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Intra-Specific Flavonoid Classes, Content and Relationships in African Yam Bean (*Sphenostylis stenocarpa* [Hochst ex A. Richmond] Harms] Tubers

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ABSTRACT

Background and Objective: Tubers of African yam bean [*Sphenostylis stenocarpa* (Hochst. ex A. Richmond) Harms], family Fabaceae, are under-exploited, especially in West Africa by humans. The tuber holds varying quantitative bioactive properties. The present study seeks to unveil inherent flavonoid compounds in different accessions of African yam bean (AYB) tubers. **Materials and Methods:** Fresh tubers were obtained from 17 accessions of AYB. The same was dried and flour samples were prepared. Flour samples from the 17 accessions were analyzed and 38 flavonoid compounds were obtained for each using High-Performance Liquid Chromatography (HPLC). **Results:** The 17 accessions differed in the proportion of the 38 flavonoid compounds. Flavonoid compounds with >1.0 g/100 g in the study were: Apigenin, didymin, daidzein, luteolin and isorharmmetic. The 38 flavonoid compounds were grouped into six sub-classes, 55% were flavones and anthocyanidin which had anthocyanin as the only member in the group, had the lowest (1%) component in the tubers of AYB. **Conclusion:** Variation in flavonoid content in AYB tuber is a function of the genotypes. Mean content is higher than most tuberous crops. The AYB tuber therefore promises to be an important industrial product for health benefits and promotion.

KEYWORDS

African yam bean, flavonoid, health benefits, nutraceutical, tubers, bioactive compounds, high-performance liquid chromatography

INTRODUCTION

Sphenostylis stenocarpa (Hochst. ex A. Rich) Harms, African yam bean (AYB) is one of the foremost indigenous tropical Africa tuberous legume[1]. This crop possesses dual edible economic products-the aerial pulse and the subterranean root tuber. Though the species is underutilized, consumption of the pulse is more popular especially, in Nigeria and West Africa[2]. Much earlier reports by Adewale *et al.*[3] and Popoola *et al.*[4] revealed outstanding food and nutritional values of AYB tubers above some common tuberous crops such as: Cassava, sweet and Irish potato. Tuberous crops are extremely important sources of carbohydrates in tropical and sub-tropical countries providing food for the ever-increasing population

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over the years[5,6]. Since AYB tubers have not been demonstrated to be equivalent to these major tuberous crops as a recipe in common meals, identifying their alternative uses rather than food would be worthwhile.

Flavonoids represent a typical secondary metabolite class of phenolic compounds generally found as glycosides in plant tissues. Furthermore, they are active nutraceutical ingredients constituting the largest group of plant phenolics[7,8]. The photosynthetic cells of the plant hosts wide varieties of flavonoid compounds[9] containing two to three aromatic C_6 rings bearing at least one hydroxyl group[10]. Flavanones, flavones, flavanols, isoflavone, flavan-3-ol and anthocyanidins are the notable sub-classes of flavonoids reported in literature[9-12]. However, Ferreyra *et al.*[13] further identified three more sub-classes, which are: Aurone, chalcone and proanthocyanidin. Major differences among the three depend on substitution patterns of the aromatic rings in their different structures[8,14]. According to Middleton[15], variation among flavonoid classes depend on oxidation and substitution pattern of the C ring and individual compounds in a class base on the substitution pattern of the A and B ring, this make them have diverse biological functions. The number of flavonoid compounds reported in the literature is staggered: 4000[15], more than 6000[16] and about 10,000[17], the number will continue to increase at more discoveries in the future.

Usefulness of flavonoids include: Possession of antioxidative activity, hepatoprotective, anti-inflammatory and anticancer activities, free radical scavenging capacity, coronary heart disease prevention, antiviral activities, combating oxidative stress and act as growth regulators[9]. Moreover, flavonoids prevent initiation and progression of atherosclerosis and cardiovascular disease[18]. Ferreyra *et al.*[13] opined that it is likely the most important mediator of plant-insect interactions.

Tubers (yam, cassava, irish and sweet potatoes) have an immense functional and nutraceutical ingredient such as saponins, phenolic, glycoalkaloids and carotenoids compounds which are exploitable for disease risk reduction and wellness[19]. The nutritional and health benefits of most of them have not been explored. Although, Ojuederi *et al.*[20] and Konyeme *et al.*[21] have attempted to unravel phytochemical compositions in tubers of AYB. The potential of natural antioxidants is rising considerably in the scientific domain[22], since every part of the plant hosts bioactive compounds in varying proportions[12-24], investigation of the flavonoid types and composition in the tuberous roots of AYB, therefore, became an interest for investigation in this study, especially because the utilization of the tuberous root of AYB for food in Nigeria is very rare.

The present study hypothesized possible identification of the quantitative resources of types of flavonoids and their sub-classes in the tuberous roots of AYB. The discovery could elucidate alternative uses to enhance awareness and productive utilization of the economic portion of the crop.

MATERIALS AND METHODS

Fifty-five African yam bean accessions for the experiment were obtained from the genetic resource centre (GRC), International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Field experiment was conducted from March to October, 2019, at the Teaching and Research Farm, Faculty of Agriculture, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria located on Latitude 4°5N and Longitude 6°5513E with an elevation of approximately 20 m above sea level. The experimental field was ploughed, harrowed and mini-mounds were made. Seeds meant for planting were treated in Macozeb (a fungicide) solution before sowing. The experiment was laid out in randomized complete block design with three replications. Planting was done at 1 m inter and intra row spacing. Two seeds were initially sown and thinning was done at two weeks after planting to reduce plant per hill to one. Seedlings were staked at three weeks after planting. Manual weeding was employed to keep the field free from weeds. At harvest, only seventeen of the accessions produced tubers (Table 1).

Table 1: Passport data of the seventeen accessions used for this study

List of accessions	Country	Name of collector
TSs 49	Nigeria	R.J Williams
TSs 57	Nigeria	L. Igbokwe
TSs 119A	NA	NA
TSs 49A	NA	NA
TSs_2015_06	NA	NA
TSs 58	Nigeria	L. Igbokwe
AYB 119A	NA	NA
TSs 10	Nigeria	C.N Aniagu
TSs 66	Bangladesh	Dr. N. Haq
TSs 6A	Nigeria	NA
AYB 44C	NA	NA
TSs 98	Nigeria	NA
TSs 84A	NA	NA
TSs 109	Nigeria	James A. Sinnar
TSs 101	Nigeria	NA
TSs 158	Nigeria	DR J. Machuka
AYB30	NA	NA

NA: Not Applicable

Preparation of plant material for quantitative determination of flavonoid: Selected tubers of the 17 AYB accessions were washed with clean water, carefully peeled and sliced with a knife and dried separately in the oven (TD-384KN model Thermotec, Tokyo, Japan) at 65°C for 48 hrs. Dried sample were milled using an electric stainless blender (Braun Multiuick Immersion Hand Blender, B White Mixer MR 5550CA, Germany).

Flavonoid analysis was done using HPLC. Flour of each accession was weighed (1.5 g) into a set of extraction tube(s) and 20 mL of boiled ultra-pure water dispensed into each extraction tubes. The setup was allowed to stand for 1.5 hrs and vortexed for 5 min. The solution was transferred to a set of centrifuge tubes, shaken for 15 min and centrifuged for 5 min at 3000 rpm. Thereafter, a set of vials were used to collect the supernatants for determination on water 616/626 HPLC. The conditions for the analysis of flavonoids were as follows:

- S23 Autosampler for AA, ICP-OES, ICP-MS, UV/Vis and FL
- An automated gradient controller
- Gradient elution HPLC pump
- Reverse-phase HPLC column, thermostatically heated in a temperature-controlled room
- Fluorescence Detector RF-20A/RF-20Axs
- Carrier gas: Nitrogen gas at flow rate of 60 mL/min
- Temperature: Detector- 147°C; Injector port- 166°C and Column: 115°C
- Computer facilities for storing data
- HP LaserJet Tank MFP 2602sdn Printer (2R7F6A) for results reporting

Statistical analysis: The generated data were subjected to statistical analysis in SAS (version 9.4, 2011). Analysis conducted include: Basic descriptive statistics and Pearson correlation. Significance of the correlation coefficients was tested at $p \leq 0.05$. Other results were presented in graphs as the mean values of the different parameters from the 17 AYB in MS Excel (version 2013) using the chart wizard option.

RESULTS

The proportion of the total flavonoid in the 17 AYB accessions is presented in Fig. 1. The mean of the total flavonoid in the 17 accessions was 10.12 g/100 g. The recorded highest (14 g/100 g) and least (6.5 g/100 g) total flavonoids were in the tubers of TSs 57 and TSs101, respectively (Fig. 1).

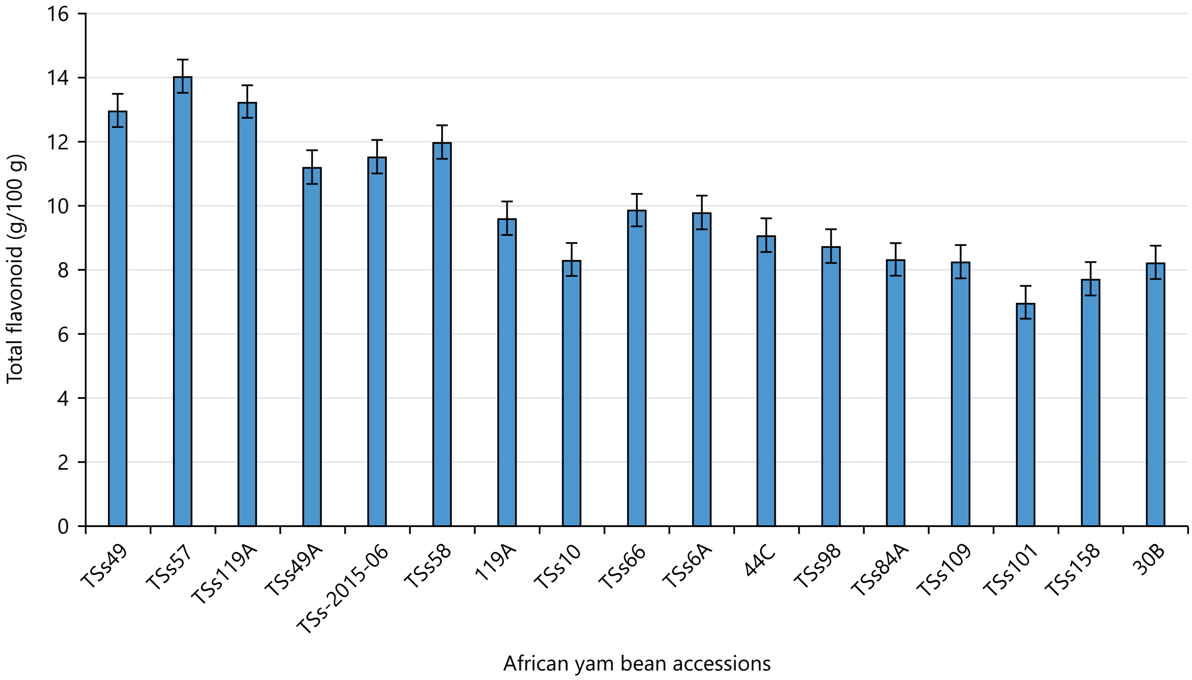

Fig. 1: Variation in the total flavonoid content of tubers of 17 African yam bean accessions

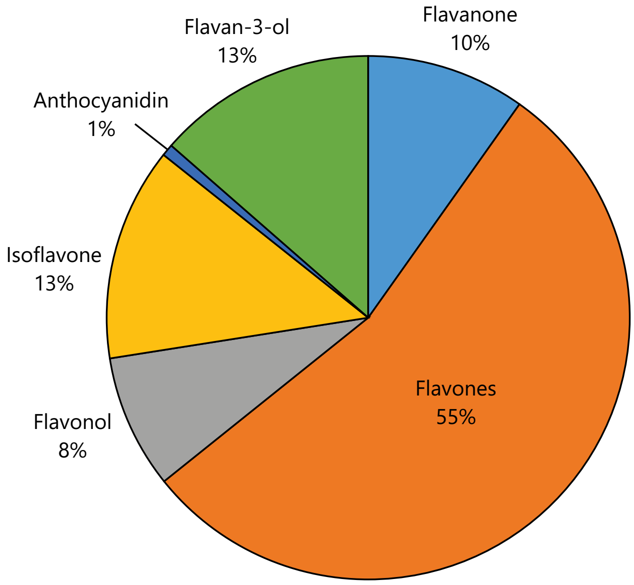

Fig. 2: Proportion of the different sub-classes of flavonoid in African yam bean tubers

In this study, six sub-classes of flavonoids were identified in the tubers of the 17 AYB accessions. The proportion of the six sub-classes in the total flavonoid varied significantly (Fig. 2). In the studied tubers of the 17 AYB, the proportion of the sub-classes in the total flavonoid was revealed as: Flavones (55%), flavan-3-ol and Isoflavone had an equal proportion of 13%, flavanone (10%), flavonol (8%) and anthocyanidin had the least (1%).

Each sub-class of flavonoid had different numbers of flavonoid compounds. However, in total, 38 flavonoid compounds were identified in the tubers of AYB (Table 2). Flavanones and flavones had 11 each, flavonols had five, isoflavones had three, anthocyanidine had just one and flavan-3-ol seven. The range for each of the sub-classes as shown in Table 2 are as follows: Flavanone (0.001-0.821), flavone (0.003-2.393), flavonol (0.002-1.37), isoflavone (0.004-2.232) and flavan-3-ol (0.008-1.194).

Table 2: Mean of flavonoid compounds and their sub-classes in tubers of 17 African yam bean accessions[9,12,13,22,25,26]

Sub-classes	Compounds	Mean (g/100 g)±SE	Minimum (g/100 g)	Maximum (g/100 g)
Flavanone	Hesperidin	0.0024±0.0002	0.001	0.004
	Nanirutin	0.1712±0.0134	0.083	0.296
	Neoriocitin	0.0157±0.0012	0.008	0.027
	Poncirin	0.0093±0.0007	0.005	0.016
	Eriocitrin	0.0098±0.0003	0.008	0.013
	Raxifolin	0.0476±0.0015	0.037	0.062
	Naringin	0.6305±0.0198	0.494	0.821
	Naringinenin	0.0252±0.0008	0.02	0.033
	Eriodictyol	0.0063±0.0002	0.005	0.008
	Taxifolin	0.0158±0.0012	0.01	0.026
	Hesperetin	0.0144±0.0011	0.009	0.024
Flavones	Didymin	1.8057±0.0568	1.414	2.353
	Rhoifolin	0.0098±0.0003	0.008	0.013
	Diosmin	0.0524±0.0016	0.041	0.068
	Nobiletin	0.9655±0.0303	0.756	1.258
	Acacetin	0.0139±0.0004	0.011	0.018
	Sinerisetrin	0.1161±0.0036	0.091	0.151
	Tangeretin	0.0477±0.0015	0.037	0.062
	Neodiosmin	0.0169±0.0006	0.013	0.022
	Apigenin	1.3844±0.1084	0.675	2.393
	Luteolin	1.0082±0.079	0.491	1.743
	Rhamnazin	0.0047±0.0004	0.003	0.008
Flavonols	Quercetin	0.0193±0.0006	0.015	0.025
	Myricetin	0.0215±0.0007	0.017	0.028
	Kaempferol	0.0076±0.0006	0.004	0.013
	Isorharmnetic	0.7927±0.0621	0.3863	1.37
	Fisetin	0.0135±0.0011	0.002	0.021
Isoflavones	Daidzein	1.3386±0.1031	0.8095	2.232
	Genistein	0.0071±0.0006	0.004	0.012
	Glycitein	0.0074±0.0006	0.004	0.012
Anthocyanidin	Anthocyanine	0.0388±0.003	0.024	0.065
Flavan-3-ol	Catechin	0.7159±0.0552	0.433	1.194
	Epicatechin	0.0126±0.001	0.008	0.021
	Theaflarins	0.0352±0.0027	0.021	0.059
	Thearubigins	0.0859±0.0066	0.052	0.143
	Epigallocatechin	0.0354±0.0027	0.021	0.059
	Epigallocatechin 3 gallate	0.4675±0.0361	0.283	0.78
	Proanthocyanidins	0.0185±0.0014	0.011	0.031

Among the seven listed medicinal plants in Table 3, the flavonoid of *Catharanthus roseus* was the highest. However, among the tuberous crops, water yam had the highest value of 410 mg/100 g, ten times higher than the content in the highest medicinal plant (Table 3). The mean value of flavonoid in the present study was from 17 accessions and it was more than twice the flavonoid contained in water yam (Table 3).

In Table 4, 55 correlation coefficients were generated from Pearson correlation among 11 flavanone compositions. Thirty-three were positive and significant ($p \leq 0.001$) with the range of 0.620 (hesperetin and raxifolin) and 0.999 (raxifolin and naringin).

Out of the total 55 correlation coefficients for flavone, 38 were positive and highly significant ($p \leq 0.05$). The least positively significant correlation coefficient (r = 0.602) was between tangeretin and rhamnazin. Didymin and nobiletin and apigenin and luteolin had a perfect unity (r = 1.000) correlation coefficient (Table 5).

Table 3: Flavanoid contents of some medicinal and tuberous crops as documented in the literature in comparison with African yam bean in the present study

Plants	Total flavanoid content (mg/100 g)	References
Medicinal plants		
Piper betel	39.84±0.15	Kaur and Mondal[24]
Asparagus racemosus	15.94±0.10	
Catharanthus roseus	41.68±0.25	
Citrus aurantifolia	39.03±0.20	
Cassia fistula	38.15±0.19	
Ocimum sanctum	20.50±0.21	
Polyalthia longifolia	27.11±0.30	
Common tuberous crops		
Water yam (*Dioscorea alata*)	410.52±20.22	Dolkar *et al.*[27] and Dilworth *et al.*[28]
Coco yam (*Xanthosoma* sp.)	145.31±5.61	
Sweet potatoes (*Ipomoea batatas*)	165.34±5.81	
Potatoes (*Solanum tuberosum*)	85.21±4.32	
Yellow yam (*Dioscorea cayenensis*)	150.67±30.34	
†*Sphenostylis stenocarpa*	1000.12±0.52	

†: Reference species, ±: A notation to depict standard error

Table 4: Correlation coefficient among flavonoid composition in flavanone sub-class

	Hesperin	Naniru	Neorio	Poncirin	Eriocitrin	Raxifolin	Naring	Naringne	Eridicty	Taxifolin
Naniru	0.946***									
Neorio	0.946***	0.998***								
Poncirin	0.936***	0.995***	0.992***							
Eriocitrin	0.453	0.336	0.357	0.346						
Raxifolin	0.36	0.26	0.284	0.268	0.981***					
Naring	0.371	0.269	0.292	0.277	0.985***	0.999***				
Naringne	0.398	0.29	0.312	0.299	0.987***	0.996***	0.997***			
Eridicty	0.209	0.093	0.113	0.108	0.915***	0.937***	0.935***	0.930***		
Taxifolin	0.709**	0.739***	0.756***	0.751***	0.664**	0.633**	0.645**	0.641**	0.445	
Hespetin	0.688**	0.715**	0.733***	0.728***	0.650**	0.620**	0.632**	0.625**	0.435	0.996***

,*Significance at p≤0.01 and p≤0.001, Hesperin: Hesperidin, Naniru: Nanirutin, Neorio: Neoriocitin, Naring: Naringin, Naringne: Naringinenin, Eridicty: Eriodictyo and Hespetin: Hesperetin

Table 5: Correlation coefficient among flavonoid composition in flavone sub-class

	Didymin	Rhoifolin	Diosmin	Nobiletin	Acacetin	Sineris	Tanger	Neodiosm	Apigenin	Luteolin
Rhoifolin	0.983***									
Diosmin	0.999***	0.984***								
Nobiletin	1.000***	0.983***	0.999***							
Acacetin	0.986***	0.966***	0.982***	0.986***						
Sineris	0.999***	0.983***	0.999***	0.999***	0.986***					
Tanger	0.999***	0.980***	0.997***	0.999***	0.985***	0.998***				
Neodiosm	0.992***	0.969***	0.990***	0.992***	0.981***	0.992***	0.992***			
Apigenin	0.268	0.324	0.262	0.268	0.311	0.274	0.252	0.279		
Luteolin	0.268	0.324	0.262	0.268	0.311	0.274	0.252	0.279	1.000***	
Rhamn	0.616**	0.641**	0.610**	0.616**	0.648**	0.621**	0.602*	0.649**	0.710**	0.710**

,*Significance at p≤0.01 and p≤0.001, Sineris: Sinerisetrin, Tanger: Tangeretin and Rhamn: Rhamnazin

Table 6: Correlation coefficient among flavonoid composition in flavonol sub-class

	Quercetin	Myricet	Kampfer	Isorharm
Myricet	0.994***			
Kampfer	0.317	0.344		
Isorharm	0.261	0.293	0.993***	
Fisetin	0.192	0.215	0.324	0.338

***Significance at p≤0.001, Myricet: Myricetin, Kampfer: Kaempferol and Isorharm: Isorharmnetic

Pairing of the five compositions of flavonol produced 10 correlation coefficients (Table 6). Only the correlation between two pairs (myricetin and quercetin) and (isorharmnetic and kaempferol) were positive and highly significant ($p\leq 0.001$). The three components of Isoflavone gave three correlation coefficients which were positively significant at $p\leq 0.001$ (Table 7).

Table 7: Correlation coefficient among flavonoid composition in isoflavone sub-class

	Daidz	Genist
Genist	0.991***	
Glycite	0.989***	0.982***

***Significance at p≤0.001, Daidz: Daidzein, Genist: Genistein and Glycite: Glycitein

Table 8: Correlation coefficient among flavonoid composition in flavan-3-ol sub-class

	Catin	Epicat	Theafl	Thearu	Egallocat	Egalocatlate
Epicat	0.995***					
Theafl	0.999***	0.994***				
Thearu	0.999***	0.995***	0.999***			
Egallocat	0.999***	0.996***	0.999***	0.999***		
Egalocatlate	1.000***	0.995***	0.999***	0.999***	0.999***	
Proantho	0.998***	0.995***	0.998***	0.998***	0.998***	0.998***

***Significance at p≤0.001, Catin: Catechin, Epicat: Epicatechin, Theafl: Theaflarins, Egallocat: Epigallocatechin, Egalocatlate: Epigallocatechin gallate and Proantho: Proanthocyanidins

In Table 8, the six components in the flavan-3-ol sub-class had 21 correlation coefficients. Each of the pairs exhibited a positive and highly significant correlation (p≤0.001). In the sub-class, catechin and epigallocatechin gallate had perfect unit (1.00) correlation coefficient.

DISCUSSION

The present study seeks to unravel possible utilities inherent in the tubers of African yam bean rather than food. The total flavonoid content it holds, though varied significantly among the studied accessions is huge, comprising many classes and holding promises for medicinal purposes. The quantity of phenolic compounds present in a given species of plant material varies with a number of factors such as cultivar, environmental conditions, cultural practices, postharvest practices, processing conditions and storage[19]. The proportions of the different flavonoids, their classes and intra-class correlation in the tubers of African yam beans were observed. Flavones were most predominant in African yam bean tubers while the proportion of anthocyanidin was very insignificant and least.

Dolkar *et al.*[27] reported that total phenolic compound and total antioxidant compound equally depend on specific plant genotype, the environment (cultivation condition) and their interaction. The range of 6.5 to 14 g/100 g is wide and it is a reflection of the possibility of wide variation in the content of total flavonoid in germplasm of African yam bean. Many authors[9-12,19] have remarked that variation in flavonoid content is dependent on many factors, cultivar type is one of such factors. The present result revealed differences in genetic resources as a factor for total flavonoid content in the tubers of African yam beans. This result was agreed with those of Haytowitz *et al.*[23] which revealed that the different occurrence of bioactive compounds e.g., flavonoids vary in different food crops. Anthocyanidin was reported by Panche *et al.*[12] to be most predominant in the vegetative portion of plants such as cranberries, red grapes, strawberries, blackberries etc. and its usefulness was most influential in fruit colors. Peeled African yam bean tubers are usually white-fleshed, justifying the low proportion of the colour-determining flavonoid in the present research.

The presence of a non-negative correlation within each of the groups of flavonoids informs that each substituent within each of the sub-classes shares relative similarities for chemical structures. Each sub-class is determined by basic chemical structure and characteristics that are unique for each group of compounds[23]. The positive status of all components in each sub-class is a reflection of harmony in the chemical structure for all components within each sub-class. Moreover, the proportion of agreements of all components within each sub-class is flavon-3-ol (100%), isoflavone (100%), flavone (69%), flavonone (64%) and flavonol (20%). The proportion of each sub-class of flavonoid varies in species and different parts of a plant. In African yam bean based on this study, flavone has the highest proportion of the total flavonoid.

From this study, the comparison of African yam bean with other tubers revealed that the content of flavonoid was about three times higher than that in water yam (*Dioscorea alata*) which had been recorded as the tuber with the highest value for total flavonoid content[28]. This highlights the possibility of the usefulness of the African yam bean as a source of raw material for the production of flavonoids on an industrial scale. Moreover and in corroboration with the report of Ojuederie *et al.*[20] and Adewale and Nnamani[29], the consumption of African yam bean tuber is safe and health-promoting. Furthermore, the intake of flavonol and isoflavonoid (major flavonoid classes discovered in African yam bean in this study) according to Panche *et al.*[12] has the potential to combat a number of diseases, especially vascular disease.

The present study considered less than 20 accessions of African yam bean, the use of more genetic resources may be more revealing in terms of type, classes and content of the flavonoid phenolic groups. It would be much more novel to harness larger genetic resources for the diversity study of flavonoids in the tubers of African yam beans.

CONCLUSION

The high flavonoid content in the tubers of African yam beans with flavone as the predominant class has confirmed that African yam bean has nutraceutical properties. Since its utilization is of poor relevance in human food especially in West Africa, tapping into its tuber production solely as an industrial product could save it from further wastages. Production of the high valued flavonoid in the tuber could rescue the crop product, its utilization could also improve human health.

SIGNIFICANCE STATEMENT

The present study seems to be the first investigation into the variability in the content of flavonoids in the tubers of African yam beans. The investigation was necessary because of the non-use and neglect of the tuber of African yam bean as food, especially in Nigeria and West Africa. To prevent its further neglect, the present study was carried out to unveil the industrial potential of the tuber. The present discovery could lead to the production and commercialization of the crop for industrial uses.

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