INTERNATIONAL JOURNAL OF NATURAL AND APPLIED SCIENCES (IJNAS), VOL. 12, Special Edition (2019); P. 39 – 46, 1 TABLE, 15 FIG.

Chemical Evaluation of Nutritional and Medicinal Potentials of Fluted pumpkin (*Telfairia occidentalis*) Seeds and Coffea robusta (*Coffea canephora*) leaves

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ABSTRACT

The evaluation of nutritional and medicinal potentials of seeds of Fluted pumpkin (*Telfairia occidentalis*) and Coffea robusta (*Coffea canephora*) leaves was carried out using various analytical techniques. Proximate parameters: ash, moisture, crude protein, crude fibre, crude fat and carbohydrate as well as phytochemicals: alkaloids, cardiac glycosides, saponins, anthranoids, flavonoids, polyphenols, tannins, anthraquinones and phlobatannins were assessed. The results of proximate analysis revealed the following compositions: ash (7.20 ±0.32%), moisture (6.40±0.41%), crude fibre (7.00±0.79%), crude lipid (6.20±0.40%), crude protein (6.10±0.43%) and carbohydrate (73.50±0.87%) for fluted pumpkin (*T. occidentalis*) seeds, while the proximate composition of the leaves of Coffea robusta revealed: ash (16.00±0.11%), moisture (26.00±0.18%), crude fibre (38.00±0.42%), crude lipid (10.00±0.05%), crude protein (1.14±0.01%) and carbohydrate (34.86±0.51%). The phytochemical screening of *T. occidentalis* showed the presence of polyphenols in moderate amount in both water extract (WE) and petroleum ether (PE) extract while flavonoids, saponins and cardiac glycosides were only present in petroleum ether but absent in water extract, while alkaloids was only present in water extract. Tannins, reducing sugar, anthraquinones and anthranoids were however absent in both extracts. For *C. canephora*, polyphenol was present in small amount in both extracts, cardiac glycosides, alkaloids, saponins and anthranoids were present in water extracts but absent in petroleum ether extract. Tannins was only present in petroleum ether extract while reducing sugar and phlobatanins were absent in both extracts. The result shows that *T. occidentalis* and *C. canephora* have good nutritional and pharmaceutical potentials.

INTRODUCTION

Chemical evaluation on plants and its significant role in medicine and health care delivery has continued to yield positive results in areas such as Malaria, HIV/AIDS, Leprosy, Tuberculosis, psychosis, Diabetes, Cellulitis, Basal cell Carcinoma, Melanoma,Squamous cell Carcinoma etc. Plants kingdom has proved to be the most useful for the treatment of diseases and provide an important source of all the world's pharmaceuticals (Ajayi *et al.* 2011). Plants provide an alternative strategy in search for new drugs which is one of the riches abundance of plants reputed in orthodox medicine to possess protective and therapeutic properties. Plants have continued to be valuable source of new molecules which may provide new and improved drugs after possible chemical manipulation. Plants have also yielded chemical drugs either as natural product molecule or as synthetic modification, particularly for chemotherapeutic treatment of cancer and malaria (Phillipson, 2007). In recent years, there have been tremendous interests in the antioxidant and health benefit of phytochemicals in foods and vegetables arising from the potential effects on human health. However, antioxidant supplements may be used to help human body to reduce oxidative damage and can function as free radical scavengers (Yang *et al.*, 2002). Thus, the unique function of plants to the sustenance of life cannot be overemphasized.

Studies have shown that *T. occidentalis* has significant pharmaceutical properties. The seed oil at about 400mg/kg can improve semen parameters but had no testicular histology and testosterone level in rat. The oil increased the levels of sperm count, sperm motility and testosterone compared to the alcohol treated rats. *T. occidentalis* component such as vitamin C, zinc and argunin have nutritional therapies in the treatment of male infertility as a result of antioxidant properties (Akang *et al.*, 2010). Several other works on Fluted pumpkin include those of (Sunday *et al.*, 1992, Fagbemi, 2005; 2007, Agatemor, 2006, , Verla *et al.*, 2014 and Udo *et al.*, 2016). Fluted pumpkin is a widely consumed vegetable by people of Akwa Ibom and Cross River States of southern Nigeria. The plant has enormous nutritional significance. For instance, Agatemor (2006) reported that the oil extracted from Fluted pumpkin seed have high iodine values compared to palm oil which indicates that the oil has high content of unsaturated fatty acids relative to palm oil, suggesting its usage for edible oil for cooking and manufacturing of margarine. Similarly, the seed oil is a rich source of edible vegetable oil due to the low free fatty acid content. It is also said to have low iodine value which suggest that the oil is non-drying oil and will have low susceptibility to

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deterioration hence having a long shelf life (Udo *et al*. 2016). Coffea robusta is a species of coffee that has its origin in central and western-sub-Saharan Africa. A part from being known as Coffea robusta, it is scientifically identified as *Coffea canephora* which has two varieties: robusta and Nganda (Dagron, 2005). It is cultivated low to medium altitude in the intertropical region of Africa, America and Asia. *C. canephora* is a self incompatible diploid species indigenous to some areas of the tropical African forest, from West Africa via Cameroon, Central African Republic, Democratic Republic of Congo and Northern Tanzania down to Northern Angola (Cubry *et al.* 2012). Coffee has been the most commercially used food product and a most widely consumed beverage in the world since the opening of the first coffee house in Meca at the end of Fifteenth century. Since then, coffee consumption has greatly increased all over the world. The coffee beverage has been consumed as a stimulant diuretic, antioxidant and antipyretic and also to relieve spasmodic asthma (Chairgulprasert and Kongsuwankeeree, 2017). In Denmark, USA, Japan, Brazil and Mediterianean countries, coffee is reported to be a major contributor to antioxidant intake (Larsson and Wolk, 2007, Ranheim and Halvorsen, 2005, and Farah, 2012). Studies have shown that moderate consumption of coffee can lead to reduced cardiovascular diseases, lower prevalence of diabetes and hyperlipideamia, lower body mass index, better renal function and higher creatinine clearance levels (ICO, 2011), while epidemiological and clinical investigations showed that independent of caffeine intake is associated with health benefits such as lower risk of type-2 diabetes (Agardh *et al.* 2004, Bravi *et al*. 2007), Parkinson and Alzheimer diseases (Lindsay *et al.* 2002) and liver cancer (Larsson and Wolk, 2007, Ranheim and Halvorsen, 2005). Coffee can reduce blood cholesterol, lower blood sugar and prevent obesity, liver and heart diseases (Chairgulprasert and Kongsuwankeeree, 2017).

In 2010, Coffee production reached 8.1million tons worldwide (ICO, 2011). Nuhu (2014) reported that coffee contains nutritive substances which may vary with the types of techniques employed in the brewing processes. It also contains mineral ingredients like Ca, Mg, Fe, Ni, K, Cu and P (Oliveiria *et al*. 2012), polyphenol, caffeine, melanoidin and carbohydrates (Vignoli *et al*., 2011 and Mussatto *et al*., 2011). The characteristics flavor and richness of coffee aroma make it a unique beverage with almost a thousand volatile compounds identified in roasted coffee (Yeretzian *et al.,* 2003). This study is basically to unravel the medicinal and nutritional potentials of *T. occidentalis* seed and *C. robusta* leaves.

MATERIALS AND METHODS

Sample collection and preparation

The Fluted pumpkin was obtained from Akpabuyo Market in Akpabuyo Local Government Area while the fresh leaves of Coffee robusta were collected from Atimbo River site in Calabar Municipality Local Government Area of Cross River State, Nigeria. Both samples were identified in the Botany Department of the University of Calabar. The seeds of Fluted pumpkin were removed from its pod and dried in an oven at 100^{o} C. The dried seeds were then reduced to the fine powder using laboratory mill while the Coffee robusta leaves were removed from the stem Coffee robusta and dried in an oven for about 8h at 200^{o} C. Both dried samples were ground to powder and stored in desiccators.

Sample extraction. Distilled water and petroleum ether were used to extract the seeds of Fluted pumpkin and leaves of Coffee robusta. In each process, 15g of the powdered samples were washed and packed into extraction thimble and fitted into the Soxhlet extractor. The samples were Soxhlet extracted for 3h. The petroleum ether and water extracts obtained were put in the reagent bottles. Each bottle was distinctively labeled and kept in the laboratory for phyto-chemical screening.

Proximate analysis. Proximate analysis involves determination of Moisture, ash, crude fibre, crude protein, fat and Carbohydrate contents (Verma, 2010). Ash, moisture, fat, fibre and crude protein were determined through methods prescribed by (AOAC, 2000). The percentage available carbohydrate (CHO) was calculated using expression:

% CHO = 100 - (% ash + % crude protein + % crude lipid + % crude fibre)

Phytochemical screening. The phytochemical screening procedures carried out on the seeds of *T. occidentalis and C. robusta* were adapted from the previous work on medicinal plants (Sofowora, 1984 and Harborne, 1973). Alkaloids, saponins, tannins, polyphenols, phlobatannins, cardiac glycosides, anthraquinones, anthranoids and reducing compounds were screened.

Test for alkaloids (Sofowora 1984). The plants extracts (2ml) were put in a test tube and treated with 10ml of 1% HCl and heated in a water bath for 10 minutes. 1ml of the filtrate was treated with few drops of Mayer's reagent and a second, 1ml portion was treated with Dragendroff reagent. Turbidity or reddish-brown precipitate with either of the reagent was taken as evidence for the presence of alkaloids.

Test for anthranoids (Sofowora 1993). A small portion of aqueous plant extract (2ml) was boiled with 5ml of KOH. The solution was filtered through glass. The filtrate was treated with 1% acetic acid and the resultant solution was mixed with toluene. The upper layer was transferred to another test tube and potassium added. The presence of a red colour indicate the presence of anthranoids.

Test for anthraquinones (Trease and Evans, 1978). A small quantity of aqueous extract (1ml) each was shaken with 10ml of benzene. This was filtered and 5ml of 1% NH_3 solution was added. The mixture was shaken and the appearance of pink red or violet colouration in ammonical (lower) layer indicates the presence of anthraquinones.

Test for cardiac glycosides (Sofowora, 1984). The plants extracts (water and petroleum ether) 2ml were separately dissolved in 2ml of Chloroform. Conc. H_2SO_4 was carefully added to form a

coloured layer. A brown ring obtained at the interface indicates the presence of a deoxysugar, a characteristics of cardiac glycosides.

Test for flavonoids (Sofowora, 1993)

A small portion of aqueous plant extract (2ml) was added to a few pieces of magnesium metal and concentrated HCl added. The formation of orange, red, crimson or magenta indicates the presence of flavonoids.

Test for polyphenols (Harborne, 1973)

The plant extracts (2ml) were treated with 5ml of distilled water and heated for 30 minutes in a water bath. 1ml of 1% $FeCl_3$ was added to the mixture and followed by addition of 1ml of 1% potassium ferro-cyanide. The formation of a green-blue colouration indicates the presence of polyphenols.

Test for reducing sugar (Sofowora, 1993)

A small portion of aqueous plant extract (2ml) was put in separate test tubes and 5ml of fehling solution added to it and heated in a water bath for 5 minutes. The formation of a brick-red precipitate or colouration indicates the presence of reducing compounds.

Test for phlobatannins(Harborne, 1973)

A small portion of aqueous plant extract (2ml) was boiled with 10% HCl. The deposition of a red precipitate or colouration indicates the presence of phlobatannins.

Test for saponins (Sofowora, 1984)

The small quantity of the extracts (2ml) were put in a test tube and treated with 10ml of 1% HCl and heated in a water bath. After heated, these were shaken vigorously; the persistent froth on warming indicates the presence of saponins.

Test for tannins (Trease and Evans, 1989)

A small portion of aqueous plant extract (2ml) was stirred with 10ml of distilled water and heated in a water bath. 1ml of 1% ferric chloride ($FeCl_3$) was added to the filtrate. Blue-black, green or blue-green precipitation or colouration indicates the presence of tannins.

RESULTS AND DISCUSSIONS

Tables 1 and 2 show the results of proximate and qualitative phytochemical analysis of Fluted pumpkin (*T. occidentalis*). Tables 3 and 4 show the results of proximate and qualitative phytochemical analysis of robusta coffea (*C. canephora*). Figures 1 and 2 show the percentage compositions of *T. occidentalis* and *C. canephora* respectively. The results of proximate analysis of Fluted pumpkin (*T. occidentalis*) as given in Table 1 and Fig. 1 showed the moisture content to be (6.40 ± 0.41%). This is slightly lower than 10.99 ± 1.34 reported on the aqueous leaf extract of *T. occidentalis* by Usunobun and Egharebva (2014) and 43.18 ± 0.59 by Akwukwaegbu *et al.*, (2016), while the proximate evaluation of the leaves of coffee robusta (*Coffea canephora*) revealed (26.00 ± 0.18%) moisture contents. This was however contrary to 12% and 8.33% moisture contents of robusta coffee seeds obtained from Rwanda and Catimor respectively by Hamid et al. (2017). The moisture content of food is usually used as a measure of shelf life, stability and susceptibility to microbial contamination (Scott, 1980). The result of this investigation shows *Tefairial occidentalis* seed can be well preserved without spoilage for export purposes, while that of *Coffea canephora* shows a lesser preservative advantage. Study on ash content of the seed of *T. occidental* was found to be (7.20 ± 0.32%). The composition corresponds to that of Akwukwaegbu *et al.* (2016) and Usunobun and Egharebva (2014) respectively, while that of coffea robusta was found to be (16.00 ± 0.11)%. The composition of *C. canephora* of the present study did not correspond to 6.66% and 4.33% ash contents obtained from Rwanda and Catimor by Hamid *et al.* (2017). The information on the ash content of any food is necessary for the evaluation of mineral value of the food.

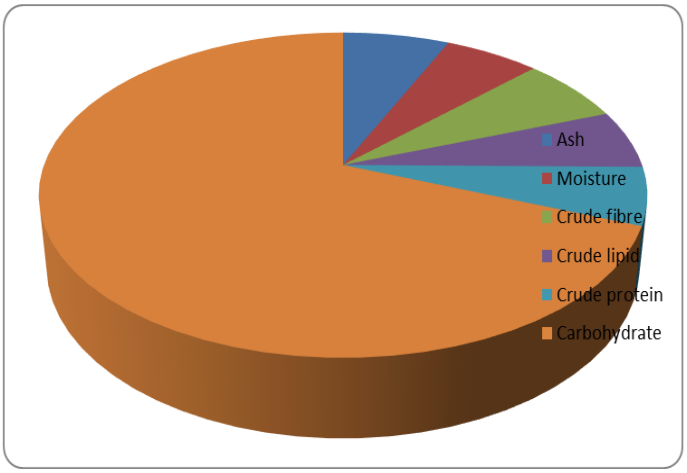

Fig. 1. Percentage composition of *Telfairia occidentalis* seeds

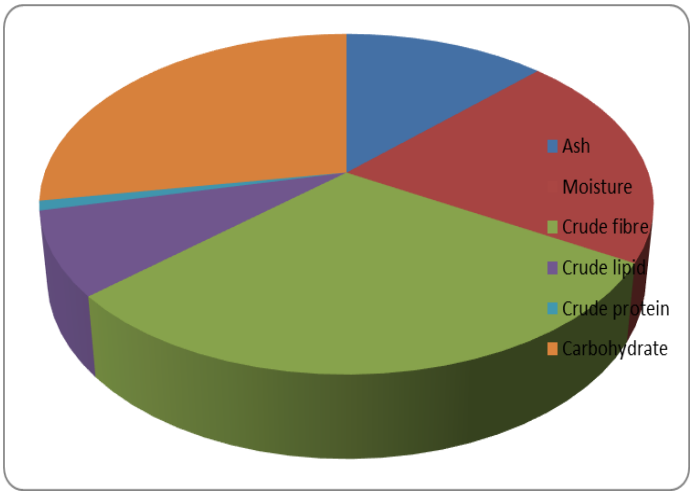

Fig. 2. Percentage composition of *Coffea canephora* leaves

The results of the analysis of crude fibre content of (*T. occidentalis*) as presented in Table 1 was found to be (7.00±0.79%). This is slightly higher than 3.44% obtained by Lennox *et al*. (2016) on the microbiological and proximate analysis of Fluted pumpkin leaves but lower than (11.56±0.68%) obtained by Usunobun and Egharebva, 2014 on aqueous leaf extract of *T. occidentalis*; and (15.43±0.01%) reported by Akwukwaegbu *et al*. (2016). However, the result of crude fibre content of *Coffea canephora* as given in Table 3 was found to be (38.00±0.42%). Crude fibre provides roughages that aid digestion and reduce the accumulation of carcinogen in the body. It helps in the maintenance of human health and has been proven to reduce cholesterol level in the body. Emphasis has been placed on the importance of keeping fibre intake low in the nutrition of infants and pre-school children (Eromosele and Eromosele, 1993). Similarly, the crude lipid content of *T. occidentalis* obtained showed (6.20±0.40%). This is significantly low (Fig 1) compared to 47.4% given by Odoemelam (2005), but the crude lipid content of *C. canephora* (Table 3) showed (10.00±0.05%). Lipids are monosaturated and considered healthy when consumed at moderate quantity. They are essential because they provide maximum energy to the body (Dreon *et al*. 1990.) The crude protein content of Fluted pumpkin seed of the present study was found to be (6.10±0.43%), while that of coffea robusta was found to be (1.14±0.01%). These compositions are very low (Fig. 1 & 2) when compared to 36.2% reported by Odoemelam, (2005) on fluted pumpkin. Protein is an essential component of diet for survival of animals and humans. The daily protein intake for children and adults are 23-26kg and 45-46g respectively (NRC, 1974). However, further investigation on the seeds of fluted pumpkin revealed a very high carbohydrate content of (73.50±0.87%) (see Table 1, Fig 1). The carbohydrate contents of *C. canephora* leaves (Table 3) revealed a moderate composition of (34.84±0.51%). The presence of carbohydrate in food prevents the unnecessary usage of protein and allows it to be used for the building of the body processes. Generally, crude protein, crude lipid, crude fibre and carbohydrate content of coffea robusta leaves of the present study were significantly low (Fig. 2).

Table 1. Results of proximate composition of *Telfairialoccidentalis* seeds

Parameters	% composition
Ash content	7.20 ± 0.32
Moisture content	6.40 ± 0.41
Crude fibre content	7.00 ± 0.79
Crude lipid conten	6.20 ± 0.40
Crude protein content	6.10 ± 0.43
Carbohydrate content	73.50 ± 0.87

Data are: mean (x) ± SD of triplicate determination

Table 2. Results of qualitative phytochemical analysis of *T. occidentalis* seeds

Plant bioactive compounds	Petroleum Ether Extract	Water Extract
Alkaloids	-	+
Anthranoids	-	-
Anthraquinones	-	-
Cardiac glycosides	+	-
Flavonoids	+	-
Polyphenols	++	++
Reducing compounds	-	-
Phlobatannins	-	-
Saponins	+	-
Tannins	-	-

KEY: - = Not present, + = Present, ++ = Excessive present

The results of phytochemical screening of the seeds of *T. occidentalis* (Table 4) show the presence of cardiac glycosides in petroleum ether extracts but absence in water extract. The present investigation on cardiac glycosides corresponds to the study of the aqueous leaf extract by (Usunobun and Egharebva, 2014), which showed the absence of cardiac glycosides, but Fluted pumpkin showed large amount of cardiac glycosides in a study conducted by Akwukwaegbu *et al*. (2016), but the phytochemical screening of the leaves of *C. robusta* (Table 4) showed the presence of cardiac glycosides in water extract (WE) and absent in petroleum ether (PE) extract. Cardiac glycosides have specific characteristics and powerful action exerted on cardiac muscles and therefore is used in congestive heart failure due to diminution of work capacity per unit weight of mycroches tissues. Medicinal interest on cardiac glycosides is because of their structure stimulation effect on heart (Olanyinka and Ononime, 1991; Osabor *et al*. 2016).

The result of phytochemical screening for phenols (Table 4) revealed that *T. occidentalis* contain polyphenols in both water and petroleum ether extracts in large amount. This study is in line with the previous studies on Fluted pumpkin pod by Akwukwaegbu *et al*. (2016) which showed the presence of polyphenols. This is indicative that Fluted pumpkin is rich in polyphenols. Similarly, the phytochemical investigations of the leaves of robusta coffea (*Coffea canephora*) (Table 4) indicated moderate composition of polyphenols in both petroleum ether and water extracts against other phytoconstituents. Polyphenols are compounds that have hydroxyl groups directly attached to a benzene ring. They are structurally similar to alcohols but are not stronger acids (Solomon and Craig, 1998). It helps in contracting the blood capillaries and thus prevents certain hemorrhages. According to Swain and Harborne, (1979) polyphenols help in the contraction of blood capillaries and thus helps certain haemorrhages. Epidemiological studies have shown that long term consumptions of diet rich in plant polyphenols offer protection against development of cancer, diabetes, cardiovascular diseases and neurodegenerative diseases (Goya, 2007). Wide use of coffee consumption is also said to be associated with lower incidences of obesity (Hamid *et al*. 2017) and type 11 diabetes (Van Dam, 2002, Bhuparthiraju *et al*. 2013). Polyphenols have also shown to protect against oxidative stress (Vitaglione, 2010). Coffea polyphenols regulate lipogenic pathways and reduce live fat accumulated in high fat fed mice (Vitaglione, 2010, Murase, 2011; 2012). This suggests that coffee polyphenol can prevent tissue diet induced ectopic lipid deposition and insulin resistance. This informed the reason coffee is a widely consumed beverage, apart from its pleasant taste and aroma (Hamid *et al*. 2017).

Table 3. Proximate composition of Coffea robusta (*Coffea canephora*) leaves

Parameters	% Composition
Ash content	16.00 ± 0.11
Moisture content	26.00 ± 0.18
Crude fibre content	38.00 ± 0.42
Crude lipid content	10.00 ± 0.05
Crude protein content	1.14 ± 0.01
Carbohydrate content	34.84 ± 0.51

Data are: mean (x) $\pm$ *SD of triplicate determination*

Table 4. Qualitative phytochemical analysis of Coffea robusta (*Coffea canephora*) leaves

Bioactive compounds	Petroleum ether extract	Water extract
Alkaloids	-	+
Athranoids	-	+
Athraquinones	-	-
Cardiac glycosides	-	+
Flavonoids	-	-
Polyphenols	+	+
Reducing compounds	-	-
Phlobatannins	-	-
Saponins	-	+
Tannins	+	-

KEY: *- = Not present, + = Present*

However, the screening for more plant phytochemicals in the seeds of *T. occidentalis* showed the presence of saponins and flavonoids in PE extract but absent in WE while alkaloids was present in WE but absent in PE (Table 4). Tannins, reducing sugar, phlobatannins, anthranoids, and anthraquinones were absent in both water and petroleum ether extracts. All investigations of the present study on Fluted pumpkin seeds correspond to the report by Usunbun and Eguarebia (2014) and Akwaukaegbu *et al*. (2016). Further studies on the qualitative phyto-constituents of *C. canephora* revealed the presence of alkaloids, anthranoids and saponins in WE and tannis in PE, while anthraquinones, reducing sugars and phlobatannins were absent in both extracts. This shows a lesser composition contrary to the report of Hamid *et al*. (2017) which revealed excessive presence of alkaloids, saponins and tannins in the extracts.

CONCLUSION

The results obtained from this study showed that Fluted pumpkin (*T. occidentalis*) and robusta coffea (*Coffea canephora*) can be used in orthodox medicine to treat cardiovascular diseases due to their richness in polyphenols and cardiac glycosides. The presence of alkaloids in water extract of both plants suggests pharmacological and physiological significance, while richness in saponins suggest their potential for liquid detergent production. However, the ash and moisture content analysis revealed that *T. occidentalis* has high storage advantage over *C. canephora*. The protein and carbohydrate contents of *C. canephora* are indicative of its wide consumption in diabetes control. Generally, both *T. occidentalis* and *C. canephora* are good sources of carbohydrate, lipid, fibre, moisture and ash with wide applications in various food productions. Both *T. occidentalis* and *C. canephora* have high nutritional and medicinal advantages. Future studies on *T. occidentalis and C. canephora* will focus on elemental

compositions and extraction of bioactive compounds for pharmaceutical, medicinal and industrial purposes.

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