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Phytochemical Content and Antioxidant Activity of Five Grain Amaranth Species

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Abstract Investigation into the antioxidant properties of plants is a very active field of research. Amaranths are underutilized pseudo-cereals with nutraceutical potentials. The phytochemical and antioxidant activity of five grain amaranth species were evaluated using standard procedures. Highest tannin content (0.14 g/100g) and Fe chelating (66.72%) capacity was recorded in *Amaranthus caudatus. Amaranthus cruentus* had the highest total flavonoid (9.93 mg CE/100g) content. *Amaranthus Hybridus* had the highest Phytate (1.58 g/100g), total polyphenol (30.79 mg GAE/100g), DPPH scavenging activity (93.35 %), ferric reducing power (0.19 g/100g), total antioxidant 199.93 mg AAE/100g) and ABTS (201.54 mmol TE/100g) content respectively. Strong correlation was observed between the phytochemicals and antioxidant tested. From the results, grain amaranth species possess antioxidant capacity and polyphenolic content. These qualities in amaranths have promising potential means of food biofortifications.

Keywords: *amaranth total polyphenol, amaranth extracts reducing power, amaranth, DPPH, ABTS*

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1. Introduction

Investigations into the antioxidant properties in plants is a very active field of research, especially for the not so well-known and underutilized plants. Phenolic compounds in plants possess antioxidant activity which help protect cells against oxidative damage caused by free radicals [1]. Diet containing whole-grain products and vegetables as primary ingredients has become one of the most important recommendation for reducing the risk of diseases caused by the increased level of free radicals [2]. Consumption of grains and vegetables has become increasingly popular for improvement and maintenance of heart health status, owing to the associated reduced risks of cardiovascular disease, and other associated chronic diseases such as diabetes and some forms of cancers [54, 55].

Amaranth is a valuable pseudo-cereal, due to its nutritional quality and nutraceutical properties, which contribute to improved human health [2,3]. Grain amaranths contain phytochemicals including flavonoids and antioxidants that help protect cells and tissues from damaging effects of free radicals and oxidative stress [4]. According to [5], the main phenolic compounds found in amaranth seeds are caffeic acid, p-hydroxybenzoic acid and ferulic acid. Some anti-nutritional phytochemicals such as tannins and phytic acid also exhibit some protective effects [6] particularly in red wine, and in some ruminants, in which higher retention of nitrogen has been observed in sheep and cattle with low to moderate levels of tannins in forages. Grain amaranth has been shown to exhibit antioxidant activity and this has been attributed to its content of polyphenols, anthocyanins, flavonoids, and tocopherols [7,8].

Amaranth of the genus *Amaranthus* L. consist of more than 60 species [9]. The three main species of grain amaranth widely cultivated are *A. caudatus, A. cruentus and A. hypochondriacus* and to a lesser extent, the leafy types *A. hybridus* and *A. tricolor* [10]. Consumption of *A. cruentus* products is advised for patients with celiac disease and also for diabetic persons [11]. *Amaranthus hybridus* has been used traditionally for the treatment of liver infections and knee pain. and for its laxative, diuretic and cicatrisation properties [12]; the products are used particularly for stomach aches, diarrhoea, and dysentery.

Amaranth cultivation remains relatively low and is not even listed in the FAO statistics on production data. Published data on the phytochemical content and antioxidant property of amaranth is limited. However, to assess their relevance as potential sources of dietary antioxidants, information on the phytochemical composition of the various species is essential. Phytochemical content and antioxidant activity of crops vary with species and are affected by environmental conditions and soil factors [8,13]. This study therefore evaluated the phytochemical profile and antioxidant activity of five grain amaranth species: *A. cruentus*, *A. hybridus, A. caudatus, A. hypochondriacus and A. hybrid*.

2. Materials and Methods

2.1. Chemicals and Reagents

Potassium ferricyanide, gallic acid, catechin, 3-(2-pyridyl)-5,6-bis-(4-phenylsulfonicacid)-1,2,4-triazine (Ferrozine), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis (3-ethylobenzothiazoline-6-sulphonic acid diammonium salt) (ABTS) and 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich. Folin–Ciocalteu reagent was from Merck (Germany). All reagents were of analytical grade.

2.2. Seed materials and Sample Preparation

Seeds of twenty nine grain amaranth accessions belonging to five species obtained from the United States Department of Agriculture, Ames, USA and National Horticultural Research Institute (NIHORT), Ibadan, Nigeria was used in this study. The seeds were planted in the experimental plot of NIHORT and harvested at maturity.

Seeds were milled into flour to obtain a homogenous particle size. Sample weighing 10g was extracted by stirring with 100 ml of methanol at 25°C at 150 rpm for 24h and filtered through Whatman No. 4 paper. The procedure was repeated twice, extracts were pooled together, evaporated at 40°C to dryness and redissolved in methanol at a concentration of 50 mg/ml and stored at 4°C prior to analyses.

2.3. Phytic Acid and Tannin Content

Phytic acid was extracted and determined according to the precipitation method of [14]. The conversion factor 3.55 for phosphorus to phytic acid was used. Tannin was determined by the acidified vanillin method of [15], using tannic acid as the standard.

2.4. Total Phenolic and Flavonoid Content

Total phenolic (TP) was determined colorimetrically using Folin-Ciocalteau reagent as described by [16]. Total phenolic assay was conducted by mixing 2.7 ml of de-ionised water, 0.3 ml of extracts, 0.3 ml 7% Na_2CO_3 and 0.15 ml Folin-Ciocalteu reagent. The mixture was vortexed and incubated at 40°C for 30min. Absorbance of mixture was measured at 725 nm. Results were expressed as gallic acid equivalent (GAE). Flavonoid content was determined by the method of [16]. Aliquots (0.5 ml) of appropriately diluted extracts or standard solutions was pipetted into 15-ml polypropylene conical tubes containing 4.5 ml of double distilled water and mixed with 0.3 ml 5% $NaNO_2$. After 5min, 0.6 ml 10% $AlCl_3.6H_2O$ solution was added, the mixture was allowed to stand for another 5min, and then 2 ml 1M NaOH was added, followed by 2.1 ml of distilled water. The reaction solution was well mixed, kept for 15 min, and the absorbance was determined at 510 nm. Total flavonoid was expressed as mg catechin equivalent (mg CE) per 100g of dry weight.

2.5. Ferric Reducing Antioxidant Power and Ferrous Ion Chelating Capacity

The ferric reducing power was determined using the method of [17]. To 1ml of the grain extract in a test tube, 0.5ml of 0.2 M phosphate buffer (pH 6.6) and 2.5ml of 1% potassium ferricyanide were added. The mixture was incubated for 20 min at 50°C. The tube was immediately cooled over crushed ice and then an aliquot of 0.5ml 10% trichloroacetic acid was added. After centrifugation at 3000 g for 10 min, 1 ml of the supernatant was mixed with 1ml of distilled water and 0.Iml of 0.1% ferric chloride. The mixture was left to stand for 10 min and absorbance was measured at 700 nm. Higher absorbance of the reaction indicates higher reducing power. Ascorbic acid was used as standard. The method described by [18] was used to determine the ferrous ion chelating capacity. Grain flour extract (1 ml), 2mM $FeCl_2.4H_2O$ (0.1 ml), 0.2 ml of 5mM ferrozine (3-(2-pyridyl)-5,6-bis-(4-phenylsulfonic acid)-1,2,4-triazine and 3.7 ml methanol were mixed in a test tube, and were reacted for 10 min. The absorbance at 562 nm was measured; a lower absorbance indicated a higher ferrous ion chelating capacity, which was calculated as follows:

$$\text{Ferrous ion chelating capacity } (\%) = \left[1-\left(A_{562}\text{nm, sample} / A_{562}\text{nm, control}\right)\right] \text{x} 100$$

Where A_{562} nm sample is the absorbance of sample at 562nm A_{562} nm control is the absorbance of control at 562nm.

2.6. Total Antioxidant Capacity

The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo V complex at acid pH [19,20]. To 0.2ml of extract was added 3.8ml reagent solution (0.6 M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The mixture was incubated at 95°C for 90 min, cooled to room temperature and the absorbance of the solution was measured at 695 nm against a blank. The total antioxidant capacity was expressed as ascorbic acid equivalent (AAE) per 100g of dry weight.

2.7. DPPH Radical Scavenging Activity and ABTS Radical Scavenging Activity

The capacity to scavenge the ''stable'' free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was monitored according to the method of Hsu et al. (2003). Methanol seed extract (1ml) was mixed with 5ml of freshly prepared methanol solution of $DPPH^{\bullet}$ radical. The mixture was shaken vigorously and left to stand for 50 min in the dark until stable absorption values were obtained. The reduction of the $DPPH^{\bullet}$ radical was measured by monitoring continuously the decrease of absorption at 517nm. Methanol (1ml), replacing the extract was used as the blank. Radical scavenging activity was expressed as inhibition percentage and calculated using the formula:

$$\%\ \text{RSA} = \left[1-\left(A_C / A_D\right)\right] \text{ x } 100$$

Where A_C is the absorbance of the solution when extract has been added and A_D is the absorbance of the DPPH solution. $ABTS^+$ radical cation was generated by the interaction of 7 mmol/L ABTS and 2.45 mmol/L potassium persulfate [21]. The solution was held at room temperature in the dark for 12-16h before use. Once the dark green solution was formed it was diluted with 95% ethanol until the absorbance read 0.7 at 734 nm [22]. For measurements, 2.5 ml of the resulting solution was mixed with 500µl of the extract. The absorbance was read after 6 min at 734 nm. The percentage decrease of the absorbance at 734 nm was calculated by the formula:

$$I = \left[(AB - AA) / AB \right] x100$$

Where I = ABTS % Inhibition
AB = Absorbance of blank sample (t=0)
AA = Absorbance of tested extract solution at the end of the reaction.

A standard curve was obtained by using Trolox solutions (0–250 mmol/mL) with ethanol. The absorbance of the reaction samples was compared to that of the trolox standard and results were expressed in terms of mmol Trolox Equivalent (TE) per 100g dry weight basis.

2.8. Statistical Analysis

All analysis was done in triplicate and results were given as means ± SD. The data were analysed by one-way ANOVA using SAS and Tukey's test was used to find significant differences between the species. Pearson correlation coefficient (r) and p-value were used to show correlations and their significance. Differences of $p \leq 0.05$ were considered significant.

3. Results and Discussion

The results of phytochemical composition and antioxidant activity of five species of grain amaranth are presented in (Table 1). The highest tannin (0.14 g/100g) and Fe chelating (66.72%) content was recorded in *A. caudatus*. *Amaranthus cruentus* had the highest total flavonoid (9.93 mg CE/100g) content. *A. Hybridus* had the highest Phytate (1.58 g/100g), total polyphenol (30.79 mg GAE/100g), DPPH scavenging activity (93.35 %), ferric reducing power (0.19 g/100g), total antioxidant 199.93 mg AAE/100g) and ABTS (201.54 mmol TE/100g) content respectively. The lowest value for all the tested parameters was recorded in *A. caudatus* except tannin and Fe chelating.

3.1. Phytochemical Content of Amaranth Species

Tannin content of the five amaranth species ranged from 0.10 – 0.14 g/100g with *A. caudatus* > *A. cruentus* > *A. hybrid* > *A. hypochondriacus* > *A. hybridus* (Table 1). Tannin content recorded in this study was lower than the maximum acceptable tannic acid daily intake for humans [23]. Values are in agreement with results of [24] who recorded tannin content of 0.12 g/100g, 0.06 g/100g and 0.87 g/100g for *A. cruentus, A. hypochondriacus* and *A. hybridus* respectively. Values obtained in this study were lower than values recorded for wheat (0.29 g/100g), Barley (0.34 g/100g) and Oat (0.62 g/100g); but higher than 0.07 g/100g obtained for rice [25]. Tannin content obtained in this study is similar to value obtained in brown rice (0.1 g/100g); but lower than values obtained in cereals such as wheat (0.4 g/100g), maize (0.4 g/100g), rye (0.6 g/100g), millet (0.6 g/100g), barley (0.7 g/100g), oat (1.1 g/100g) and sorghum (1.5 g/100g) [26]. Finger millets have been reported to contain high amounts of tannin ranging from 0.04 to 3.74 g/100g [27,28]. It was also reported by [29] that the tannin content in brown finger millet is 0.36 g/100g, these results is higher than values obtained in this study. Put together, grain amaranth can be seen as a potential source of dietary component for easy digestibility with mild to moderately low tannin content compared to other well-known cereals.

Table 1. Phytochemicals and antioxidant contents of five grain amaranth species

Phytochemicals				
Amaranth species	Tannin g/100g	Phytate g/100g	Total Flavonoid (mg CE/100g)	Total Polyphenol (mg GAE/100g)
A. Caudatus	0.14±0.002^{a}	1.16±0.07^{c}	8.91±0.16^{a}	27.52±1.14^{b}
A. Cruentus	0.13±0.004^{a}	1.33±0.02^{b}	9.93±0.31^{a}	30.48±0.60^{a}
A. Hybrid	0.12±0.004^{a}	1.41±0.04^{b}	8.96±0.37^{a}	29.65±0.47^{a}
A. Hypochondriacus	0.12±0.003^{a}	1.42±0.04^{b}	9.50±0.27^{a}	30.03±0.71^{a}
A. Hybridus	0.10±0.002^{b}	1.58±0.04^{a}	9.56±0.38^{a}	30.79±1.35^{a}

Antioxidant					
Amaranth species	DPPH scavenging activity (g kg $^{-1}$)	Ferric Reducing Power (g/100g)	Total Antioxidant (mg AAE/100g)	Fe chelating (g kg $^{-1}$)	ABTS (mmol TE/100g)
A. Caudatus	89.53±0.45^{b}	0.14±0.01^{b}	140.22±4.92^{b}	66.72±6.38^{a}	169.6±3.77^{b}
A. Cruentus	90.15±0.85^{b}	0.16±0.01^{b}	145.33±8.40^{b}	64.08±2.09^{ab}	179.3±2.83^{b}
A. Hybrid	91.19±0.53^{b}	0.16±0.01^{b}	183.08± 8.4^{a}	62.33±1.48^{ab}	179.1±2.67^{b}
A. Hypochondriacus	91.40±0.55^{ab}	0.16±0.01^{b}	147.54±8.35^{b}	57.52±1.77^{c}	179.2±3.98^{b}
A. Hybridus	93.35±1.27^{a}	0.19±0.02^{a}	199.93±16.5^{a}	59.19±3.25^{bc}	201.5±4.04^{a}

Values are mean ± SE. Means with the same letter in each column are not significantly different ($p<0.05$).

Phytate content of the five grain amaranth species ranged from 1.16 – 1.58 g/100g, with *Amaranthus hybridus* > *A. hypochondriacus* > *A. hybrid* > *A. cruentus* > *A. caudatus* (Table 1). Values are higher than 0.03 – 0.80 g/100g obtained for raw amaranth grains [30], 0.00079 g/100g obtained for grain amaranth [31]; but lower than values obtained for wheat (4.17 g/100g), rice (4.28 g/100g), Barley (4.03 g/100g) and Oat (2.77 g/100g [25]. Phytate content obtained in finger millet ranged from 0.679 to 0.693 g/100g [28]; and 0.149 to 0.150 g/100g [27]. These are lower than values obtained in this present study. Phytic acid is a known anti-nutrient which forms insoluble complexes with minerals such as zinc, calcium, magnesium and iron in the body. [32] reported that the presence of phytic acid below the level of 6 g/100g will not pose any health risk to the body and concluded that dietary intake of low level phytate may protect against a fatty liver resulting from elevated hepatic lipogenesis. The anti-nutrient effect of phytic acid on mineral absorption will only occur at 10 fold higher levels of what was obtained in this study.

Total flavonoid content ranged from 8.91 to 9.93 mg CE/100g (Table 1), with highest value observed for *Amaranthus cruentus* followed by *A. hybridus* and *A. hypochondriacus then A. Hybrid and A. Caudatus.* The result for total flavonoid obtained in this study is lower than 13.4 to 14.3 mg CE/100g obtained for *A. hypochondriacus* and 17.7 mg/100g obtained for oat [33]. It was reported [7] that there were no quantifiable amount of flavonoids in *Amaranthus caudatus* seeds, only traces of quercetin was found. Low levels of quercetin glycoside, rutin (4.0-10.2 µg/g) was detected in *A. hypochondriacus* seeds [34]. These values corroborate findings from this present study in which low level of flavonoid was obtained in all the five species. The flavonoid content of quinoa (41.7 to 72.6 mg/100g) and *Chenopodium* (36.2 to 144.3 mg/100g) species [7] were also higher than results obtained for amaranth in our study.

Total Polyphenol content of the five grain amaranth species ranged from 27.52 – 30.79 mg GAE/100g (Table 1). *Amaranthus hybridus* had the highest value (30.97 mg GAE/100g), this is higher than 14.72 to 14.91 mg/100g GAE obtained for *A. hypochondriacus* and 19.61 mg/100g GAE obtained for oat [33]. *Amaranthus caudatus* had the lowest total polyphenol content (27.52 mg GAE/100g), this is higher than 21.20 mg/100g GAE obtained for *Amaranthus caudatus* [35]; but comparable to values (16.8 to 32.9 mg GAE/100g) obtained for some *A. caudatus* genotypes [7]. Values obtained in this study were lower than values obtained for other pseudocereals like Quinoa (71.7 mg GAE/100g), buckwheat (323.0 mg GAE/100g) and wheat (53.1 mg GAE/100g) [35]. They are also significantly lower than values obtained for amaranth v. Rawa (295 mg GAE/100g) and amaranth v. Aztek (300 mg GAE/100g) [2]. Total polyphenol content of 15.5, 25 and 29 mg GAE/100g was obtained in amaranth, quinoa and buckwheat, respectively [3]. The total phenol content of the five amaranth species were also lower than values obtained for Tumeric (119 mg GAE/100g), wheat (53 mg GAE/100g), corn (49 mg GAE/100g), sesame (42 mg GAE/100g), soybean (37 mg GAE/100g), oatmeal (35 mg GAE/100g) and linseed (31 mg GAE/100g) [36]. Whole grain flour of rye and wheat have been reported to contain phenolic acids of 137 and 134 mg/100g, respectively [37]. The polyphenol content of amaranth seeds is comparable to that of oat, barley, rice; but lower when compared with common cereals like wheat, maize and rye.

3.2. Antioxidant Activity of Amaranth Species

DPPH activity ranged from 89.53% – 93.35% with highest value observed in *A. hybridus* > *A. hypochondriacus* > *A. hybrid* > *A. cruentus* > *A. caudatus*. The DPPH activities obtained in *A. hypochondriacus* (91.40%) and *A. cruentus* (90.15%) in this study are higher than 86.93% obtained in *A. hypochondriacus* seeds [38] and 84.67% obtained in *A. cruentus* [39]; respectively. Result from this study is similar to values obtained in grain amaranth [40] but higher than values obtained for turmeric (31.5%), wheat (10.7%), corn (13%), soybean (12.7%), oatmeal (2.3%) and linseed (5.7%) [36]. Ferric reducing power ranged from 0.14 to 0.19 g/100g with *A. hybridus* and *A. Caudatus* having the highest and lowest values. High values obtain in this study for all the Amaranth indicates that they are a potent source of antioxidant since the reducing capacity of a compound is usually an indicator of its potential antioxidant activity.

Total antioxidant activity of the five grain amaranth species ranged from 140.22 to 199.93 mg AAE/100g. These are significantly higher than values obtained for certain cereals such as rice (85.49 mg AAE/100g), maize (26.94 mg AAE/100g), wheat (14.17mg AAE/100g) and barley 3.46 mg AAE/100g [41].

Iron chelating activity of the five amaranth species ranged from 57.52 to 66.72 %. Iron chelating activity obtained in methanol and hexane extract of *A. cruentus* leaves were 64% and 54% respectively; [42]. Earlier report had indicated that polyphenols exhibit potent iron chelating ability [43]. Data variation in the antioxidant activity are to be expected due to sample type, differences in the methodology employed, standard used to express results and experimental conditions [22,44].

ABTS antioxidant activity of the five amaranth species ranged from 169.6 to 201.5 mmol TE/100g; with *A. hybridus* having the highest value. The ABTS activity (179.3 mmol TE/100g) obtained for *Amaranthus cruentus* is higher than values obtained for *Amaranthus cruentus* varieties: Aztek (127 mmol TE/100g) and Rawa (114 mmol TE/100g) [2]. ABTS obtained for *Amaranthus caudatus* (169.6 mmol TE/100g) is much higher than values obtained for two varieties of *A. caudatus*: Centenario (82.76 mmol TE/100g) and Oscar Blanco (67.01 mmol TE/100g) [45]. The ABTS value is lower than value obtained for quinoa seeds (272 mmol TE/100g) [2] but higher than values obtained for rye [46]; barley, oat, buckwheat [47] and wheat bran [48].

4. Correlation

The result for Pearson correlation analysis is presented in (Table 2) while the linear relationship between individual significant parameters is presented in Figure 1.

Pearson's correlation analysis (Table 2) revealed strong correlation between total polyphenol vs. DPPH (r = 0.678) (Figure 1A) and total polyphenol vs. ABTS (r = 0.688) (Figure 1B). This is in agreement with report of [2], who observed strong correlation between total polyphenol content vs. DPPH (r = 0.98); and ABTS (r = 0.98) in amaranth seeds. Strong correlation was also observed between total phenolic content and DPPH (r = 0.99) in *A. caudatus* seeds [35]. These findings suggest that total polyphenols content is a good predictor of *in vitro* antioxidant activity. Strong correlation was observed between total polyphenol vs. Reducing power (r = 0.88) (Figure 1C). Similar result was observed in some Mexican maize phenotypes in which total polyphenol showed strong correlation with DPPH, ABTS and Reducing power [49]. Strong correlation was observed between DPPH and ABTS (r = 0.921) (Figure 1D), DPPH vs. ferric reducing power (r = 0.851) (Figure 1E) and ferric reducing power vs. ABTS (r = 0.735) (Figure 1F). Strong correlation was observed between ABTS and DPPH (r = 0.98) [2], this corroborates result of this study. Significant ($P < 0.05$) correlation was also observed between phytate and total polyphenol (0.862), DPPH (0.946), Ferric reducing power (0.930) and ABTS (0.946).

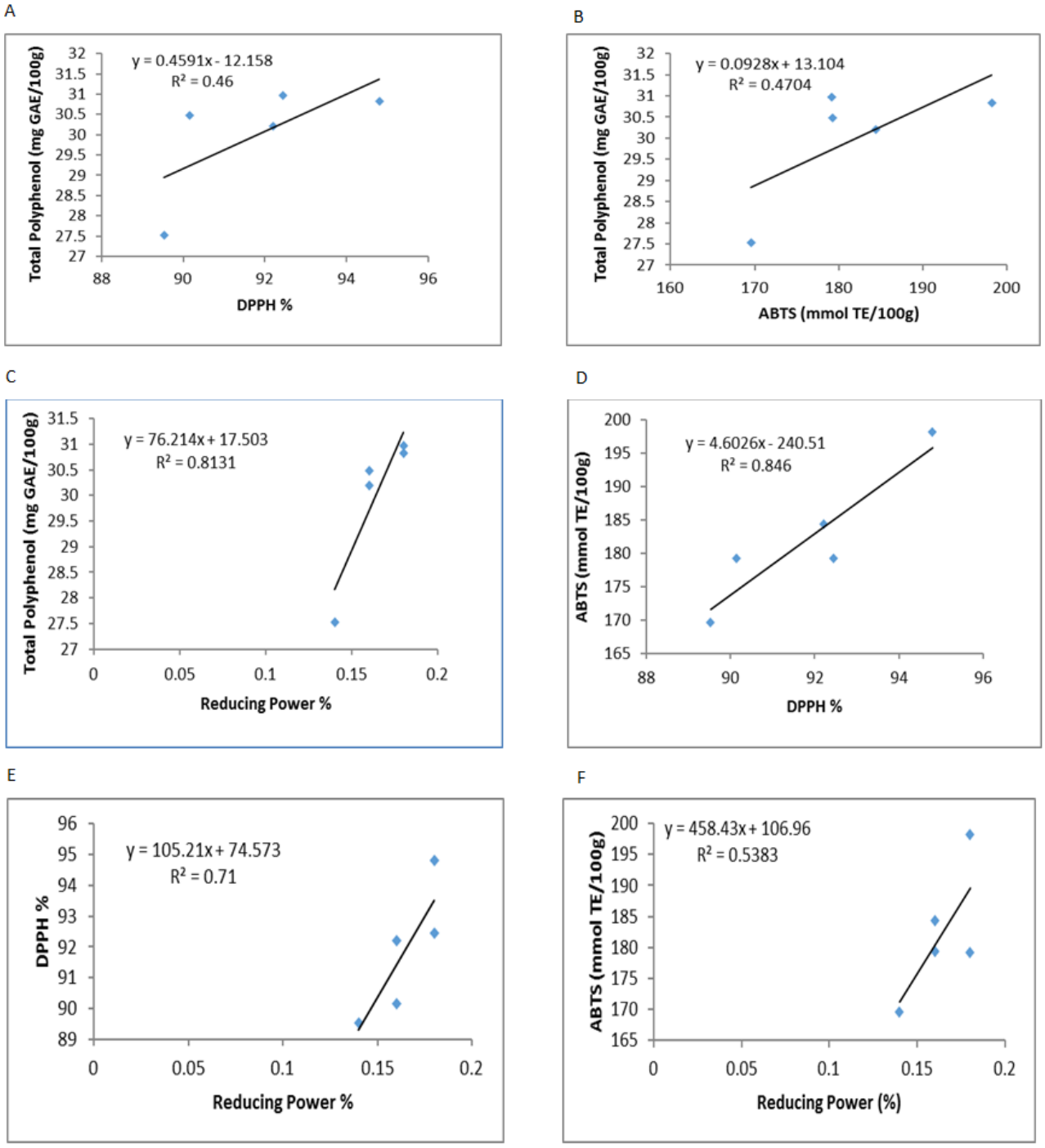

Figure 1. Linear relationship between the parameters determined: (A) Total Polyphenol vs. DPPH (B) Total Polyphenol vs. ABTS (C) Total Polyphenol vs. Reducing Power (D) DPPH vs. ABTS (E) DPPH vs. Ferric reducing power (F) ABTS vs. ferric reducing power

Table 2. Pearson's correlation matrix of phytochemical and antioxidant activity of five Amaranth species

Amaranth species	Tannin	Phytate	Total Flavonoid	Total Polyphenol	DPPH	Ferric Reducing Power	Total Antioxidant	Fe Chelating	ABTS
Phytate	-0.856								
Total Flavonoid	0.124	0.068							
Total Polyphenol	-0.479	0.862**	0.311						
DPPH	-0.959	0.946**	0.073	0.678*					
Fe Reducing Power	-0.739	0.930**	0.315	0.882**	0.851**				
Total Antioxidant	-0.396	0.043	0.410	-0.258	0.339	0.081			
Fe Chelating	0.581	-0.741	0.025	-0.694	-0.597	-0.846	0.224		
ABTS	-0.805	0.861**	0.256	0.688**	0.921**	0.735**	0.376	-0.321	

* = significant (P<0.05); ** = very significant (P<0.01).

Weak correlation was observed between total polyphenol and total antioxidant activity (r = -0.258). This is not in agreement with result of [50] in which strong correlation was observed between total polyphenol and total antioxidant activity (r = 0.97) in amaranth; and between total polyphenol and antioxidant activity (r = 0.96) in rice [51,52]. A significant correlation (r = 0.96) was observed between total phenolic content and antioxidant activity in buckwheat extract [53].

The correlation discrepancies found in literature could be explained on the basis of differences in the interpretation of the results by individual methods. Also, the antioxidant activity of a substance can vary from method to method depending on factors such as antioxidant solubility, oxidation state and medium of pH [44,46].

5. Conclusion

These results highlight the significance of grain amaranth as potential source of phytochemicals and antioxidants. *Amaranthus hybridus* had most of the phytochemicals and antioxidant activity in higher levels when compared with the other species examined in the study. The five grain amaranth species showed higher antioxidant activity and phytochemical content when compared to cereals such as oat, barley, wheat, corn, millet and rice. Amaranth is therefore a good substitute for traditional cereals and has potential as source of health-promoting bioactive compounds. As these grains are affordable and widely available, efforts to promote their consumption for health benefits, its use in food biofortification applications and pharmaceutical industries should be encouraged.

References

[1] Kirakosyan, A., Seymour, E., Kaufman, O.B, Warber, S., Bolling, S. and Chang, S.C. "Antioxidant capacity of polyphenolic extracts from leaves of *Crataegus laevigata* and *Crataegus monogyna* (Hawthorn) subjected to drought and cold stress". *Journal of Agriculture and Food Chemistry* 50. 3713-3717. 2003.

[2] Pasko, P., Barton, H., Zagrodzki, P., Gorinstein, S., Fołta, M. and Zachwieja, S. "Anthocyanins, total polyphenols and antioxidant activity in amaranth and quinoa seeds and sprouts during their growth. *Food Chemistry*. 115. 994-998. 2009.

[3] Gorinstein, S., Vargas, O.J.M., Jaramillo, N.O., Salas, I.A., Ayala, A.L.M. and Arancibia, A.P. "The total polyphenols and the antioxidant potentials of some selected cereals and pseudocereals. *European Food Research and Technology*. 225. 321-328. 2007.

[4] Majewska, M., Skrzycki, M., Podsiad, M. and Czeczot, H. "Evaluation of antioxidant potential of flavonoids: An *in vitro* study". *Acta Poloniae Pharmaceutica.* 68. 611-615. 2011.

[5] Klimczak, M., Malecka, M. and Pacholek, B. "Antioxidant activity of ethanolic extracts of amaranth seeds". *Nahrung/Food*, 46, 184-186. 2002.

[6] Aletor, V.A. and Adeogun, O.A. "Nutrients and antinutrient components of some tropical leafy vegetables". *Food Chemistry*. 54. 375-379. 1995.

[7] Repo-Carrasco-Valencia, R., Hellstrom, J.K., Pihlava, J. and Mattila, P.H. "Flavonoids and other phenolic compounds in Andean indigenous grains: Quinoa (*Chenopodium quinoa*), kaniwa (*Chenopodium pallidicaule*) and kiwicha (*Amaranthus caudatus*)". *Food Chemistry*. 120. 128-133. 2010.

[8] Escudero, N.L., Alabarracın, G.J., Lopez, L. and Gimenez, M.S. "Antioxidant activity and phenolic content of flour and protein concentrate of *Amaranthus cruentus* seeds". *Journal of Food Biochemistry*. 35. 1327-1341. 2011.

[9] Ray, T. and Roy, S.C. "Genetic diversity of *Amaranthus* Species from the Indo-Gangetic Plains revealed by RAPD analysis leading to the development of Ecotype-Specific SCAR marker". *Journal of Heredity* 100(3). 338-347. 2009.

[10] Shukla, S., Bhargava, A., Chatterjee, A., Pandey, A.C. and Mishra, B.K. "Diversity in phenotypic and nutritional traits in vegetable amaranth (*Amaranthus tricolor*), a nutritionally underutilized crop". *Journal of the Science of and Food Agriculture*. 90. 139-144. 2010.

[11] Guerra-Matias, A.C. and Arêas, J.A.G. "Glycemic and insulinemic responses in women consuming extruded amaranth (Amaranthus cruentus L.)". *Nutritionnal Research*. 25. 815-822. 2005.

[12] Nacoulma, O.G. "Plantes médicinales et pratiques médicales traditionnelles au Burkina Faso: cas du plateau central". Thèse d'état, Université de Ouagadougou. p. 574. 1996.

[13] Sudhir, S., Vibha, P., Pachauri, G., Dixit, B.S., Banerji, R. and Singh, S.P. "Nutritional contents of different foliage cuttings of vegetable amaranth". *Plant Foods for Human Nutrition*. 58. 1-8. 2003.

[14] Wheeler, E.L. and Ferrel, R.E. "A method for phytic acid determination in wheat and wheat fractions". *Cereal Chemistry.* 48. 312-316. 1971.

[15] Burns, R.E. "Method of estimation of tannin in the grain sorghum". *Agronomy Journal.* 63. 511-519. 1971.

[16] Bao, J.S., Cai Y., Sun, M., Wang, G.Y. and Corke, H. "Anthocyanins, flavonols and free radical scavenging activity of Chinese bayberry (*Myrica rubra*) extracts and their color properties and stability". *Journal of Agricultural and Food Chemistry*. 53. 2327-2332. 2005.

[17] Oyaizu, M. "Studies on the products of browning reaction prepared from glucose amine". *Japanese Journal of Nutrition.* 44. 307-315. 1986.

[18] Hsu, C.L., Chen, W., Weng, Y.M. and Tseng, C.Y. "Chemical composition, physical properties and antioxidant activities of yam flours as affected by different drying methods". *Food Chemistry*. 83. 85-92. 2003.

[19] Prieto, P., Pineda, M. and Anguilar, M. "Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdaenum complex: specific application to the determination of vitamin E". *Analytical Biochemistry.* 269(2). 337-341. 1999.

[20] Banerjee, A., Dasgupta, N. and Bratati, D. "*In vitro* study of antioxidant activity of *Syzygium cumini* fruit". *Food Chemistry*. 90. 727-733. 2005.

[21] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. "Antioxidant activity applying an improved ABTS radical cation decolorisation assay". *Free Radica Biology and medicine.* 26. 1231-1237. 1999.

[22] Huang, D.J, Chen, H.J, Lin, C.D. and Lin, Y.H. "Antioxidant and antiproliferative activities of water spinach (*Ipomoea aquatica* Forsk) constituents". *Botanical Buletin of the Academia Sinica.* 46. 99-106. 2005.

[23] Fekadu, H., Beyene, F., and Desse, D. "Effect of traditional processing methods on nutritional composition and anti-nutritional factors of Anchote (Coccinia abyssinica) tubers grown in western Ethiopia". *Journal of Food Processing Technology*. 4(7): 249-256. 2013.

[24] Gorinstein, S., Lojek, A., Cízhacek, M., Pawelzik, E., Delgado, L.E. and Medina, O.J. "Comparison of composition and antioxidant capacity of some cereals and pseudocereals". *International Journal of Food Science and Technology*. 43. 629-637. 2008.

[25] Satinder, K., Savita, S. and Nagi, H.P.S. "Functional properties and anti-nutritional factors in cereal bran". *Journal of Food and Agro Industry.* 4(2). 122-131. 2011.

[26] Juliano, B.O. "Production and utilization of rice. In Rice Chemistry and Technology". *American Association of Cereal Chemist.* 2nd ed. St. Paul, Minnesota, USA. 774. 1985.

[27] Rao, P.U. "Evaluation of protein quality of brown and white ragi (*Eleusine coracana*) before and after malting". *Food Chemistry*. 51. 433-436. 1994.

[28] Anthony, U. and Chandra, T.S. "Enzymatic Treatment and Use of Starters for the Nutrient Enhancement in Fermented Flour of Red and White Varieties of Finger Millet (Eleusine coracana)". *Journal of Agricultural and Food Chemistry*. 47. 2016-2019. 1999.

[29] Rao, B.S.N. and Prabhavati, T. "Tannin content of foods consumed in India and its influence on ionisable iron". *Journal of the Science of and Food Agriculture*. 33. 89-96. 1982.

[30] Whittaker, P. and Ologunde, M.O. "Study of Iron Bioavailability in a Native Grain Amaranth Cereal for Young Children using a rat model". *Cereal Chemistry*. 67(5). 505-508. 1990.

[31] Njoki, J.W., Sila, DN. and Onyango, A.N. "Impact of Processing Techniques on Nutrient and Anti-Nutrient Content of Grain Amaranth (*A. albus)"*. *Food Science and Quality Management.* 25, ISSN 2224-6088 (Paper) ISSN 2225-0557 Online. 2014.

[32] Onomi, S., Okazaki, Y. and Katayama T. "Effect of dietary level of phytic acid on hepatic and serum lipid status in rats fed a high-sucrose diet". *Bioscience, Biotechnology and Biochemistry*. 68(6). 1379-81. 2004.

[33] Czerwinski, J., Bartnikowska, E., Leontowicz, H., Lange, E., Leontowicz, M. and Katrich, E. "Oat (*Avensa sativa* L.) and amaranth (*Amaranthus hypochondriacus*) meals positively affect plasma lipid profile in rats fed cholesterol-containing diets'. *The Journal of Nutritional Biochemistry*. 15. 622-629. 2004.

[34] Barba de la Rosa, A.P., Fomsgaard, I.S., Laursen, B., Mortensen, A.G., Olvera-Martinez, L. and Silva-Sanchez, C. "Amaranth (*Amaranthus hypochondriacus*) as an alternative crop for sustainable food production: Phenolic acids and flavonoids with potential impact on its nutraceutical quality". *Journal of Cereal Science*. 49. 117-121. 2009.

[35] Alvarez-Jubete, L., Wijngaard, H., Arendt, E.K. and Gallagher, E. :Polyphenol composition and *in vitro* antioxidant activity of amaranth, quinoa buckwheat and wheat as affected by sprouting and baking". *Food Chemistry.* 119. 770-778. 2010.

[36] Camatari, F.O.S., Lopes, K.H., Valentim, B., Xavier, J.A., da Costa, J.G., Santana, A.E.G. and Goulart, M.O.F. "Antioxidant Potential of Flours from Cereals, Tubers, Beans and Seeds Chemical Profile of Curcuma longa Flour". *Journal of Nutrition and Food Science*. 6. 2. 2016.

[37] Mattila, P., Pihlava, J.M. and Hellstrom, J. "Contents of phenolic acids, alkyland alkenylresorcinols, and avenanthramides in commercial grain products". *Journal of Agricultural and Food Chemistry*. 53. 8290-8295. 2005.

[38] López, V.R.L., Razzeto, G.S., Giménez, MS. and Escudero, N.L. "Antioxidant Properties of Amaranthus hypochondriacus Seeds and their Effect on the Liver of Alcohol-Treated Rats". *Plant Foods for Human Nutrition*. 66(2). 157-162. 2011.

[39] Kunyanga, C.N., Imungi, J.K., Okoth, M.W., Biesalski, H.K. and Vadivel, V. "Total phenolic content, antioxidant and antidiabetic properties of methanolic extract of raw and traditionally processed Kenyan indigenous food ingredients". *Food Science and Technology,* 45(2). 269-276. 2012.

[40] Kunyanga, C.N., Imungi, J.K., Okoth, M., Momanyi, C., Biesalski, H.K. and Vadivel, V. "Antioxidant and antidiabetic properties of condensed tannins in acetonic extract of selected raw and processed indigenous food ingredients from Kenya". *Journal of Food Sci*ence 76. 560-567. 2011.

[41] Goufo, P. and Trindale, H. "Rice antioxidants: phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol and phytic acid". *Journal of Food Science and Nutrition*. 2(2). 75-104. 2014.

[42] Oboh, G., Raddatz, H. and Henle T. "Antioxidant properties of polar and non-polar extracts of some green leafy vegetables". *Journal of the Science of and Food Agriculture* 8. 2486-92. 2008.

[43] Omololu, P.A., Rocha, J.B.T. and Kade, I.J. "Attachment of rhamnosyl glucoside on quercetin confers potent iron-chelating ability on its antioxidant properties". *Experimental and Toxicological Pathology* 63(3). 249-245. 2011.

[44] Stratil, P., Klejdus, B. and Kubánhacek, V. "Determination of total content of phenolic compounds and their antioxidant activity in vegetables-evaluation of spectrophotometric methods". *Journal of Agricultural and Food Chemistry*. 54. 607-616. 2006.

[45] Repo-Carrasco-Valencia, R., Pena, J., Kallio, H. and Salminen, S. "Dietary fiber and other functional components in two varieties of crude and extruded kiwicha (*Amaranthus caudatus*)". *Journal of Cereal Science*. 49. 219-224. 2009.

[46] Gallardo, C., Jimenez, L. and Garcia-Conesa, M.T. "Hydroxycinnamic acid composition and *in vitro* antioxidant activity of selected grain fractions". *Food Chemistry*. 99. 455-463. 2006.

[47] Zieliński, H. and Kozłowska, H. "Antioxidant Activity and Total Phenolics in Selected Cereal Grains and Their Different Morphological Fractions". *Journal of Agricultural and Food Chemistry.* 48(6), 2008-2016. 2000.

[48] Yu, L., Haley, S., Perret, J., Harris, J.W. and Qian, M. "Free radical scavenging properties of wheat extracts". *Journal of Agricultural and Food Chemistry*. 50. 1619-1624. 2002.

[49] Lopez-Martine, L.X., Oliart-Ros, R.M., Valerio-Alfaro, G., Lee, CH., Parkin, K.L. and Garcia, H.S. "Antioxidant activity, phenolic compounds and anthocyanins content of eighteen strains of Mexican maize". Food Science and Technology. 42(6). 1187-1192. 2008.

[50] Nsimba, R.Y., Kikuzaki, H. and Konishi Y. "Antioxidant activity of various extracts and fractions of *Chenopodium* quinoa and *Amaranthus* spp. Seeds". *Food Chemistry.* 106. 760-766. 2008.

[51] Adom, K.K. and Liu, R.H. "Antioxidant activity of grains". *Journal of Agricultural and Food Chemistry.* 50. 6182-6187. 2002.

[52] Choi, Y., Jeong, H.S. and Lee, J. "Antioxidant activity of methanolic extracts from some grains consumed in Korea". *Food Chemistry*. 103. 130-138. 2007.

[53] Sun, T. and Ho, C. "Antioxidant activities of buckwheat extracts". *Food Chemistry*. 90, 743-749. 2005.

[54] Lillioja, S., Neal, A.L., Tapsell, L. and Jacobs, D.R., Jr. "Whole grains, type 2 diabetes, coronary heart disease, and hypertension: links to the aleurone preferred over indigestible fiber". *Biofactors* 39. 242-258. 2013.

[55] Jones, J. M. and Engleson, J. "Whole grains: benefits and challenges". *Annu Rev Food Sci. Technol.* 1: 19-40. 2010.