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Some Phytochemicals and Hydrophilic Vitamins of *Anacardium occidentale*

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ABSTRACT

Plants are composed of vast array of phytochemicals and vitamins that characterize their pharmacologic properties and nutritional values. The present study sought to investigate, using standard methods, the concentrations of some phytochemicals and hydrophilic vitamins, viz., alkaloids, saponins, flavonoids, tannins, HCN, phenols, anthocyanin, ascorbic acid, niacin, riboflavin and thiamine, in the fruit, leaf, stem bark and root of red and yellow fruited species of *Anacardium occidentale* L. The leaf of the Red Fruited Species (RFS) and Yellow Fruited Species (YFS) of *A. occidentale* L. gave the highest concentration of alkaloids, whereas the level of alkaloids was lowest in the fruits of both species. The fruit and root of the RFS as well as the fruit and stem bark of the YFS gave the lowest concentrations of flavonoids. The fruit and leaf of the RFS and YFS gave relatively high concentrations of anthocyanin compared to other parts of the plant. The concentrations of saponins in stem bark of the RFS was 10 folds approximately. higher than the concentration of anthocyanin and 9 folds than that of anthocyanin in the YFS. HCN was absent in the fruits and roots of the RFS and YFS. The leaf of the RFS and YFS gave the highest concentrations of phenolics. The stem bark of both the RFS and YFS gave the highest concentration of tannins, whereas the lowest concentrations of tannins occurred in the fruits of both species. The concentrations of the four hydrophilic vitamins in the fruit of the RFS were in the order: Ascorbic acid = 134.20±0.12 mg/100 g>thiamine = 15.50±0.11 mg/100 g >riboflavin = 2.90±0.05 mg/100 g>niacin = 0.23±0.05 mg/100 g. The present study has substantiated and justified the application of extracts of this multipurpose economic tree in ethnomedicinal practices and herbal remedies.

Key words: *Anacardium occidentale* L., anthocyanin, thiamine, phytochemicals, vitamins

INTRODUCTION

Plants are composed of vast array of phytochemicals and vitamins that characterize their pharmacologic properties and nutritional values. Phytochemicals are also known to play critical roles in defense and signaling at the cellular and organismic levels (Bonjar *et al.*, 2004; Lila and Raskin, 2005; Dhawale, 2013). Phyto-constituents are generally classified into primary and secondary metabolites. The primary metabolites include metabolic carbohydrates, proteins/amino acids, lipids, vitamins and minerals. Few examples of plant secondary metabolites are alkaloids,

anthocyanin, flavonoids, Cyanogenic Glycosides (CGs), phenolic, saponins and tannins. Although, alkaloids are naturally occurring amine of plant origin, studies have shown that amines produced by animals and fungi are also classified as alkaloids. Several types of alkaloids are present in biologic systems that are sub-classified on the basis of the chemical nature of their nitrogen containing rings and structural characteristics. Alkaloids possess a characteristic bitter taste with accompanied toxicity to insects and herbivores. However, alkaloids could serve as stimulants and psychotropic agent (http://www.herbs2000.com/h_menu/alkaloids). Anthocyanins and other flavonoids are hydroxylated phenolic substance present in all tissue types of higher plants, such as leaves, stems, roots, flowers and fruits. They are water-soluble vacuolar pigments, which may appear red, purple or blue depending on prevailing cellular pH.

According to reports of FSANZ (2004), there are about 25 CGs known, with the major CGs found in the edible parts of plants; notable are amygdalin (almonds), dhurrin (sorghum), linamarin (cassava, lima beans), lotaustralin (cassava, lima beans), prunasin (stone fruit) and taxiphyllin (bamboo shoots). The biosynthesis of CGs involves hydroxylation of L-amino acids, which yields N-hydroxylamino acids that are converted to aldoximes. The aldoximes are transformed to nitriles/α-hydroxynitriles and subsequently glycosylated, usually β-linked to D-glucose, to form CGs (Vetter, 2000). Cellular damage caused by maceration of plant tissues engenders the interaction between β-glycosidases and cyanogenic glycoside.This action is followed by hydrolysis of CGs to generate glucose and cyanohydrin, which rapidly decomposes to hydrocyanic acid (HCN) and aldehyde or ketone (Moller and Seigler, 1999). The catalytic hydrolysis of CGs that yields HCN is a chemical defense mechanism by which cyanogenic plants are protected against herbivores (Moller and Seigler, 1999).

Saponins are amphipathic glycosides of steroids, steroid alkaloids or triterpinoids (Prabhakar and Doble, 2008) with characteristic soap-like foam forming property when shaken in aqueous environment; commonly referred to as the frothing test for saponins. The glycoside-free saponins portion, termed sapogenins, can be attached to varied number of saccharides resulting to structural varieties of saponins. Also, nitrogen can be incorporated into the aglycone derivatives eliciting pharmacologic characteristics similar to the alkaloids. Tannins are classified into two categories; viz., condensed tannins and hydrolyzed tannins (Clinton, 2009). Structurally, they are polyphenols present in abundance in tree bark, wood, fruit, fruit pod, leaf and root. High levels of tannins in tree bark serve as barrier to invading micro-organisms such as bacteria and fungi. Apart from its utilization in the tanning process, tannins are applied in the dyeing, photography, brewery and wine industries as well as astringent in medicinal preparations.

The cashew (*Anacardium occidentale* L.) tree is a member of the Anacardiaceae family that contains 73 genera and about 600 species (http://elkinvanaeon.net). Records have it that the tree is native to tropical America, often found growing wild in the central plains of Brazil and later cultivated in many parts of the Amazon rainforest (Red River Foods Inc., 2010). The trees are now widely grown in tropical climates of Africa especially Ivory Coast, which was the world's largest producer of cashew 2010 (Red River Foods Inc., 2010). The tree is a source of cashew apples and nuts renowned for their international appeal and market value as food. Raw materials for industrial uses have their origin from the cashew tree. Also, evidence from worldwide ethnomedicinal practices reveals a multifaceted therapeutic value of different parts of cashew tree. Herbal remedies sourced from extracts of *A. occidentale* L. have been used from time immemorial to present day for the treatment of several pathologic conditions; notable are malaria, bronchitis, dyspepsia, eczema, psoriasis, syphilis, urinary insufficiency and nasal congestion among others (http: //www.rain-tree.com/cajueiro.htm).

A compendium of information on the distribution of phytochemicals in plant systems will serve as a benchmark for precise extraction targets for phytochemicals of interest from amongst the various plant parts, with a view to achieving maximum therapeutic benefits and well informed administration of plant materials. However, the multiplicity of phytochemicals and biodiversity of plants has largely been responsible for the handicap encountered by investigators to predict the phytochemical composition of plant systems or make comprehensive reports on their structural and functional properties, despite significant advances in analytical and screening technology (Raskin *et al.*, 2002; Milugo *et al.*, 2013). Therefore, the present study sought to investigate, in comparative terms, the concentrations of some phytochemicals and hydrophilic vitamins, viz., alkaloids, saponins, flavonoids, tannins, HCN, phenols, anthocyanin, ascorbic acid, niacin, riboflavin and thiamine, in the fruit, leaf, stem bark and root of red and yellow fruited species of *A. occidentale* L. The outcome of the present study will serve to validate the ethnomedicinal usefulness of *A. occidentale* L.

MATERIALS AND METHODS

Collection and preparation of plant extracts: Different parts of *A. Occidentale* L. tree, namely, the fruit, leaf, stem bark and root were harvested, between the months of March and April, 2012, from two species of *A. occidentale* L. trees {Red Fruited Species (RFS) and Yellow Fruited Species (YFS)} growing in the wild along Uturu/Okigwe Express Road, Okigwe, Imo State, Nigeria. The specimens were authenticated by Dr. F.N. Mbagwu at the Herbarium of the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. Voucher specimens were deposited at the Herbarium for reference purposes. The separate plant parts were washed under continuous flow of tap water to remove dust/debris and air-dried at room temperature (25±4°C) for 5 h. The specimens were cut into bits and further dried at 60°C in an oven (WTC BINDER, 7200 Tuttlingen, Germany) for 3 days (Neogi *et al.*, 2007) till sufficiently devoid of moisture. The specimens were ground into powdered form using the Thomas-Willey milling machine.

Phytochemical and vitamin analyses: Using standard methods, quantitative analyses were carried out to measure the concentrations of phytochemicals and vitamins, viz., alkaloids, saponins, flavonoids, tannins, HCN, phenols, anthocyanin, ascorbic acid, niacin, riboflavin and thiamine.

Alkaloids: The gravimetric method according to Harborne (1973) was used for determination of alkaloids in the various specimens. Five grams (5 g) of each prepared samples and 100 mL of 10% CH_3COOH in EtOH were measured into 250 mL capacity conical flask and mixed properly by vortex. The mixture was allowed to stand for 4 h at 25±4°C with continuous agitation (shaking bath; Precision Scientific Inc., Chicago, IL). The blend was separated by simple filtration methods using the Whatman No. 42 filter paper and the filtrate was concentrated by reducing the volume by evaporation to ¼ of its original volume in a steam bath. Next, concentrated NH_4OH was added in droplets to the filtrate to precipitate alkaloids out of solution, which was subsequently separated by filtration. The precipitate was washed with 1% NH_4OH, dried in an oven until devoid of moisture and weighted after cooling in a dessicator.

Flavonoids/anthocyanin: Concentrations of flavonoids/anthocyanin were measured according to the method of Harborne (1973). Briefly, 5 g sample, 100 mL of 2 M HCl was boiled under reflux for 35 min, cooled to 25±4°C and filtered with Whatman No. 42 filter paper. The filtrate was treated with equal volume of $CH_3COOC_2H_5$, which was added in droplets until flavonoids were completely

precipitated. The precipitate was recovered by simple filtration. The filtrate was reserved for determination of anthocyanin concentration, whereas the residue was dried in an oven, cooled in adessicator and weighted.

Anthocyanin was precipitated from the filtrate (obtained as described above) by the addition, in drops, of 0.1 mL EtOH until in excess. The precipitate was harvested by simple filtration, dried in an oven, cooled in a dessicator and weighted.

Saponins: The method as described by Obadoni and Ochuka (2002) was used for the measurement of saponins concentration. Five grams (5 g) of the sample was mixed with 200 mL of 20% EtOH. The mixture was boiled in a water bath while stirring for 4 h, allowed to cool to 25±4°C and filtered with Whatman No. 42 filter paper. The filtrate was concentrated by reducing the total filtrate volume to 40 mL by evaporation in a steam bath. In a 250 mL capacity separating funnel, the 40 mL concentrated filtrate was mixed properly with 20 mL of $C_2H_5OC_2H_5$. The aqueous layer was recovered, whereas the organic layer was decanted. The purification and separation procedure was repeated twice. Finally, 60 mL of n-C_4H_9OH was added to the extract followed by 10 mL of 5% NaCl to precipitate the saponins. The solution was evaporated to dryness in a water bath, dried in an oven and cooled in a dessicator and weighted.

Calculations: The concentrations of alkaloids, flavonoids and anthocyanin (mg/100 g sample weight) in the various samples were calculated thus:

$$C_{AFAS} = \frac{(W2 - W1)}{Wt} \times 100 \qquad (1)$$

Where:
C_{AFAS} = Concentrations of alkaloids, flavonoids, anthocyanin and saponins
W2 = Weights of filter paper + precipitate
W1 = Weight of filter paper
Wt = Weights of samples

HCN: Cyanide content of sample was measured by alkaline titration method as described by Kamalu and Oghome (2012) with minor modifications according to Chikezie and Ojiako (2013). Fifteen gram (15 g) sample was measured into 800 mL Kjedahl flask containing 200 mL of distilled water and allowed to stand for 3 h at 25±4°C. Autolysis was carried out with the apparatus connected to a distiller. A 150 mL of distillate was collected in 20 mL 25% NaOH solution and further diluted to 250 mL with distilled water. Next, 100 mL of the diluted distillate was mixed with 8.0 mL of 6.0 N NH_4OH and 2.0 mL of 5% KI indicator solution and titrated against 0.02 N $AgNO_3$. The end point was indicated by a faint permanent turbidity appearance. The cyanide content (mg/100 g sample weight) of the sample was evaluated from the expression: 1.0 mL 0.02 N $AgNO_3$ = 1.08 mg HCN.

Tannins: The Van Burden and Robinson (1981) method was used to measure the concentration of tannins. A 5 g of the sample was mixed with 50 mL of distilled water for 1 h in a 50 mL capacity plastic bottle on a mechanical shaker. The mixture was filtered with Whatman No. 42 filter paper. A 5 mL aliquot of the filtrate was mixed with 2 mL of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M $K_4(Fe)CN_6$ in a test tube. The absorbance of the analyte was measured at maximum absorptivity (λ_{max}) = 605 nm within 10 min and compared with the standards.

Phenolic: The Folin-Ciocalteau method as described by Waterhouse (2002) was used for the measurement of total phenolic content in the various samples. A 0.2 g of the ground sample was added to 10 mL of concentrated MeOH and agitated for 1 h for proper mixing. The mixture was separated by simple filtration to harvest the extract. The residue was further rinsed with concentrated MeOH to obtain 10 mL mark of extract. Next, 3 test tubes each containing 1.0 mL aliquot of the extract (diluted in 4-folds; D_F = 4 or as desired), standard phenol solution and Folin-Ciocalteau reagent (reagent blank) were measured for their corresponding absorbance at λ_{max} = 520 nm. The concentration of phenol (mg/100 g sample weight) was calculated thus:

$$C_P = \frac{100 \times A_u \times D_F \times C}{Wt \times A_e} \tag{2}$$

Where:
C_p = Concentration of phenol
Wt = Weight of sample
A_u = Absorbance of standard phenol solution
C = Concentration of standard phenol solution
D_F = Dilution factor
A_e = Absorbance of extract

Ascorbic acid: The concentrations of ascorbic acid in the samples were measured by the methods according to Barakat *et al.* (1973). Twenty grams (20 g) of the sample was weighted into an extraction tube with 100 mL of EDTA and mixed properly by agitation for 30 min at 25±4°C. Next, the mixture was centrifuged at 3000 rpm for 20 min. The supernatant was introduced into a 100 mL volumetric flask and made up to the 100 mL mark with EDTA solution. Twenty milliliter (20 mL) of the extract was pipetted into a volumetric flask and 1% starch solution (indicator) was added followed by titration with 20% $CuSO_4$ solution to obtain a dark end point.

Calculations: The concentration of ascorbic acid (mg/100 g sample weight) was calculated thus:

$$C_{ac} = \frac{100 \times 0.88 \times V_E \times T}{W \times V_A} \tag{3}$$

Vitamins B analyses: Vitamins B (riboflavin, thiamine and niacin) contents of the samples were measured by the methods as described by Okwu and Okwu (2004).

Riboflavin: Five grams (5 g) of the sample was extracted with 50% EtOH solution for 1 h at 25±4°C by continuous agitation. The suspension was filter with Whatman No. 42 filter paper. A 10 mL aliquot of the filtrate was mixed with equal volume of 5% $KMnO_4$ solution and 10 mL of 30% H_2O_2 and allowed to stand for 100°C water bath for 30 min. Next, 40% Na_2SO_4 solution was added, mixed properly by continuous agitation for 5 min and the absorbance of the analyte was measured at λ_{max} = 510 nm. Meanwhile, 10 mL riboflavin solution was used as standard and the absorbance measured accordingly.

Thiamine: A 5 g sample was extracted with 50 mL of 1N ethanolic NaOH solution for 30 min. The suspension was filtered with Whatman No. 42 filter paper. A 10 mL aliquot of the filtrate was

treated with 10 mL of 1N $K_2Cr_2O_7$ solution. A 10 mL thiamine solution was used as standard. The absorbance of the test and standard assays were measured at λ_{max} = 360 nm accordingly.

Niacin: In a 100 mL conical flask, 5 g of the sample was mixed with 50 mL $(NH_4)_2SO_4$,followed by the introduction of 0.3 mL of NH_4OH. The suspension was mixed properly at 25±4°C for 30 min by continuous agitation. The mixture was separated by simple filtration using Whatman No. 42 filter paper. A 5 mL 1 N KCN solution was added to10 mL aliquot of the filtrate and acidified with 5 mL of 0.02N H_2SO_4 and mixed properly. A 10 mL niacin solution was used as standard. The absorbance of the test and standard assays were measured at λ_{max} = 470 nm accordingly.

Calculations: The concentrations of riboflavin thiamine and niacin (mg/100 g sample weight) were calculated thus:

$$C_{rtn} = \frac{100 \times A_t \times V_E \times STD_{rtn}}{W \times Astd \times V_A} \qquad (4)$$

Where in Eq. 3 and 4:

Cac	=	Concentration of ascorbic acid
Crtn	=	Concentrations of riboflavin thiamine and niacin
V_E	=	Total extract volume
T	=	Concentration of standard ascorbic acid
W	=	Weight of sample
V_A	=	Volume of extracted analyzed
At	=	Absorbance of test sample
Astd	=	Absorbance of standard solutions of riboflavin, thiamine and niacin
STD_{rtn}	=	Concentrations of standard solutions of riboflavin, thiamine and niacin

Statistical analyses: The data collected were analyzed by the analysis of variance procedure while treatment means were separated by the Least Significance Difference (LSD) incorporated in the statistical analysis system package of 9.1 version 2006.

RESULTS

The leaf of the RFS and YFS of *A. occidentale* L. tree gave the highest concentration of alkaloids, whereas the level of alkaloids was lowest in the fruits of both species. In addition, the results showed that the various plant parts exhibited the same order of concentrations of alkaloids. Similarly, the leaf of the RFS and YFS registered the highest concentration of flavonoids. The fruit and root of the RFS as well as the fruit and stem bark of the YFS gave the lowest concentrations of flavonoids. Conversely, the fruit and leaf of the RFS and YFS gave relatively high concentrations of anthocyanin compared to other parts of the plant.

A close inspection of Table 1 showed that the concentrations of saponins were comparatively, the highest occurring phytochemicals in the various parts of the RFS and YFS of *A. occidentale* L. tree, except in the fruits of both species. For instance, the concentrations of saponins in stem bark of the RFS was 10 folds approximately. higher than the concentration of anthocyanin and 9 folds than that of anthocyanin in the YFS.

Table 1: Concentrations of some phytochemicals of *A. occidentale* L. tree

Phytochemicals	Phytochemicals mg/100 g sample							
	RFS				YFS			
	Fruit	Leaf	S/bark	Root	Fruit	Leaf	S/bark	Root
Alkaloids	0.08 ± 0.01^d	0.46 ± 0.02^a	0.36 ± 0.01^b	0.18 ± 0.01^c	0.06 ± 0.01^d	0.38 ± 0.02^a	0.32 ± 0.01^b	0.18 ± 0.01^c
Flavonoids	0.14 ± 0.05^c	0.22 ± 0.01^a	0.16 ± 0.01^b	0.14 ± 0.01^c	0.12 ± 0.01^c	0.22 ± 0.05^a	0.12 ± 0.01^c	0.16 ± 0.01^b
Anthocyanin	0.16 ± 0.01^d	0.12 ± 0.05^b	0.06 ± 0.01^a	0.04 ± 0.01^b	0.14 ± 0.01^c	0.10 ± 0.01^d	0.06 ± 0.05^a	0.05 ± 0.01^b
Saponins	0.08 ± 0.01^d	0.52 ± 0.01^b	0.58 ± 0.01^a	0.22 ± 0.05^c	0.08 ± 0.01^d	0.46 ± 0.01^b	0.54 ± 0.05^a	0.18 ± 0.01^c
HCN	0.00 ± 0.00^b	$0.12\pm0.15^{b,a}$	0.14 ± 0.01^a	0.00 ± 0.00^b	0.00 ± 0.00^c	0.19 ± 0.01^a	0.19 ± 0.26^b	0.00 ± 0.00^c
Phenolic	0.25 ± 0.01^d	0.49 ± 0.03^a	0.39 ± 0.01^b	0.29 ± 0.05^c	0.23 ± 0.05^d	0.43 ± 0.01^d	0.38 ± 0.05^b	0.27 ± 0.01^c
Tannins	0.12 ± 0.05^d	0.16 ± 0.01^c	0.32 ± 0.05^a	0.24 ± 0.05^b	0.12 ± 0.01^d	0.14 ± 0.01^c	0.26 ± 0.01^a	0.22 ± 0.05^b

Values are Means±Standard error of mean of triplicate determinations. Means in the rows with the same letters are not significantly different at $p>0.05$ according to LSD. S/bark: Stem bark

Table 2: Concentrations of four hydrophilic vitamins of *A. occidentale* L. tree

Vitamins	Vitamins mg/100 g sample							
	RFS				YFS			
	Fruit	Leaf	S/ bark	Root	Fruit	Leaf	S/bark	Root
A/acid	134.20 ± 0.12^a	28.60 ± 0.09^b	14.30 ± 0.12^c	3.30 ± 0.08^d	135.08 ± 0.10^a	$26..40\pm0.08^b$	13.20 ± 0.02^c	4.40 ± 0.03^d
Niacin	0.23 ± 0.05^a	0.12 ± 0.05^b	0.12 ± 0.02^b	0.12 ± 0.07^b	0.12 ± 0.05^a	0.06 ± 0.03^b	0.12 ± 0.02^a	0.12 ± 0.02^a
Riboflavin	2.90 ± 0.05^b	5.90 ± 0.03^a	2.90 ± 0.02^b	2.00 ± 0.07^b	3.90 ± 0.05^a	4.90 ± 0.05^a	2.00 ± 0.10^b	2.01 ± 0.06^b
Thiamine	15.50 ± 0.11^a	10.50 ± 0.02^b	7.25 ± 0.03^c	3.32 ± 0.06^d	16.10 ± 0.04^a	11.00 ± 0.05^b	5.25 ± 0.04^c	2.25 ± 0.04^d

Values are Means±Standard error of mean of triplicate determinations. Means in the rows with the same letters are not significantly different at $p>0.05$ according to LSD. S/bark: Stem bark, A/acid: Ascorbic acid

HCN was absent in the fruits and roots of the RFS and YFS. Again, the leaf of the RFS and YFS gave the highest concentrations of phenolics. Furthermore, the concentrations of phenolics in the fruits and roots of both species were not significantly different ($p > 0.05$). The stem bark of both the RFS and YFS gave the highest concentration of tannins, whereas the lowest concentrations of tannins occurred in the fruits of both species.

Table 2 showed that the concentrations of the Four Hydrophilic Vitamins (FHV) in the fruit of the RFS were in the order: Ascorbic acid = 134.20±0.12 mg/100 g >thiamine = 15.50±0.11 mg/100 g>riboflavin = 2.90±0.05 mg/100 g>niacin = 0.23±0.05 mg/100 g. in addition, the order of concentration of the FHV in the fruits of the RFS was analogous to the order of concentrations of the FHV in the leaf, stem bark and root of both the RFS and YFS.

Generally, the fruits of the RFS and YFS contained the highest levels of the FHV, except riboflavin concentrations, which were highest in the leaves the RFS and YFS, compared to other parts of *A. occidentale* L. trees, viz., the leaf, stem, bark and root. Finally, an overview of Table 2 showed that the relative abundance of the FHV in the various parts of the RFS and YFS were comparable and not significantly different ($p>0.05$) from each corresponding concentrations of the two species of *A. occidentale* L.

DISCUSSION

The present study revealed the presence of uneven distribution of phytochemicals, namely, alkaloids, flavonoids, anthocyanins, saponins, CGs, phenolics and tannins in the various parts

(fruits, leaf, stem bark and roots) of the RFS and YFS of *A. occidentale* L. In the same vein, Milugo *et al.* (2013), had reported a non-uniform distribution of phytochemicals in methanol leaves and stem bark extracts of quinine tree (*Rauvolfia caffra*). Furthermore, they noted that genetic variation among plant species may elicit variation in phytochemical composition, thus affecting the relative abundance of bioactive compounds among different plant species and various organs of the same plant species. These opinions were indications that individual plant organs and tissues do not share the same capacity to synthesize and/or store phytochemicals.

Phytochemicals are sources of dietary supplements for animal nutrition and have been demonstrated to exhibit medicinal properties (Edeoga and Eriata, 2001; Lila and Raskin, 2005; Hussain *et al.*, 2011). However, there are safety concerns about the medicinal use of crude plant extracts, especially in situations when some of these phytochemicals occur at toxic levels (Tedong *et al.*, 2007; Milugo *et al.*, 2013), some of which have also been known to possess certain antinutritional qualities (Makkar and Becker, 1999; Singh *et al.*, 2003). Alkaloids possess significant therapeutic potentials, which are partly or wholly, ingredients of many important medicines applied as analgesic, antispasmodic and antibacterial agents (Okwu and Okwu, 2004; Ayepola and Ishola, 2009; Yadav and Agarwala, 2011; Penecilla and Magno, 2011). Research findings according to Ntsoelinyane and Mashele (2014) stated that free radical scavenging property of alkaloids was responsible for its antiinflammatory, anticarcinogenic, antibacterial and antiviral activities, which was in concord with the opinions of Sala *et al.* (2002). Accordingly, Brazilian traditional medicinal practice uses stem bark of *A. occidentale* L. for the treatment of gastric and inflammatory disorders, of which the efficacy has been clinically affirmed in the reports by Vanderlinde *et al.* (2009). Also, medicinal plants that contain relatively strong antioxidant compounds such as alkaloids have been applied in therapy against cardiovascular diseases (CVDs) and cancer (Trevisanato and Kim, 2000) and age related conditions such as Alzheimer's disease or dementia (Commenges *et al.*, 2000).

The present study showed that the leaf and stem bark of the RFS and YFS of *A. occidentale* L. tree are rich sources of alkaloids compared to the other plant parts investigated. Accordingly, previous reports have validated the efficacy of *A. occidentale* L. extracts in the treatment of several pathologic conditions, in which alkaloids, among other bioactive agents, were considered to be the contributing agents to the therapeutic actions of *A. occidentale* L. For instance, previous investigations have shown that alkaloids in the extract of *A. occidentale* L. are cytotoxic to certain cancerous cells (http://www.rain-tree.com/cajueiro.htm). Also, there are several reports on the antimicrobial activity of *A. occidentale* L. against *Escherichia coli* (Kudi *et al.*, 1999; Akinpelu, 2001), *Pseudomonas* (Kudi *et al.*, 1999) and *Helicobacter pylori* (Ofusori *et al.*, 2008). According to reports by Ababutain (2011), several phytochemicals from different plants species inhibit bacterial activity by membrane disruption, intercalation of DNA/cell wall components and inactivation of enzyme complexes.

The flavonoids have been widely reported for their antioxidant potentials (Bors *et al.*, 1990; Pietta, 2000). Very recently, Rehman *et al.* (2013) demonstrated the antioxidant potentials of aqueous-methanolic extract of *Suaeda fruticosa*, which was averred to be due to the presence of flavonoids and tannins. They noted that paracetamol-induced hepatotoxicity was ameliorated by the free radical scavenging effect of flavonoids, which promoted the regeneration of hepatic cells and stabilization of plasma membrane of hepatocytes in experimental rabbits. In another report, extracts of *A. occidentale* L. have been empirically demonstrated to exhibit membrane stabilization activity and amelioration of oxidative stress of human sickle erythrocyte *in vitro* (Chikezie and

Uwakwe, 2011; Chikezie, 2011). Barcelos *et al.* (2007) had earlier shown that stem bark methanolic extract of *A. occidentale* L. exhibited anti-mutagenic and anti-genotoxic effects on Chinese hamster lung fibroblasts V79, which may not be unconnected with the therapeutic action of flavonoids of *A. occidentale* L. Paradoxically, Konan *et al.* (2012) reported that cashew flavonoids are toxic towards malignant cell by inducing apoptosis in Jarkat (acute lymphoblastic leukemia) cells.

In another study, Kurowska and Manthey (2004) showed that polymethoxylated flavones and flavanone glucosides have cholesterol and triacylglycerol-lowering potential in hamsters with diet-induced hypercholesterolemia. In a related study, Larson *et al.* (2012) reported the therapeutic potentials of quercetin (flavonol) to reduce blood pressure and risk of CVD, which was in conformity with earlier reports in this regard (Geleijnse *et al.*, 2002; Knekt *et al.*, 2002). Findings from the present investigations showed that the fruit, leaf, stem bark and root of the RFS and YFS of *A. occidentale* L. contained approximately equivalent quantities of flavonoids. Expectedly, *A. occidentale* L. is commonly used for the treatment of hypertension in Côte d'Ivoire and investigations showed that aqueous *A. occidentale* L. bark extract applied intravenously caused dose-dependent decrease in blood pressure of previously normotensive rabbits (Tchikaya *et al.*, 2011). Their report further noted that action of extracts of *A. occidentale* L. on heart contractile activity in modified heart culture media elicited cardio-inhibitory activity. Similar reports have revealed anti-diabetic and renal protective potentials of leaf extract of *A. occidentale* L. (Adjanohoun *et al.*, 1988; Ojewole, 2003; Tedong *et al.*, 2006; Saidu *et al.*, 2012). Diabetes and renal insufficiency are risk factor for hypertension, which can precipitate CVD.

Anthocyanins also act as powerful antioxidants (Williams *et al.*, 2004; Lotito and Frei, 2006; Lachman *et al.*, 2009) with similar capability to neutralize free radicals linked to oxidative stress disorders. For instance, anthocyanins possess neuro-protective, anti-inflammatory and anti-atherogenesis activities (Del Bas *et al.*, 2005; Cazarolli *et al.*., 2008; Korte *et al.*, 2009). The anti-inflammatory actions of stem-bark of *A. occidentale* L. has been documented in traditional medicinal healing practice in Nigeria (Ojewole, 2004). Also, anthocyanins constituent of *A. occidentale* L. is a subset of the various contributing bioactive principles responsible for the anti-diabetic property of *A. occidentale* L. in line with previous reports of Ogbede *et al.* (1986) with respect to anti-diabetic properties of *Cnestis ferruginea* extracts. Precisely, the fruit and leaf of the two species of *A. occidentale* L. are particularly rich in anthocyanins compared to the stem bark and root.

The antimicrobial activity of *A. Occidentale* L. has been attributed to its saponins content (Concalves *et al.*, 2005). Fittingly, extracts of *A. occidentale* L. have effectively been applied for the treatment of diarrhea and bacterial infections (Ayepola and Ishola, 2009). In addition, the capability of phytosterols e.g., β-sitosterol (saponins) to impede intestinal absorption of cholesterol could be one among other several factors that are responsible for plasma cholesterol lowering effect of *A. occidentale* L. as previously reported (Kurowska and Manthey, 2004). It is important to note that non-cardioactive steroidal glycosides of saponins present in Alfalfa, cockles and English ivy can provoke gastric upset (Manahan, 2003) and therefore, could be administered as purgative. The aglycone saponins are powerful antioxidant that can curb CVD (Oakenfulll and Sidhu, 1990). Aqueous leaf extract of *A. occidentale* L. has been reported to inhibit polymerization of sickle cell haemoglobin *in vitro* (Chikezie, 2011). Specifically, the role of saponins in preventing sickle cell crisis has been demonstrated by the works of Imaga (2010). In addition to the medicinal benefits of saponins, studies have established that saponins extracted from *Medicago truncatula* seeds exhibited strong toxic activity towards the adults of the rice weevil *Sitophilus oryzae* (Coleoptera)

(Da Silva *et al.*, 2012). On the grounds of results of the present study, in spite of the presence of relatively high concentrations of saponins in the leaf and stem bark of the two species of *A. occidentale* L. the insecticidal potentials of extracts of *A. occidentale* L. have not been evaluated and exploited.

More than four decades ago, tannins containing beverages, especially green teas and red wines had been suggested to having the capability to cure or prevent CVD (Van Burden and Robinson, 1981). Therapeutic tannins are administered as antimicrobial, protein synthesis inhibitors and in the treatment of non-specific diarrheas (Scalbert, 1991; Shimada, 2006; Westendarp, 2006). Specifically Mota *et al.* (1985), reported that tannins extracted from the stem bark of *A. occidentale* L. possess analgesic and anti-inflammatory activities and these findings were reaffirmed by Vanderlinde *et al.* (2009). *In vitro* studies have shown the antimicrobial activity of phenols against wide array of microorganisms (Ntsoelinyane and Mashele, 2014). The present report showed that stem bark and leaf of both the RFS and YRV of *A. occidentale* L. are rich sources of tannins and phenolics.

HCN is highly toxic and there are no therapeutic values attached to the administration of HCN. The negligible HCN contents in the leaf and stem bark of *A. occidentale* L. conformed to the reports of Okonkwo *et al.* (2010) and affirmed the unreported incidence of cyanide toxicity following the consumption of plant materials derived from *A. occidentale* L. In animals, the lethal doses of HCN are generally between 0.66 and 15 mg kg^{-1} b.wt. (bw) for various species, whereas acute lethal dose of HCN for human beings is between 0.5-3.5 mg kg^{-1} b.wt. (FSANZ, 2004). Intuitively, the absence of HCN in the fruit and root of the RFS and YRV of *A. occidentale* L. was an indication that liberation of HCN does not serve as a primary chemical defense arsenal against invading animals.

Ascorbic acid is a low molecular weight antioxidant required by biologic systems for the neutralization of free radicals that are associated with degenerative/pathologic conditions occasioned by the process of ageing. A related study according to Kubo *et al.* (1994) showed that extracts of *A. occidentale* L., curbed the darkening effect associated with aging by inhibiting tyrosinase activity. The fruits of the RFS and YRV of *A. occidentale* L. are particularly rich in ascorbic acid and therefore recommended for rapid wound healing and prevention of scurvy (Hunt *et al.*, 1980; Hussain *et al.*, 2011). Generally, plant materials rich in ascorbic acid are administered as herbal remedies for common cold and certain cancers (Okwu and Okwu, 2004; Njoku and Akumefula, 2007). Niacin is an integral component of the reducing equivalents, NADH+H$^+$ and its phosphate derivative-NADPH+H$^+$, which are indispensible cofactors of cellular metabolism. Adequate dietary intake of niacin prevents pellagra, a disease involving the gastrointestinal tract and central nervous system. Based on the relative abundance of ascorbic acid, niacin, thiamine and riboflavin in the RFS and YRV of *A. occidentale* L. the fruit extract in particular of the plant could serve as source of dietary supplements for these vitamins. In the same vein, Makkar and Becker (1996) had previously proposed that *Moringa oleifera* leaves are ideal dietary supplement for essential amino acids and vitamins.

Toxicological studies pertaining to acute and sub-chronic administration of *A. occidentale* L. extracts have previously been reported elsewhere (Tedong *et al.*, 2007; Okonkwo *et al.*, 2010). According to Okonkwo *et al.* (2010) serum indicators showed that sub-chronic administration of inner stem bark extract of *A. occidentale* L. did not significantly (p<0.05) depress liver function in Wistar rats. However, Tedong *et al.* (2007) noted that toxic effects of *A. occidentale* L. hexane leaf extract occurred in mice at higher doses than those used in cameroon folk medicine. Other relevant toxicological properties of the bark and fruits extracts of *A. occidentale* L. were also mentioned in

the reports of Tedong *et al.* (2007). Although, these studies provided limited insights into the safety concerns that are pertinent to the administration of *A. occidentale* L. extracts, the outcome of the present study has substantiated and justified the application of extracts of this multipurpose economic tree in ethnomedicinal practices and herbal remedies.

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