are sun dried. Comparatively, the vegetables used for this research work were not sun dried.

Conclusion

It was found that the different seeds and vegetables were remarkably different in antioxidant capacity. *V. amygdalina* was the highest in total FRAP value among all the vegetables analyzed. *T. africana* seed was higher than *T. occidentalis* seed fraction in total FRAP value. *T. occidentalis* leaf had high antioxidant capacity than its corresponding seed. All water extracts were higher in FRAP value than the acetone extract. Ascorbic acid and flavonoid contributed most significantly to the antioxidant capacity of the vegetables and seeds in the water extracts.

This study is a systematic comparison of antioxidant capacity among different African seeds and vegetables. Further studies should be carried out on other fractions of these vegetables to investigate the specific components

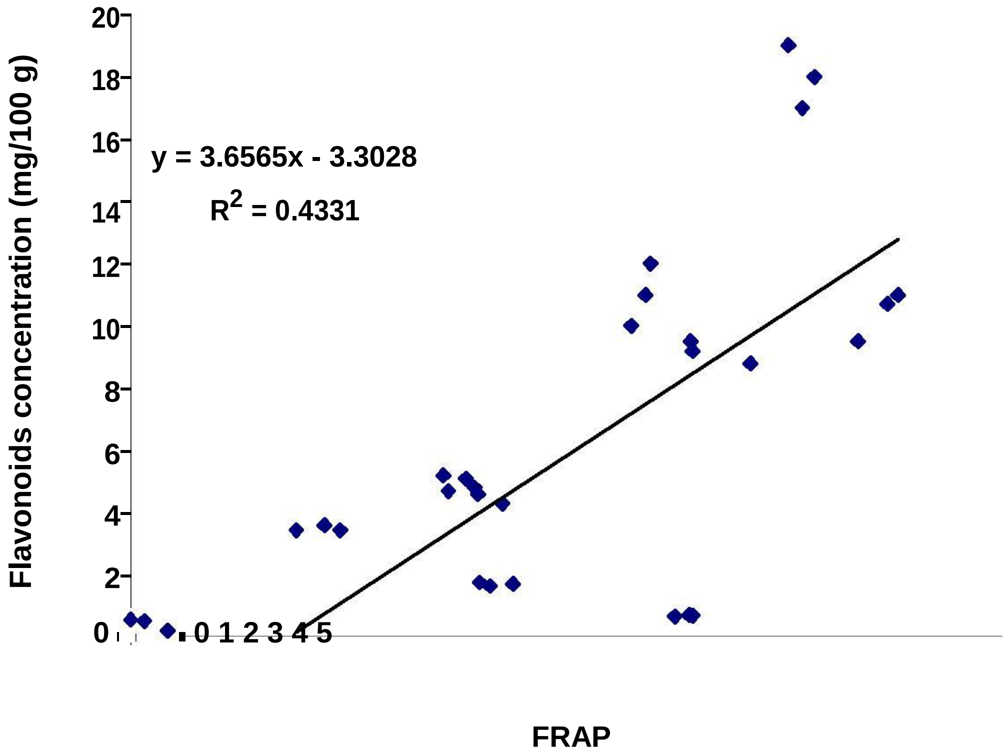

Figure 2. Relationship between FRAP and flavonoids content of water extract.

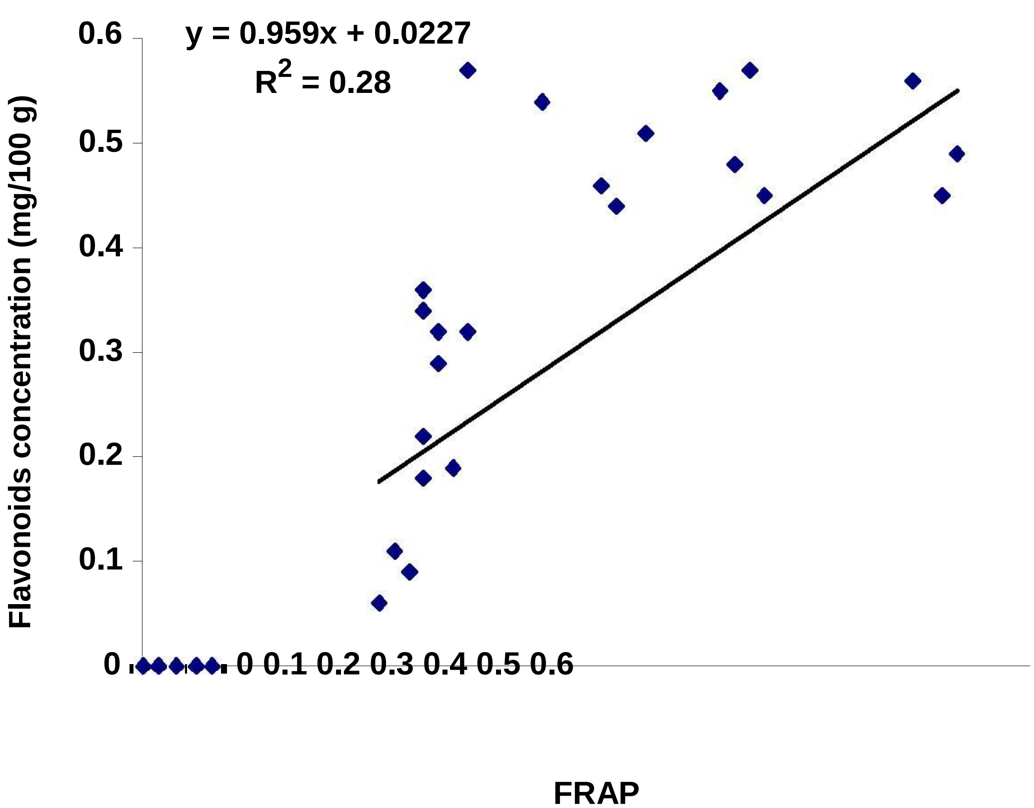

Figure 3. Relationship between FRAP and flavonoids content of acetone extract.

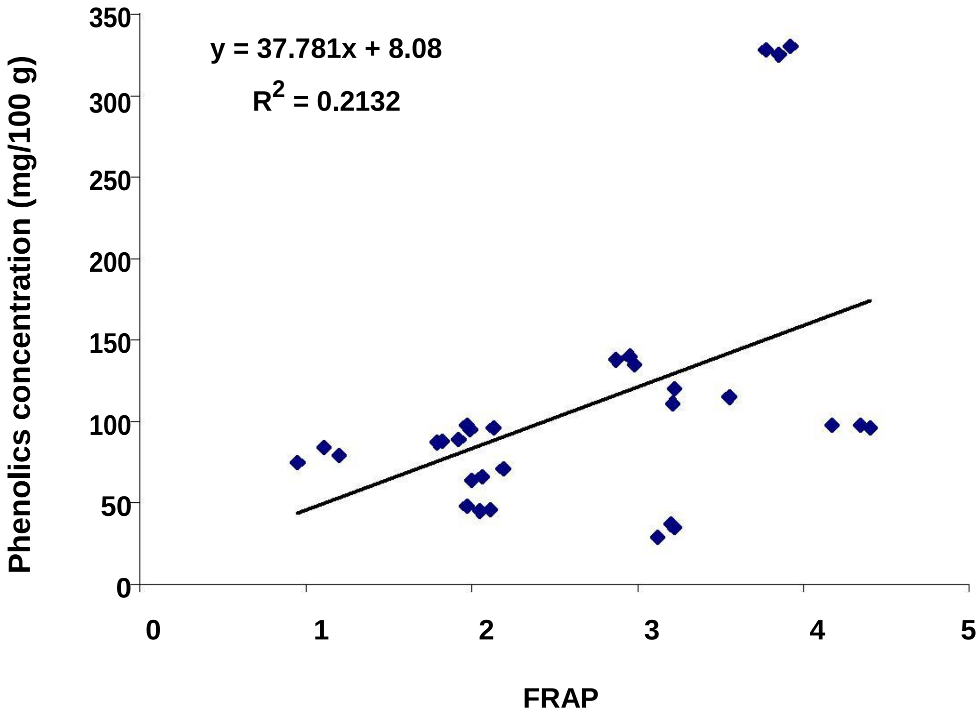

Figure 4. Relationship between FRAP and phenolics content of water extract.

responsible for their high antioxidant capacity and their possible health effects.

Conflict of Interest

The author(s) have not declared any conflict of interest.

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