COMPARATIVE *IN VITRO* ANTIOXIDANT ACTIVITIES OF SIX ACCESSIONS OF AFRICAN YAM BEANS (*SPHENOSTYLIS STENOCARPA* L.)

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Abstract
African yam bean (AYB) is an underutilized legume with numerous food and medicinal properties, which are yet to be fully exploited for the benefit of human and animals. The effects of extraction medium on the total phenolic components and antioxidant activity of six accessions of Africa Yam Beans were investigated. The different accessions of AYB used were TSS-10, TSS-57, TSS-84, TSS-95, TSS-96 and TSS-111. Antioxidant activities were determined using 2, 2-diphenyl -1- picrylhydrazyl (DPPH) scavenging effect, reducing power, total antioxidant capacity and antioxidant-related Phytochemical: total phenolics (TPC) and total flavonoid content (TFC) using standard methods. Phytochemical screening showed the presence of different photochemical like alkaloids, cardenolides, saponins and flavonoid at different levels in the AYB accessions. The results showed that 70% acetone exhibited higher TPC and TFC yield than the aqueous extract. TSS-10 had the highest TPC, DPPH scavenging and reducing power while TSS-96 and TSS-57 had the highest TFC and TAC respectively for aqueous extracts. For the 70% acetone, TSS-84 had the highest TPC, TFC and reducing power while TSS-10 and TSS-96 had the highest DPPH and TAC respectively. The results obtained in this study indicate that AYB is rich in antioxidants and can serve as nutraceutical. In addition, solvents of extraction had significant effects on TPC, TFC and antioxidant activities.

***Key words*:** African yam beans, Antioxidant activity, Phenolics and Flavonoid

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1. INTRODUCTION

Antioxidants are substances that scavenge or slow down the activities of ROS and RNS which inhibit oxidative mechanisms that lead to chronic and degenerative diseases (Cardador-Martinez *et al*., 2002; Olaiya *et al*., 2016). Recently, the use of antioxidants has been encouraged due to the implication of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in virtually all diseases. Phytochemicals such as phenol and flavonoids which are widely present in foods have been shown to possess anti-oxidative power thereby making food the natural source of these antioxidants.

Legumes are prominent sources of proteins and are rich in essential amino acids, lysine and tryptophan. They are cost-effective because they are cheaper than animal products (Oboh, 2006). They are the next important food crop after cereals (Uzoechina, 2009) and an important source of affordable protein especially to people with low income in many tropical countries where it is predominantly eaten (Ihekoronye and Ngoddy, 1995). Apart from their nutritional value, legumes are also functional foods in that they promote good health and have therapeutic properties (Geil and Anderson, 1994; Xu *et al*., 2007)**.**

The African yam beans *(Sphenostylis stenocarpa* L.) is one of the underutilized legumes of tropical origin and underutilized legumes contribute to food security in a significant way (Adebowale *et al*., 2009). Underutilized legumes increase the demand for plant protein in lieu of expensive animal protein (Adebowale *et al*., 2009). The different accessions of African yam beans are shown in Figure 1. AYB ranks well among neglected crops and can contribute to food security if its genetic resources are saved for utilization in

breeding and improvement (Adewale *et al*., 2012).The seed and tuber of AYB contain different food fractions and minerals that are comparable to other food legumes. The seed is a highly priced food legume in south eastern Nigeria (Asoiro and Ani, 2011), owing to high crude protein content. In Nigeria, AYB has an enduring reputation in the food and culture of the Igbos and the Yorubas. It is a very significant substitute for cowpea (*Vigna unguiculata*) in the rainforest belt of Nigeria (Okpara and Omaliko, 1995). The Igbos extensively explored the crop as a good source of dietary protein in feeding the displaced and the severely malnourished refugees during the Nigerian civil war of 1967–1970 (Nwokolo, 1996).

There is a dearth of information on the antioxidant activity of these accessions of *Sphenostylis stenocarpa* L. (AYB). Therefore, the aim of this study is to evaluate the antioxidant ability of aqueous and 70% acetone extracts of six accessions of *Sphenostylis stenocarpa L.*

Figure 1: The six accessions of African yam bean (*Sphenostylis stenocarpa* L.)
Source: Soetan, 2017

2.MATERIALS AND METHODS

Sample Materials

The six accessions of (AYB) (*Sphenostylis stenocarpa L*) namely TSS-10, TSS-57, TSS-84, TSS-95, TSS-96 and TSS-111 used in this work were supplied by the Genetic Resources Centre (GRC) of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria.

Sample Preparation

Dried AYB seeds were ground into powdery form with Blender/Miller III Model MS-223 Taiwan, China. Two extracts were prepared from 1g each of the powdered sample (aqueous and 70% acetone). 1g of each legume accessions were soaked differently in 10ml extraction solvent. The solution was left overnight for 12 hours and centrifuged at 3000 rpm for 10 minutes. The supernatant was stored in the refrigerator and later used for the analysis.

Phytochemical Screening

Phytochemical analyses on the aqueous extracts of the powdered samples of African Yam Bean (*Sphenostylis stenocarpa L*) were carried out using standard procedures to identify the phytochemical constituents as described by (Harborne, 1973; Trease and Evans, 1989; Sofowora, 1993).

Antioxidant Assays

DPPH radical scavenging activity

DPPH (2, 2-diphenyl -1- picrylhydrazyl) radical scavenging activity of each AYB accessions was estimated according to the method of (Gyamfi *et al*., 1999). 1.0ml (100µg/ml – 400µg/ml) was added to 4.0 ml of DPPH (25mg/l). The samples were mixed thoroughly and allowed to stand in a dark room for 30 minutes and absorbance was read at 520 nm. Percentage inhibition of DPPH scavenging ability was calculated using:

Percentage inhibition of
DPPH = (Absorbance of control-Absorbance of sample) / (Absorbance of control) x 100

DPPH solution without sample served as control.

Total phenolic content

The total phenolic content of each AYB accessions was determined by spectrophotometric method (Kim *et al*., 2003). 1.0ml of the samples was mixed with 1 ml (10%) of Folin-Ciocalteu phenol reagent. After 5 minutes, 5 ml of 7% Na_2CO_3 solution was added to the mixture followed by the addition of 5ml of distilled water. This was mixed thoroughly. The mixture was kept in the dark

for 90 minutes at 25^0C after which the absorbance was read at 750 nm. The total phenolic content was evaluated from a Gallic acid standard curve and expressed as Gallic acid equivalent (GAE).

Total flavonoid content

The total flavonoid content of each AYB accessions was determined using the method of Park *et al*. (1999). 1.0ml of the sample was mixed with 3.4ml (30%) of methanol, 0.15ml (0.5M) of $NaNO_2$ and 0.15ml (0.3M) of $AlCl_36H_20$. After 5 minutes, 1ml of 1M NaOH was added. The absorbance was read at 506nm and determined from quercetin calibration curve and expressed as quercetin equivalent (QUE).

The reducing power

The reducing power of each AYB accessions was determined according to the method of Oyaizu (1986). 1.0ml (100µg/ml -500µg/ml) of sample was mixed with 1.0ml phosphate buffer (0.2 M, pH 6.6) and 0.5 ml (0.1%) potassium ferricyanide followed by incubation at 50^0C for 20 minutes. After which 0.5 ml of 10% trichloroacetic acid was added to terminate the reaction. Upper portion of the solution (1 ml) was taken, mixed with 1 ml distilled water and 0.1 ml (0.01%) of Iron (III) chloride solution was added. The reaction mixture was allowed to stand 10 minutes at room temperature before the absorbance was read at 700 nm.

Total antioxidant capacity

The total antioxidant capacity (TAC) of each AYB accessions was determined by the method of Prieto *et al*. (1999). About 0.2ml of the sample was mixed with 2ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The mixtures were incubated in a water bath at 95^0C for 90 minutes. The samples were allowed to cool and absorbance was read at 765nm against a reagent blank. The TAC was calculated from ascorbic acid calibration curve and expressed as Ascorbic acid equivalent.

Statistical analysis

All data were given as the mean ± SD of three measurements. The significance of the differences between the means of the samples were established by analysis of variance, ANOVA ($p<0.05$) and charts were drawn with graph pad prism 5.

3. RESULTS AND DISCUSSIONS

Phytochemical Screening

The Phytochemical screening revealed the presence of alkaloids, flavonoids and saponins in all the six accessions of AYB. Cardenolides were present only in TSS-96 while anthraquinones and tannins were absent in all the accessions of AYB (Table 1).

The presence of some phytochemicals like alkaloids, flavonoids and saponins in all the accessions of AYB indicate that the AYB phytochemicals could be a rich source of materials for drug production in treating diseases.

Alkaloids play active roles in defending plants against herbivores and pathogens and are widely utilized as pharmaceuticals because of their biological activities (Madziga *et al*., 2010; Doughari, 2012). Flavonoids are widely distributed in plants and several reports confirm their use as antioxidants or free radical scavengers and as quenchers of singlet oxygen formation (Kar, 2007; Ghasemzadeh and Ghasemzadeh, 2011). Antioxidant-rich foods, including flavonoids, have been reported to inhibit LDL oxidation and thus prevent the formation of cell-to-cell adhesion factors which are implicated in the damage to the arterial endothelium and in formation of blood clots (Beretz and Cazenave, 1988). Saponins have been reported to exhibit antioxidant activities (Chan *et al.*, 2014; Chen *et al*., 2014; Soetan *et al*., 2015).

Phenols play important roles in defending plants against pathogens and herbivore predators and are thus applied in the control of human pathogenic infections (Doughari, 2012).

The medicinal properties of plants' secondary metabolites are very numerous and have been well documented (Banso and Adeyemo, 2007; Soetan, 2008 and Akpan *et al*., 2012).

Phenolics, when present in food, exert their health benefits mainly through their antioxidant capacity (Fang *et al*., 2002). These compounds can decrease oxygen concentration, scavenge

singlet oxygen and are powerful chain breaking antioxidants (Shahidi and Wansundeara, 1992).

TABLE 1: Phytochemical Screening of Six Accessions of African Yam Bean (*SphenostylisstenocarpaL*)

	Alkaloids	Tests	
AYB Accessions	Dragenduff's	Meyer	Wagner
TSS-10	++	++	++
TSS-57	+	+	+
TSS-84	+	+	+
TSS-95	+	+	+
TSS-96	+	+	+
TSS-111	++	++	++
	Cardenolides	Tests	
	Keller-Killiani	Kedde	
TSS-10	-	-	
TSS-57	-	-	
TSS-84	-	-	
TSS-95	-	-	
TSS-96	+	+	
TSS-111	-	-	
	Anthraquinones		
	Chloroform/Ammonia		
TSS-10	-		
TSS-57	-		
TSS-84	-		
TSS-95	-		
TSS-96	-		
TSS-111	-		
	Saponins		
	Frothing		
TSS-10	+		
TSS-57	+		
TSS-84	+		
TSS-95	+		
TSS-96	+		
TSS-111	+		
	Tannins		
	Ferric Chloride		
TSS-10	-		
TSS-57	-		
TSS-84	-		
TSS-95	-		
TSS-96	-		
TSS-111	-		
	Flavonoids		
	Magnesium Turning/Conc. H_2SO_4		
TSS-10	++		
TSS-57	+		
TSS-84	+		
TSS-95	++		
TSS-96	+		
TSS-111	+		

+ = Positive ++ = Strongly Positive - = Negative

The total phenolics contents of the different accessions of AYB are shown in Figure 2.

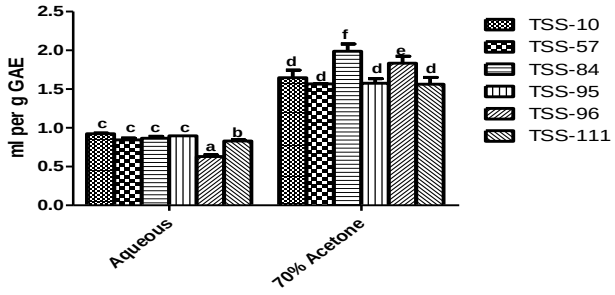

Figure 2: Total phenolics contents of African yam beans. Bars with different letters are significantly different (P < 0.05).

The results of the total phenolic contents (expressed as GAE) of the samples showed that African yam beans is rich in phenolics (Figure 2). The extractable total phenolic content was affected by the solvent medium used. 70% acetone was significantly (P>0.05) higher than aqueous. The TPC also differ significantly across legume accessions. However, TSS-10 had the highest value (0.925mgGAE/ml) of TPC and TSS-96 (0.630mgGAE/ml) had the lowest in aqueous extracts. However in 70% acetone extracts, TSS 84(1.989mgGAE/ml) had the highest value while TSS-111(1.645mgGAE/ml) had the lowest value though the value forTSS-57,TSS-95 and TSS-111 accessions were not significantly different.The total phenolic content of AYB accessions determined in our study was slightly higher than the results obtained for the AYB (Enujiugha *et al*., 2012).

The total flavonoids contents of the different accessions of AYB are shown in Figure 3.

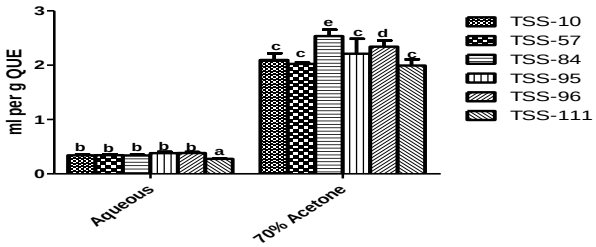

Figure 3: Total flavonoids contents of African yam beans. Bars with different letters are significantly different (P < 0.05).

Flavonoids are plant secondary metabolites commonly found in human diets. They exhibit several health benefits such as antioxidant,

anti-inflammatory, antiviral and anticancer effects (Umamaheswari and Chatterjee, 2008). Several studies have shown that the consumption of flavonoid-rich foods protect against diseases associated with oxidative stress. The mechanism of the anti-oxidative ability of flavonoids include scavenging of free radicals, chelation of metal ions and inhibition of enzymes responsible for free radical generation (Benavente-Garcia *et al*., 1997). The result of total flavonoids content (TFC) expressed as QUE of the legume accessions was shown in Figure 3. The TFC of the extracts from different extraction solvents differ significantly ($P < 0.05$). Extracts of 70% acetone had higher extractable TFC. The values for aqueous extracts range from TSS-96 (0.383mgQUE/ml) to TSS-111 (0.276 mgQUE/ml) while for 70% acetone extracts, the values range from TSS-84 (2.535mgQUE/ml)to TSS-111 (1.995mgQUE/ml) in both extracts. TSS-111 had the lowest value.

The DPPH scavenging abilities of the different accessions of AYB are shown in Figure 4.

DPPH assay showed the ability of the extracts to convert the DPPH to its reduced form Diphenylpicrylhydrazine with the loss of its violet colour.

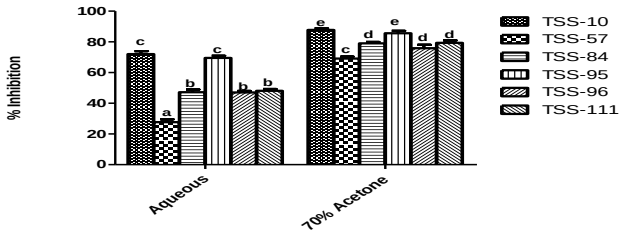

Figure 4: DPPH scavenging abilities of African yam beans. Bars with different letters are significantly different (P < 0.05)

The violet colour of DPPH is lost whenever it mix with a sample that can donate a hydrogen atom. It is converted to its reduced form with the loss of its violet colour (Alam *et al*., 2013; Olaiya *et al*., 2016). The result of the DPPH scavenging ability of our legume samples reported as percentage inhibition was showed in Figure 4.The values range from (22.72-72.05) for aqueous extracts and (69.16- 87.83) for 70% acetone extracts. This result showed that 70% acetone extracts had higher DPPH scavenging activity than aqueous extracts which is statistically different ($P < 0.05$). TSS-10 had the highest DPPH scavenging ability closely followed by TSS-95 when compared across legume accessions in both extracts (Figure 4).

The total antioxidants contents of the different accessions of AYB are shown in Figure 5.

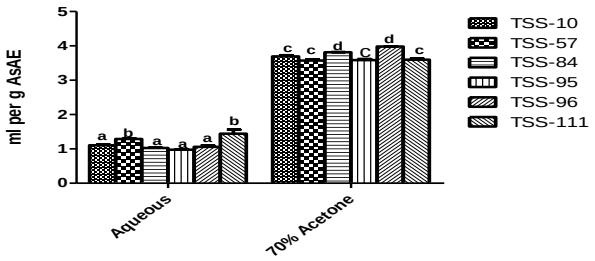

Figure 5: Total antioxidants contents of African yam beans. Bars with different letters are significantly different (P < 0.05)

Total antioxidant capacity assay is an important quantitative method of determining antioxidant capacity through the formation of phosphomolybdenum complex. The assay is based on the reduction of Mo (VI) to Mo (V) by the donation of electron by the antioxidant sample and subsequent formation of a green phosphate Mo (V) complex at acidic pH (Alam *et al*., 2013). The result of total antioxidants capacity (TAC) of our study reported as AsAE was showed in Figure 5. In the result, 70% acetone extracts had a significant ($P < 0.05$) higher value when compared with aqueous extracts. Comparison across the accessions showed that in aqueous extracts, TSS-111(1.444 mgAsAE/ml) had the highest values but was not significantly different ($P < 0.05$) from TSS-57(1.29 mgAsAE/ml). In 70% acetone, TSS-96 (3.9795 mgAsAE/ml) had the highest value which was significantly different from TSS-84(3.813 mgAsAE/ml).

The reducing capacities of the different accessions of AYB are shown in Figure 6.

Reducing power activity is usually used to evaluate the ability of natural antioxidants such as food produce to donate electrons (Dorman *et al*., 2003), and has always served as a significant indicator of antioxidant activity. It is based on the ability of the antioxidant to

form coloured complex with potassium ferricyanide, trichloroacetic acid and ferric chloride.

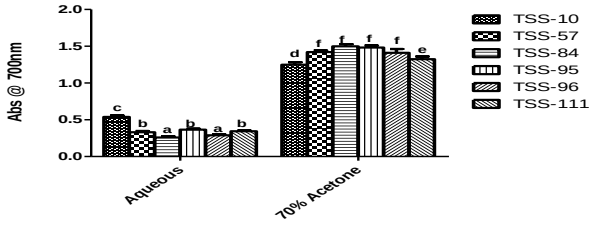

Figure 6: The reducing capacities of African yam beans. Bars with different letters are significantly different (P < 0.05)

The ability of antioxidants to donate electron would result in the reduction of Fe^{3+} to Fe^{2+} (Ebrahimzadeh *et al*., 2008; Olaiya *et al*., 2016). The result of reducing capacity was shown in Figure 6. The demonstrated reducing ability of aqueous extracts was far lesser than the 70% acetone extracts and it was statistically different (P < 0.05). In the aqueous extracts, TSS-10 (0.5395) exhibited the highest ability to reduce Fe^{3+} while the lowest was exhibited by TSS-84 (0.26). In the 70% acetone, TSS-84 (1.4985) exhibited the highest activities but the value was not statistically significant compared to those of TSS-57(1.4215), TSS-95(1.4825) and TSS-96 (1.410).

4. CONCLUSIONS

From the results obtained in the study, it was clearly shown that the accessions of AYB examined possess antioxidant-related phytochemicals: phenolics and flavonoids content which resulted in appreciable antioxidant activities. However, the extraction of these antioxidant-related phytochemicals was affected by the solvent used. The most effective solvent for antioxidant-related phytochemicals of these AYB accessions from our study was 70% acetone.

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