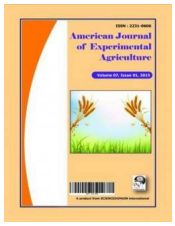

American Journal of Experimental Agriculture
12(5): 1-10, 2016, Article no.AJEA.18087
ISSN: 2231-0606

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Phytochemical Constituents and Antioxidant Activities in Leaves of 14 Breeding Lines of Cassava (*Manihot esculenta* Crantz)

E. K. Quartey[1*], H. M. Amoatey[1,2], E. Achoribo[3], M. Owusu-Ansah[1], W. Nunekpeku[1], S. Donkor[3], A. S. Appiah[1] and E. S. K. Ofori[1]

[1]Biotechnology and Nuclear Agriculture Research Institute, Ghana Atomic Energy Commission, P.O.Box LG 80, Legon-Accra, Ghana.
[2]Graduate School of Nuclear and Allied Sciences, University of Ghana, P.O.Box AE 1, Atomic Energy-Accra, Ghana.
[3]Radiological and Medical Sciences Research Institute, Ghana Atomic Energy Commission, P.O.Box LG 80, Legon-Accra, Ghana.

Authors' contributions

This work was carried out in collaboration between all authors. Authors EKQ, WN, ASA, MOA, SD, EA and ESKO designed the study, wrote the protocol, managed the analyses of the study and wrote the first draft of the manuscript. Author HMA reviewed the experimental design and all drafts of the manuscript. Authors EKQ and HMA performed the statistical analysis. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJEA/2016/18087
Editor(s):
(1) Lanzhuang Chen, Laboratory of Plant Biotechnology, Faculty of Environment and Horticulture, Minami Kyushu University, Miyazaki, Japan.
Reviewers:
(1) Anonymous, National Root Crops Research Institute, Nigeria.
(2) Brahmadeo Dewprashad, City University of New York, USA.
(3) Preeya P. Wangsomnuk, Khon Kaen University, Thailand.
Complete Peer review History: http://sciencedomain.org/review-history/14724

Original Research Article

Received 2nd April 2015
Accepted 8th October 2015
Published 22nd May 2016

ABSTRACT

Two (2) month-old leaves of fourteen (14) breeding lines of cassava, consisting of five (5) parental lines and nine hybrids, were evaluated for their phytochemical constituents. The objective of the study was to determine total flavonoid, phenolic and antioxidant activity in the leaves of the breeding lines. The 14 breeding lines were grown in the research farm of the Biotechnology and nuclear Agriculture Research Institute between March and September 2011. Analyses were carried

**Corresponding author: E-mail: emmaquart@yahoo.com;*

out at the laboratories Ghana Atomic Energy Commission (GAEC) between July and August 2011. The randomized complete block design, with three replicates, was used. Results indicate statistically significant differences in Total Flavonoid Contents (TFCs), Total Phenolic Contents (TPCs) and Total Antioxidant Activities (TAAs) recorded for both the ethanolic and aqueous extracts of the breeding lines. Hyb-9 gave the highest total flavonoid content of 179.90±0.21 mg/g/QE (ethanol extract) as well as total phenolic contents of 128.25 mg/g/GAE and 95.33+3.61 mg/g/GAE for both ethanol and aqueous extracts, respectively, while Security gave the highest value for total flavonoid content of 96.7+0.03 (aqueous extract). Similarly, Larbi recorded the highest TAA in ethanolic extract (82.88+3.07%), while Hyb-15 gave the highest value of aqueous extract (80.92+2.79%). In general there was strong positive correlation among the TFC, TPC and TAA. Most hybrids exhibited higher TFCs and TPCs than their parents in the ethanolic extracts.

Keywords: Cassava; breeding lines; antioxidants; phenolics; flavonoids; ethanolic extract; aqueous extract.

1. INTRODUCTION

Cassava (*Manihot esculenta* Crantz), is a perennial shrub of the family Euphorbiaceae, cultivated mainly for its starchy roots [1]. It is the world's sixth most important crop in terms of production and is a vital staple food to over 500 million in the humid tropics [2]. The tuber is popularly used for production of tapioca chip and starch. Starch is used mainly in the manufacture of monosodium glutamate, glucose and paper products. Tubers are also processed as feed for livestock. New food uses of cassava include flour in gluten free or gluten-reduced products [3].

Ghana is currently the 4th largest producer of cassava in Africa and 6th in the world [4]. The crop is cultivated in all eight out of ten regions with a prevalence in the Eastern and Central regions. The crop contributes 22 percent of Ghana's Agricultural Gross Domestic Product (GDP) and it is a major staple crop with an annual production above 10 million metric tonnes (MT) in the last decade [5]. Per area harvested, cassava is currently the second largest crop as it has been recently superseded by maize. Yields show a slight increase starting from 2008 up to 15 MT per hectare in 2010 following the introduction of high yielding and disease resistant varieties [6], though still below the achievable level of 28 MT [5].

In Ghana, consumption of cassava leaves as a vegetable is low compared to other African countries such as Congo DRC or Tanzania [5]. However, with current trends of food insecurity and severe malnourishment in sub-Saharan Africa [7], this multi-purpose crop can play a significant role in ameliorating these conditions in the country.

Cassava leaves contain an average of 21% crude protein, but values ranging from 16.7 to 39.9% have been reported [8]. This wide variability is related to differences in cultivars, stage of maturity, sampling procedure, soil fertility and climate [9]. They are rich in iron, zinc, manganese, magnesium, calcium, vitamins B1, B2 and C, and carotenoids [7,10]. Furthermore, the essential amino acid profile of the leaves of cassava is higher than the Food and Agriculture Organization's recommended reference protein intake and to that of soybean protein [1,11]. Cassava leaves also contain moderate levels of phytochemicals that are important as natural antioxidant components of plant food products [1].

Natural antioxidants, particularly in fruits and vegetables have gained increasing interest among consumers as antidotes to aging and associated chronic diseases [12]. Flavonoids are phenolic substances isolated from a wide range of vascular plants, and more than 8150 different types have been reported [13]. They act in plants as antioxidants, antimicrobials, photoreceptors, visual attractors, feeding repellents, and for light screening [14].

Mechanisms of antioxidant action include serving as physical barriers to prevent reactive oxygen species (ROS) generation or ROS access to important biological sites; chemical traps/sinks that "absorb" energy and electrons, quenching ROS; catalytic systems that neutralize or divert ROS [15]. They also bind and/or inactivate metal ions to prevent generation of ROS and serve as chain-breaking entities which scavenge and destroy ROS [16].

In the past, breeding efforts in cassava have primarily focused on high yield, earliness and

wide adaptation (photoperiod insensitivity, resistance to insects and diseases) [17]. However, the importance of the quality of food source for improving the health of mankind is gaining prominence. Hence, enhancing nutritional quality through plant breeding would have great impact on the livelihoods of people living in sub-Saharan Africa.

Hybridization is an important technique used in transferring genes among intra-and inter-specific plant species to generate genetic variability towards selection for improved traits such yield, adaptation and nutritional quality. Cassava has traditionally been improved through hybridization [18-22]. Nunekpeku [23] crossed nine parental lines of cassava to generate several hybrid lines out of which a number were selected for evaluation with respect to yield and adaptation to cultivation under variable agro-ecological conditions. However with the increasing use of cassava leaves as leafy vegetables, it is important to conduct phytochemical evaluation to ascertain their nutritional status towards enhanced usage. The objective of the study was therefore to assess some phytochemical constituents (namely total flavonoids, phenolics and antioxidant activity) in the leaves of fourteen (14) breeding lines of cassava, comprising of five (5) parental lines and nine (9) hybrids.

2. MATERIALS AND METHODS

2.1 Experimental Area

The experiment was conducted at the Nuclear Agriculture Research Centre (NARC), Applied Radiation Biology Centre (ARBC) and the Ghana Research Reactor-One (GHARR-1), all of the Ghana Atomic Energy Commission, Kwabenya (Accra), Ghana. Fourteen (14) breeding lines (Table 1), comprising five (5) parental lines and nine (9) hybrids and were planted on a plot of land measuring 60 m x 89 m using the Randomized complete Block design with three replications at the research fields of the Biotechnology and Nuclear Agriculture Research Institute of the Ghana Atomic Energy Commission (GAEC) in July 2011.

2.2 Sample Preparation

Two (2) months after planting (MAP) (i. e. September 2011), 10 mature leaves were harvested separately from each breeding line (one per plant) and put into Zip-lock bags and transferred to the laboratory of the Applied Radiation Biology. The samples were washed, air-dried, and lyophilized at the Ghana Research Reactor-One (GHARR-1) of GAEC. Dried samples were homogenized in a stainless steel Waring blender to obtain powdery forms. Two grams (2 g) of each powdered sample were weighed into a centrifuge tube. 30 ml of distilled water was added and stirred on a mechanical agitator for 2 hours. This was topped up with 20 ml of distilled water and agitated for additional 2 hours, making a total volume of 50 ml. The supernatant was then stored and kept in the freezer till needed.

Table 1. Cassava breeding lines used in the study

Accession	Source	Pedigree
Afisiafi	BNARI Field Gene Bank	-
Asare	- do -	-
BS-1	- do -	-
Larbi	- do -	-
Security	- do -	-
Hyb-2	F_1 Derived Hybrids	AF x SE
Hyb-8	- do -	AF x SE
Hyb-9	- do -	AF x SE
Hyb-13	- do -	AF x SE
Hyb-14	- do -	AF x SE
Hyb-15	- do -	AF x SE
Hyb-17	- do -	SE x BS-1
Hyb-24	- do -	AF x AS
Hyb-35	- do -	LA x BS

Note: AF=Afisiafi, SE=Security, BS= BNARI Selection, AS=Asare, LA=Larbi

2.3 Determination of Total Flavonoid Content

The aluminium chloride colorimetric assay method (Zhishen et al. 1999) was employed to evaluate total flavonoid content using Quercetin as a standard. An aliquot of 500 µL extract was mixed with the following: 1500 µL of 99.9% ethanol, 100 µL of 1 M potassium acetate, 100 µL of 10% Aluminium Chloride and 3000 µL of distilled water. The resulting mixture was incubated for 30 minutes at room temperature and corresponding absorbance measured at 415 nm. All determinations were carried out in triplicates.

A standard calibration curve was constructed using Quercetin standard solutions of 25 µg/mL, 50 µg/mL, 75 µg/mL, 100 µg/mL and 125 µg/mL each time the samples were analysed. 500 µL of

each standard was treated in the same manner as the samples above and a calibration linear regression equation of y = 141.9x was obtained, (where x = mg per Quercetin), r^2 = 0.996, where *r* is the coefficient of the regression line. Total flavonoid content of each extract was determined from the curve and expressed as milligram Quercetin equivalent per gram sample (mg QE/g) according to the formula by [24]:

$$\text{Total Flavonoid Content} = \frac{(c\ x\ df\ x\ v)}{w}$$

where; *c* = concentration obtained from the standard curve; *df* = dilution factor; *v* = volume of stock solution; *w* = weight of cassava leaf extract used in the experiment.

2.4 Determination of Total Phenolic Content

Polyphenolic contents of the extracts were determined by a modified Folin-Ciocalteau method using Gallic acid as standard [25]. A 50 µL portion of each of the extracted sample was mixed with 3 mL of distilled water (dH_2O) and 250 µL of a 1 in 10 diluted Folin-Ciocalteu phenol Reagent. The mixtures were allowed to stand for 5 minutes, after which 750 µL of 20% Na_2CO_3 was added to each. They were thoroughly mixed and incubated for 30 minutes at room temperature in a dark place. Absorbance was measured at 760 nm using a UV-VIS Spectrophotometer (Shimadzu, 1201, Japan). All determinations were performed in triplicate. A calibration curve was prepared using serial dilutions of 1 mg/mL Gallic acid dissolved in water to the following concentrations, 0.2 mg/mL, 0.4 mg/mL, 0.6 mg/mL, 0.8 mg/mL and 1 mg/mL each time the samples were analysed. A regression equation for the two curves used was determined as $y = 1.0938x + 0.0378$ with a regression factor of r^2=, 0.9954 for when the 22℃ and 30℃ extract were analysed respectively. Total phenolic content in each extract was determined from the respective curves and expressed as milligram Gallic acid equivalent per gram sample (mg GAE/g) using the formula below:

$$\text{Total Phenolic Content} = \frac{c\ x\ v}{m}$$

Where ***c*** = the concentration of Gallic acid established from the calibration curve in mg/g; ***v*** = the volume of cassava leaf extract in micro litres; ***m*** = the weight of cassava leaf extract in grams.

2.5 Free Radical Scavenging Assay

The free radical scavenging activity was determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay as described by Brad-William et al. [26]. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases. The free radical scavenging activity was measured in terms of hydrogen donating or radical scavenging ability, using stable radical DPPH. DPPH is suggested by many authors because this method is repeatable and provides a precise assay for measuring the antioxidant activity [26-28]. 200 µl of extracts were each added to 3800µl of 0.004% DPPH in methanol. Concentrations of 0.01, 0.02, 0.03, 0.04, 0.05, 0.06 and 0.07 mg/ml of Gallic Acid were used to plot the standard curve. After 60 minutes of incubation at room temperature in the dark, the absorbance was measured at 517 nm. A blank sample containing only methanol was used to correct the spectrophotometer reading to zero. Ascorbic acid (Vitamin C) was used for comparison. Each experiment was performed in triplicate. The activity of the test sample was determined in terms of the percentage reduction of the DPPH (sometimes referred to as "inhibition" or "quenching"). Radical scavenging activity, Q, was calculated as follows:

$$Q = \frac{(Abs0 - Abs1)}{Abs0} \text{ x } 100$$

where; Abs_0 = absorbance of 0.004% DPPH without analyte, Abs_1 = absorbance of 0.004% DPPH plus the test compound.

2.6 Data Analyses

Spectrophotometric readings were evaluated for statistical significance using one-way analysis of variance (ANOVA) and means separated by the new Duncan's multiple range tests (Statgraphics Centurion XVI, version 16.1.11, USA) expressed as the Mean **±** SE(standard error of the mean) upon three independent analyses. A p-value of 0.05 or less was considered as statistically significant.

3. RESULTS AND DISCUSSION

3.1 Total Flavonoid Content in Leaves of Cassava Breeding Lines

Variation in total flavonoid contents (TFCs) of the ethanolic and aqueous extracts of leaves of the

cassava breeding lines are shown in Table 2. In the ethanol extract, the highest TFC was recorded by Hyb-9 (179.9±0.21 mg/g/QE) while Afisiafi, with a TFC of 103.6±0.10 mg/g/QE, recorded the least. In the aqueous extract however, Security recorded the highest TFC (96.7±0.03 mg/g/QE) with Hyb-24 recording the least (55.9±0.01 mg/g/QE). Mean TFCs were 131.3 and 84.5 mg/g/GAE in the ethanolic and aqueous extracts respectively.

In both extraction methods, there were significant differences in the TFCs of the leaves of the cassava breeding lines. Higher TFCs in the ethanolic extracts were observed compared to the ethanolic extracts, indicating that the ethanolic extraction method was more efficient than the aqueous extraction method. The results also indicate that some hybrid lines (Hyb-8, Hyb-9, Hyb-17 and Hyb-35) contained more TFCs in the ethanolic phase than in their parental lines. This could be attributed to heterosis (hybrid vigour). TFC values reported in this study are within the range reported by other workers [28-30] – all for cassava leaves, [31] – for moringa leaves) but far below that of Ahiapka et al. [32] in okra.

3.2 Total Phenolic Content in Leaves of Cassava Breeding Lines

The total phenolic contents (TPCs) of the ethanolic and aqueous extracts of leaves of the cassava breeding lines are also presented in Table 2. The highest TPC of 128.25±0.60 mg/g/GAE was recorded in Hyb-9 while Afisiafi recorded the least (62.12±0.95 mg/g/GAE) in the ethanolic extract. Again, Hyb-9 with TPC of 95.33±3.61 mg/g/GAE (which was not statistically different from Hyb-15), recorded the highest TPC in the aqueous extract, while Hyb-24 recorded the lowest TPC of 39.07±0.73 mg/g/GAE. Mean TPCs were 85.80 mg/g/GAE and 66.44 mg/g/GAE in the ethanolic and aqueous extracts respectively. Hyb-8, Hyb-9 and Hyb-35 recorded higher values for both TFC and TPC in the ethanolic phase than their parental lines. Hyb-9 recorded higher TPC values than all the breeding lines in both extraction methods. Hyb-14 and Hyb-15 recorded higher TPCs in the aqueous extracts than in the ethanolic extracts indicating that phenolic contents in the leaves of these lines are more soluble in water than in ethanol.

Generally, however, TPCs for all the accessions (except Hyb-14 and Hyb-15) were generally higher in the ethanolic extracts than in the aqueous extracts, signifying that phenolic compounds in cassava leaves are more soluble in ethanol than in water. These results are inconsistent with the report of Ahiakpa et al. [32] and Owusu-Ansah et al. [31] who, working on okra and moringa leaf respectively, reported higher TPCs in water than in organic solvents. TPC values recorded in this study are higher

Table 2. Total flavonoids and phenolics in leaves of cassava breeding lines

Breeding line	Total flavonoid content (mg/g/QE)		Total phenolic content (mg/g/GAE)	
	Ethanol extract	Aqueous extract	Ethanol extract	Aqueous extract
Afisiafi	**103.6±0.10^{i}**	85.7±0.04^{d}	**62.12±0.95^{g}**	55.37±1.39^{e}
Asare	122.3±0.04^{f}	58.1±0.18^{h}	73.88±3.10^{ef}	42.52±3.27^{f}
BS-1	112.9±0.19^{gh}	70.6±0.02^{g}	75.84±1.47^{ef}	52.24±2.26^{e}
Larbi	116.3±0.04^{g}	90.8±0.02^{c}	98.60±1.18^{b}	79.87±0.75^{bc}
Security	141.4±0.11^{c}	**96.7±0.03^{a}**	86.13±1.67^{d}	78.68±2.32^{c}
Hyb-2	132.0±0.18^{d}	96.0±0.02^{ab}	88.32±0.81^{cd}	65.55±3.09^{d}
Hyb-8	151.2±0.18^{b}	80.5±0.04^{e}	102.33±2.60^{b}	56.35±1.98^{e}
Hyb-9	**179.9±0.21^{a}**	94.7±0.06^{b}	**128.25±0.60^{a}**	**95.33±3.61^{a}**
Hyb-13	127.3±0.05^{e}	94.6±0.02^{b}	92.63±0.07^{c}	77.17±1.26^{c}
Hyb-14	111.5±0.12^{h}	95.7±0.01^{ab}	78.28±0.65^{e}	85.30±2.88^{b}
Hyb-15	104.7±0.13^{i}	95.8±0.02^{ab}	77.69±2.94^{e}	92.74±0.22^{a}
Hyb-17	153.7±0.18^{b}	77.9±0.01^{f}	71.36±4.15^{f}	40.97±0.14^{f}
Hyb-24	128.4±0.01^{de}	**55.9±0.01^{i}**	62.25±0.45^{g}	**39.07±0.73^{f}**
Hyb-35	153.6±0.19^{b}	89.5±0.03^{c}	103.46±1.71^{b}	68.98±3.07^{d}
Mean	**131.34**	**84.46**	**85.80**	**66.44**

±SE=standard error, means with same letters in a column are not statistically different (p≥0.05) from each other according to Duncan's multiple range test. Values bolded and underlined refer to accessions with the highest concentration; Bolded values represent accessions with the lowest concentration

than those reported by Simao et al. [29] and Faezah et al. [28] also working on leaves of some accessions of cassava.

Similarly, low TPC values has been reported for some vegetables, such as broccoli (0.68 mg/g), onion (1.13 mg/g) and cabbage (0.67 mg/g) and for some fruits, such as pineapple (0.85 mg/g), banana (2.16 mg/g), orange (1.14 mg/g), papaya (0.15 mg/g) and mango (1.10 mg/g) [33]. Ethanolic TPC values however are in the range reported by Suganyadevi et al. [30] (64.0-164.0 mg/g/GAE). From the analysis of variance, statistically significant differences were observed among the 14 breeding liness of cassava in terms of TPCs in both the ethanolic and aqueous extracts. Many studies have reported that environmental, climatic, or geographic factors as well as extraction techniques may significantly influence the quality as well as the quantity of phenolic components [34-36].

3.3 Total Antioxidant Activity in Leaves of Cassava Breeding Lines

Presented in Table 3 is the total antioxidant activity (TAA) of leaves of the 14 breeding lines of cassava in the ethanolic and aqueous extracts. Larbi exhibited the highest scavenging activity (82.88±3.07%) in the ethanolic extract while Hyb-17 (48.95±0.22%) recorded the least. On the other hand, the highest scavenging activity in the aqueous extract was recorded in Hyb-15 (80.92±2.79%) which was not statistically different from values recorded by Larbi, Hyb-2, Hyb-9 and Hyb-14. Security recorded the least TTA (45.78±1.32%) in aqueous extract. Means of the TAA in the leaves differed from each other significantly in both extraction states. In a similar study, Faezah et al. [28] and Simao et al. [29] reported TAA within the ranges 44-67% and 79-94%, respectively in the leaves of some cassava varieties. Abdelhady et al. [37] have also reported TAA in leaves of some *Callistemon* species to vary between 49-91%. Results reported in this study are similar to those reported by these earlier researchers.

Phenolic compounds of plants are very important because their hydroxyl groups confer scavenging ability. Phenolic compounds of plants fall into several categories; chief among these are the flavonoids which have potent antioxidant activities [38]. Flavonoids are naturally-occurring in plants and have positive effects on human health through a wide range of antibacterial, antiviral, anti-inflammatory, anticancer, and anti-allergic activities of their derivatives [39,40]. Flavonoids have been shown to be highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various free radicals implicated in several diseases [37].

Table 3. Total antioxidant activity (TAA) in ethanol and aqueous extracts of leaves of cassava breeding lines

Breeding line	Inhibition (%)	
	Ethanol extract	Aqueous extract
Afisiafi	64.63±0.22^{fg}	52.29±0.36^{cd}
Asare	62.60±0.27^{g}	56.36±1.71^{b}
BS-1	59.44±0.20^{h}	50.53±1.19^{cde}
Larbi	**82.88±3.07^{a}**	79.57±0.85^{a}
Security	68.43±0.70^{de}	**45.78±1.32^{e}**
Hyb-2	72.28±0.09^{bc}	78.37±0.28^{a}
Hyb-8	74.31±0.11^{b}	50.11±0.62^{de}
Hyb-9	71.77±0.38^{bc}	78.01±1.91^{a}
Hyb-13	67.22±0.03^{ef}	68.56±2.19^{b}
Hyb-14	67.97±1.07^{de}	80.48±0.26^{a}
Hyb-15	71.64±1.20^{bc}	**80.92±2.79^{a}**
Hyb-17	**48.95±0.22^{j}**	52.18±5.19^{cd}
Hyb-24	53.81±0.24^{i}	47.34±0.69^{de}
Hyb-35	70.22±1.15^{cd}	69.34±2.34^{b}
Mean	**66.87**	**63.56**

±SE=standard error, means with same letters in a column are not statistically different (p≥0.05) from each other according to Duncan's multiple range test. Values bolded and underlined refer to accessions with the highest concentration; Bolded values represent accessions with the lowest concentration

In general, the results of this study indicate clearly that ethanolic extracts gave significantly higher TPC, TFC and TAA values compared with water extracts for most of the lines investigated. This can be attributed to the fact that ethanol as an organic solvent was able to denature polyphenol oxidases and was more efficient in degrading cell wall, thereby enhancing extraction of more endocellular materials compared to water [41]. Similar reports have been made by Ao et al. [42].

3.4 Association between Total Flavonoid, Total Phenolics and Total Antioxidant Scavenging Activity in Leaves of Cassava Breeding Lines

Table 4 shows the association between pairs of total flavonoid content, phenolics and total antioxidant scavenging activity in the leaves of the cassava breeding lines following ethanolic and aqueous extractions. Total flavonoid content (TFC) showed a strong and positive correlation

Table 4. Correlation coefficients between total flavonoid, total phenolics and free radical scavenging activity in leaves of cassava genotypes

Parameter	TFC		TPC		TAA	
	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous
TFC						
Ethanolic	1					
Aqueous		1				
TPC						
Ethanolic	0.681 (0.00)		1			
Aqueous		0.831 (0.000)		1		
TAA						
Ethanolic	**-0.014 (0.933)**		0.625 (0.000)		1	
Aqueous		0.617 (0.00)		0.704 (0.000)		1

with total phenolic content (TPC), but negatively correlated with total antioxidant scavenging activity in the ethanolic extract. TFC was also positive and strongly correlated with TPC and TAA in the aqueous extract. Similarly, TPC was positive and strongly correlated with TAA in both ethanolic and aqueous extracts. Several researchers found strong positive correlation between TPC and TAA for different plants [43,44,45]. However, some studies found no correlation between scavenging activity and TPC [46,47].

The significant positive correlation shown between total flavonoid and phenolic compounds indicates that an increase in total flavonoids corresponds an increase in total phenolics. Similarly, results from the current study also imply that the higher the total phenolic content, the higher the anti-oxidant scavenging activity. Indeed, polyphenols are reported to be responsible for the TAA of plant extracts, usually showing a positive correlation [48].

The flavonoid family consists of six (6) sub-groups: flavonols, flavones, isoflavones, flavanols (catechin, gallocatechin), flavanones and flavanonols [41,49] and these react differently to different organic solvents. Results indicate that in the ethanolic extract, antioxidant activity in the leaves analysed did not depend on the flavonoid content but rather on the phenolic content. In the aqueous extracts however, the results seek to suggest that flavonoids and phenolic compounds may be the major contributors for the antioxidant activity of the leaves of the cassava breeding lines investigated. These results are in consonance with findings of other workers using extracts of other plant products [50,51].

4. CONCLUSION

High levels of Total Flavonoids and Total Phenolics were found in the both ethanolic and aqueous extracts of the leaves of the 14 breeding lines of cassava. Total flavonoid content (TFC) was in the ranges 103.6±0.10 - 179.9±0.21 mg/g/QE and 55.9±0.01 and 96.7±0.03 mg/g/QE, respectively for ethanolic and aqueous extracts. Similarly, Total phenolic content (TPC) fell within the ranges 62.12±0.95 – 128.25±0.60 mg/g/GAE and 39.07±0.73 - 95.33±3.61 mg/gGAE for ethanolic and aqueous extracts, respectively. Hyb-9 gave the highest TFC in ethanolic extract of 179.9±0.21 mg/g/QE while Security (a parental line) gave the highest TFC in aqueous extract of 96.70.03 mg/g/GAE.

For TPC, Hyb-9 gave the highest values (128.25±0.60 mg/g/QE and 95.33±3.61 mg/g/QE) for both ethanolic and aqueous extracts. Total antioxidant activity (TAA) was in the ranges 48.95±0.22% – 82.88±3.07% and 45.78±1.32% - 80.92±2.79% for ethanolic and aqueous extracts, respectively.

Breeding lines Larbi (82.88%) and Hyb-15 (80.92%) emerged as fore-runners with respect to TAA following extraction in ethanol and water, respectively. There was high variability among all 14 cassava breeding lines used for the study with respect to total flavonoids, phenolics and antioxidant activities. Ethanol as an extraction solvent, yielded higher mean total flavonoids, phenolics and antioxidant activity than water. In

the aqueous extracts, there were highly positive interactions among TFC, TPC and TAA.

The four breeding lines Hyb-9, Security, Larbi and Hyb-15 may be employed in any future breeding work aimed at improvement of phytochemical constituents of cassava leaves.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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