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Comparative studies on the radical scavenging capacity of polyphenol extracts from white and red varieties of pigeon pea (*Cajanus cajan*)

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Abstract
The aim of this work is to evaluate the phenolic content and antioxidant activity of red and white types of pigeon pea extracts obtained using two different solvents; methanol and acetone. Two different solvents were utilized in extracting the total phenol and flavonoid present in the two varieties. The content of total phenol of both acetone and methanol derivatives of red variety in pigeon pea were found to be higher (101.01±0.03 and 79.00±0.10) than the white variety (95.01±0.02 and 73.02±0.02) respectively. No significant difference existed in total flavonoid content found in both acetone and methanol derivatives from white variety. Acetone extracts from red variety had higher amount of total flavonoid (99.01±0.10) than its methanol extracts (71.11±0.00). In red variety of acetone extracts, high activities of antioxidants were observed.

Keywords: Acetone, methanol, pigeon pea, phenol and flavonoid

Introduction
Excessive radical production and lipid peroxidation underline the pathogenesis of diseases like atherosclerosis, carcinogenesis, diabetes, and ageing (Pourmorad *et al*., 2006) [16]. Antioxidants scavenge reactive oxygen species and free radicals and can be hugely important in restricting oxidative mechanisms, which cause chronic ailments (Cardador *et al*., 2002) [7]. The antioxidant content of almost all sources of plant food is often connected with phenolic contents obtained from them. They are ascertained useful as natural antioxidants in scavenging deleterious free radicals released in the body by fat metabolism (Enujiugha *et al*., 2012) [10]. Therefore, concerns have been stated regarding synthetic antioxidants such like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) for their possible activeness as boosters of carcinogenesis (Atiqur *et al*., 2008) [3]. There is increasing awareness towards antioxidants of natural phenolics from herbal sources like vegetables, fruits, cereals grains and legumes. High concentration of phenolics with strong free radical scavenging potentials was found in a tropical underutilized legume seed (Babak *et al*., 2012) [4]. Epidemiological and in vitro analyses on medicinal plants and vegetables potently have confirmed the idea that plant components with antioxidant activity are able to exercise protective effects against oxidative stress in biological system (Block *et al*., 1992) [5]. Pigeon pea (*Cajanus cajan*) is locally obtainable and cheap but has been one of the most underutilized legume of the tropics and sub-tropics. Different kinds of pigeon pea have protein substance in the scope of 23 - 26% (Oshodi *et al*., 1985) [14]. The protein content is comparable with those in other legumes like cowpea and groundnut which have been utilized in complementing maize. It is rich in mineral and fibre content. Pigeon pea grows well in Nigeria but the hard-to-cook phenomenon and the presence of antinutrient have limited its utilisation (Nene *et al*., 1990) [13]. A group of varieties of pigeon pea have been developed combining diverse plant types, different maturity periods and resistance to several diseases, insect pests, parasitic weeds and possessing other good agronomic traits (Adebowale *et al*., 2011) [1]. There is no known work in its innate anti-oxidative properties. This study's objectives were to analyze and compare the phenolic content and antioxidant capability of red and white forms of pigeon pea extracts obtained using two different solvents: (methanol and acetone).

Materials and Method
Dried red and white kinds of pigeon pea (*Cajanus cajan*) were purchased from Ogbete main market in Enugu - Nigeria. The immature and damaged seeds were discarded and the mature seeds were sun- dried for 24 h, sieved (0.18 mm sieve) and ground to obtain the flour which was packaged until further use.

Extraction
200 g of flour sample was defatted by using different solvents, 100 ml of 70% (methanol and acetone) individually and with continous agitation for 24 h at 28 ^{o}C. The extracts were filtered through Whatman No. 4 filter paper, following the procedures of Sowndhararajan *et al*., (2011) [18], with slight modification. The residues were further defatted with an additional 50 ml of 70% of the same solvents (methanol and acetone) described above, for 3 h. The solvents of the extracts were evaporated at 40 ^{o}C under reduced pressure, using a rotary vacuum evaporator (RE 300, Yamato, Tokyo, Japan) and the remaining water was removed by lyophilization (4KBT x L - 75; virtis Benchtop k, New York, USA). The obtained dry powers was stored in an air tight polythene bag at 0 ^{o}C till they were utilized. Total Phenol, Total Flavonoid, DPPH (1,1, Dipheny1-2-picrylhydrazyl) and Reducing power assay were determined as described by Makkar *et al*., 1993 [11]., Chang *et al*., 2002 [8], Blois, 1958 [6] and Pin-Der *et al*. [15], 2001 respectively. Data was subjected to analysis of variance with the statistical package for social science (SPSS), version 15.0. Results were presented as mean ± standard deviations. One way analysis of

variance (ANOVA) was utilized for comparison of the means. Deviations between means were considered to be significant at P<0.05 using the Duncan Multiple Range Test. Values are mean of triplicate experiments ± standard deviation.

Results and Discussion

Total phenol

Phenol and other antioxidants found in fruits, legumes and vegetables are bioactive compounds capable to neutralize free radicals, and many play a role in the prevention of certain ailments (Doss *et al*., 2010) [9]. The total phenol content of extracts gotten from different solvents for red and white variety pigeon pea is shown in Table I. Phenolic compounds of the highest extraction rate for both red and white variety of pigeon pea was obtained by acetone. This is in agreement with Maryam *et al.*, (2012) [12] whom recorded highest extraction rate of phenolic compounds with acetone compared to other solvents used. Statistical analysis indicated significant difference (p<0.05) between total phenolic content of red and white variety. The total phenolic substance of both acetone and methanolic derivatives of red variety of pigeon pea was higher (101.01±0.03 and 79.00±0.10) than white variety (95.01±0.02 and 73.02±0.02) respectively. This can be due to high concentration of benzoic acids protocatechuic, gentisic and vanillic, a dominant phenolic acid detected in the coloured seed coat (Agnieszka *et al*., 2002) [2].

Table 1: Antioxidants/Antioxidants activity of acetone methanolic extracts of white and red variety of pigeon pea.

Antioxidants and Antioxidants Sample Activity		Solvent	
		Methanol	Acetone
Total Phenol (mg/kg)	White variety pigeon pea	73.02±0.02^{a}	95.01±0.02^{b}
	Red variety pigeon pea	79.00±0.10^{c}	101.01±0.03^{d}
Total flavonoid (mg/kg)	White variety pigeon pea	58.31±0.13^{a}	59.03±0.11^{a}
	Red variety pigeon pea	71.11±0.00^{b}	99.02±0.10^{c}
DPPH (%)	White variety pigeon pea	52.01±0.04^{a}	59.00±0.05^{b}
	Red variety pigeon pea	64.11±0.02^{c}	72.00±0.01^{d}
Reducing Power (%)	White variety pigeon pea	0.51±0.11^{a}	0.53±0.00^{a}
	Red variety pigeon pea	0.81±0.03^{b}	0.99±0.15^{c}

The values are expressed as mean ± standard derivation, n = 3. There are no significant differences between similar letters (p<0.05)

Total Flavonoid

Researchers have demonstrated that intake of food rich in flavonoid protects human against diseases linked with oxidative stress. The mechanisms of action of flavonoids are through free-radical chelating or scavenging process and protection against oxidative stress. Shahidi *et al*., (1995) [17]. There was no significant difference in total flavonoid content of both Acetone and methanolic extracts from the white variety (Table 1), Acetone extracts from the red variety had higher amount of Total flavonoid (99.01±0.10) than its methanolic extracts (71.11±0.00). This can be due to difference in solvent polarity. The acetone and methanolic extracts of red variety pigeon pea seeds indicated higher flavonoids (99.01±0.10 and 71.11±0.00) than that of white variety (59.03±0.11 and 58.31±0.13) respectively. This might be due to high amounts of proanthocyanins found in darker seed coats.

DPPH Assay

DPPH has been used for assessing the antioxidant activity of food products. Acetone and methanolic extracts of Red variety of pigeon pea seed indicate higher radical scavenging activity of 72% and 64% than that of the white variety 59% and 52% respectively, this might be due to the alternation in solvents polarity and as a result of the type of extracted compounds. There was a significant difference between Acetone and methanolic extracts (p<0.05).

Reducing Power Assay

The results of the present study showed that the extracts of Red variety of pigeon pea which contained increased amounts of flavonoid and phenolic compounds, exhibited greater reducing power than the derivatives of white variety of pigeon pea (Table 1). This is in agreement with the work done by Agnieszka *et al.*, (2002) [2] whom concluded that the concentration of free phenolic acids was about six times higher (46.36µg/g) in the coloured seed coat than in the white one (7.75µg/g) while that of ester-bound acids was about three times higher (16.45µg/g) in the coloured seed coat than in the white one (5.31µg/g).

Conclusion

Pigeon pea varieties can display different antioxidant activity. In conclusion, it was found that phenol and flavonoid acetone extract of red variety pigeon pea exhibited a remarkable antioxidant activity. The findings in this study will provide information for the selection of suitable pigeon pea variety as an origin of natural antioxidants in functional foods, supplements and some medicine formulation.

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