Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
NAAS Rating: 3.53
JMPS 2018; 6(6): 162-170

Received: 22-09-2018
Accepted: 23-10-2018

Njoya HK
Department of Biochemistry, School of Basic Medical Sciences, College of Health Sciences, Igbinedion University Okada, Edo, Nigeria

Chukwu E Onyeneke
Department of Biochemistry, Faculty of Life Sciences, University of Benin, Edo, Nigeria

Okwuonu CU
Department of Anatomy, School of Basic Medical Sciences, College of Health Sciences, Igbinedion University Okada, Edo, Nigeria

Erifeta GO
Department of Biochemistry, School of Basic Medical Sciences, College of Health Sciences, Igbinedion University Okada, Edo, Nigeria

Correspondence
Njoya HK
Department of Biochemistry, School of Basic Medical Sciences, College of Health Sciences, Igbinedion University Okada, Edo State Nigeria

Phytochemical, proximate and elemental analysis of the African mistletoe (*Tapinanthus preussii*) crude aqueous and ethanolic leaf extracts

Njoya HK, Chukwu E Onyeneke, Okwuonu CU and Erifeta GO

Abstract
In ethnotherapy, the African mistletoe (*Tapinanthus preussii*) is implicated as being medicinal, without any scientific validation. Consequently, to ascertain this claim, this study was designed to investigate its phytoconstituents. The phytochemical analysis revealed that varied significant concentrations of alkaloids, carotenoids, flavonoids, anthraquinones, tannins, steroids, antioxidants and saponins were present in both the aqueous and ethanolic extracts; however terpenoids, phlobatannins, phenolics and cardiac glycosides were in trace concentrations. In the proximate analysis, moisture and protein content was significantly (p<0.05) higher in the aqueous extract compared to the ethanolic extract, carbohydrate and lipid contents were significantly higher in the ethanolic extract while trace amounts of the ash content was present in the aqueous extract. The essential minerals Fe^{2+}, Ca^{2+}, K^{+}, Na^{+}, Mg^{2+} and PO_4^{2-} were significantly (p<0.05) higher in the aqueous extract relative to the ethanolic extract.. These findings, strongly suggest that *Tapinanthus preussii* leaves are relevant both nutritionally and medicinally.

Keywords: Ethnotherapy, phytochemistry, proximate, essential minerals, *Tapinanthus preussii*

Introduction
Mistletoes are aerial semi/quasi -parasitic ever-green shrubs which depend on their host tree for minerals and water supply. The leathery green and oblong leaf of this plant contains chlorophyll that enables it to photosynthesize carbohydrate from carbon dioxide and water, like all other green plants hence eliminating it is as true parasite. Its root system called haustoria anchors and invades the internal tissues of the host tree, which could be either edible or non edible evergreen and deciduous trees all year round (Gill, 1973; Judd *et al*., 2002) [1, 2], basically extracting water and minerals, at its expense (Griggs, 1991) [3]. This plant is quite versatile with over 700 species known world-wide (Gill, 1973) [1] belonging to one of several types based on its location e.g. European, Korean, American, African, Japanese or Argentinean mistletoe.

According to ethno-botanical information, more than eight hundred medicinal plants and their products including the African mistletoe (*Tapinanthus preussii*) are increasingly gaining grounds as alternatives to orthodox medicine especially in developing countries (Murray, 1998) [4]. Their use as decoction, extract or infusion is a widespread practice as sources of prophylaxis, treatment and management in myriads of abnormal conditions, some of which include cardiovascular and neurological disorders, antitumor, antimicrobial activities (Owolabi *et al*., 2007) [5] as well as their complications traditionally (Iwu, 1980; Alarcon-Aguilara *et al*., 1998) [6, 7]. Although scientific evaluations of some of these plants have been carried out using several experimental models of varied disorders; clinical studies are rare. Hence these medicinal herbs have not gained enough momentum in medicine due to the absence of specific standardized raw materials and extracts for formulation of phytomedicines as well as content uniformity and therapeutic effectiveness. With proper investigations these may serve as a source of modern drugs since quinine, digitalis, atropine, etc were obtained from medicinal plants. They may also serve as a source of intermediate compounds for synthesizing analogue drugs with more desirable properties. In developing countries their use has helped to substitute imports of some drugs, thus boosting economic self-reliance.

Medicinal importance of plants has been attributed to the presence of secondary metabolites (phytochemicals) because they are believed to activate, catalyze or initiate some curative reactions in humans (Dwuma-Badu *et al*., 1986) [8].

These phytochemical compounds can he concentrated in any plant part like the leaves, roots, bark, stem, flowers and rhizome. These phytocompounds include alkaloids, flavonoids, tannins, terpenes, sterols, saponins, glycosides, lycopene, Isoflavones, B- carotene etc (Ray *et al*., 2006) [9] flavours (e.g. taxol, artemisinin) and cidal agents (e.g. thiophenes, thiarubrimes) which are responsible for the pharmacological activity of the plant (Gill and Akinwumi, 1986) [10]. They are non nutrient natural bioactive compounds produced by biological synthesis in very minute concentrations of the dry material content found in fruits, vegetables, grains and other plant foods (Honork, 1992) [11] responsible for protecting the plant against pathogenic fungi, herbivorous vertebrates, herbivorous insects, bacterial and viral infestations (Ajaiyeoba *et al*., 2005; El – Mahmood *et al*., 2008; Doughari *et al*., 2008) [12, 13, 14]. These secondary metabolites are bioactive, work with nutrients and dietary fibres and have been linked to protect/reduce the risk of major human diseases (Hark and Deen, 2006) [15]. Studies which address the effectiveness of different traditional medicinal treatments generally evaluate their potential for pharmaceutical development.

Medicinal plants are known to be important sources of new chemical substances with potential therapeutic effects (Farnsworth, 1989) [16], such as antioxidants, antimicrobials, anti-inflammatory and anticarcinogens. Others are biocides against a broad range of organisms (Kalemba and Kunica, 2003) [17]. The chemical constituents in medicinal plants usually explain the rationale for the use of the plant in traditional medicine. The trend now is that phytochemists exploit medicinal plants and isolate bioactive compounds from which different analogues are synthesized with the aim of obtaining agents with better actions or even different biological properties. Plant active constituents thus serve as templates for future drug development (Cline, 1985) [18]. Studies which address the effectiveness of different traditional medicinal treatments generally evaluate their potential for pharmaceutical development. Thus, information can be found in chemical and pharmacological studies which analyze the phytochemistry of different species. Most however, do not link the presence of a particular substance (e.g. flavonoids) with its traditional use (Dwuma-Badu *et al*., 1986) [8].

Some phytochemicals with physiological properties may be elements rather than complex organic molecules. Abundant in many fruits and vegetables, selenium, for example, is involved with major metabolic pathways, including thyroid hormone metabolism and immune function (Brown and Arthur, 2001) [19]. Selenium is particularly important as an essential nutrient and co-factor for enzymatic synthesis of glutathione, an endogenous antioxidant (Papp *et al*., 2007) [20]. Therefore this study was carried out in order to investigate the phytochemistry, proximate and elemental composition of the African mistletoe *Tapinanthus preussii* in order to establish its medicinal potentials.

Materials and Methods

Reagents/chemicals

All reagents and chemicals used were of analytical grade and products of Aldrich Laboratory, Germany,), British Drug House (BDH) England, E. Merck, Darmstadt, Germany and Aldrich Chemical Company, USA.

Methods

Collection, identification and authentication of *Tapinanthus preussii* leaves

A reasonable quantity of fresh *Tapinanthus preussii* leaves; parasitic on cocoa (*Theobroma cacao*) was harvested from a cocoa plantation located in Kumba, Meme Division, South west region of Cameroon at midday, January 2017. They were identified and authenticated by Mr.

Litonga Ndive Elias a botanist in the Limbe Botanic gardens in the South West Region of Cameroon and a voucher specimen was deposited in the herbarium for future reference.

Extraction of *Tapinanthus preussii* leaves

The harvested *Tapinanthus preussii* leaves were thoroughly rinsed with distilled water to remove debris. Thereafter, air dried in the shade for four days at room temperature and further oven dried at 40^{o}C until a constant weight was obtained. The dried leaves were then pulverized into very fine powder using an electric grinder. (Harborne, 1998; Sofowara, 1993) [21, 22] and eventually cold macerated in two different extraction solvents: 95% ethanol and distilled water for 72 hours respectively according to standard methods (Williams *et al.*, 1996) [23].

Proximate analysis of *Tapinanthus preussii* leaves

The determination of crude fat, protein and crude fibre in the whole crude, aqueous and ethanolic extracts of the *Tapinanthus preussii* leaves were carried out by the methods of Association of official Analytical Chemist (A.O.A.C. 1996) [24] while the carbohydrate content was calculated by difference.

Estimation of minerals/elements in *Tapinanthus preussii* leaves

The estimation of sodium and potassium in the whole crude, aqueous and ethanolic extracts of *Tapinanthus preussii* leaves was done by flame emission photometry using the Corning 421 flame emission photometer (Isaac and Kerber, 1972) [25]. Whereas the estimation of the other microminerals/elements like zinc, magnesium and iron was carried out by atomic absorption spectrophotometry using the Perkin-Elmer model 403 atomic absorption spectrophotometer (Perkin-Elmer Corp. Notwalk Connecticut) with acetylene/air flame (Perkin-Elmer, 1997) [26].

Estimation of phytochemical constituents in *Tapinanthus preussii* leaves

Qualitative phytochemical tests for the presence of tannins, anthraquinones, steroids, alkaloids, terpenoids, cyanogenic glycosides, phlobatannins, saponins, anthocyanins, flavonoids, and cardiac glycosides, were carried out on the aqueous extracts, ethanolic extracts and the powdered specimens of *Tapinanthus preussii* leaves using standard conventional procedures as described by Trease (1989) [27] and Sofowara (1993) [22].

A Spectroscopic technique using the ultra violet-visible spectrophotometer (cyberlab USA), at a range of 200-800 nm was employed to quantitatively detect and record the characteristic peaks of the wavelengths to confirm the phytochemicals present in the whole crude, aqueous and ethanolic leaf extracts (Harborne, 1984) [28].

Statistical analysis

Data collected from this study were subjected to statistical analysis using the Statistical Package for Social Sciences (SPSS) version 20 (IBM, 2013) [29]. The results are expressed as mean ± SEM. One-way Analysis of variance (ANOVA) was also used to compare the means of the parameters measured and where significant differences were observed, at 95% confidence level (p<0.05) Duncan's New Multiple

Range test was used to separate the significantly different means.

Results

Yield of *Tapinanthus preussii* crude leaf extracts.

The aqueous extract gave a yield of 20% w/w while the ethanolic extract gave a yield of 12.06% w/w.

Proximate composition of *Tapinanthus preussii* leaves

The proximate composition of the whole crude, aqueous and ethanolic extracts of *Tapinanthus preussii* leaves expressed as percentage of dry matter is represented in Table 1. It was observed that there were discrepancies in the percentage of all the substances determined. The moisture content was seen to be significantly ($p<0.05$) higher in the aqueous extract (94.6 ± 0.1%) compared to the whole crude and ethanolic extract which were 8.0 ± 0.04% and 6.4 ± 0.09% respectively. The protein content was significantly ($p<0.05$) higher in the whole crude (14.7 ± 0.29) compared to that obtained in the aqueous and ethanolic extracts which were as low as 0.5% and 0.2 ± 0.03% respectively. The total lipid content (ether extract) was completely absent in the aqueous extract but present in the whole crude and ethanolic extracts only in very minute quantities i.e. 0.2 ± 0.03% and 0.4 ± 0.03% respectively. The Ash content was significantly ($p<0.05$) higher in the whole crude (3.6 ± 0.06%) than in the aqueous extract (0.1%) but was completely absent in the ethanolic extract. The crude fibre was present only in the whole crude dry matter and completely absent in both the aqueous and ethanolic extracts. The carbohydrate content was significantly ($p<0.05$) higher in the ethanol extract (93.0 ± 0.1%) than that of the dry matter and aqueous extracts which were 69.3 ± 0.05% and 4.7 ± 0.1% respectively.

Table 1: Proximate analysis of the whole crude dry matter, aqueous and ethanolic extracts of T*apinanthus preussii* leaves

Parameter	Content (%)		
	Whole crude	Aqueous extract	Ethanolic extract
Moisture Content	8.0 ± 0.04^{b}	94.6 ± 0.1^{a}	6.4 ± 0.09^{c}
Protein	14.7 ± 0.29^{a}	0.5 ± 0.00^{b}	0.2 ± 0.03^{c}
Ether Extract (Fat)	0.2 ± 0.03^{b}	0.00 ± 0.00^{c}	0.4 ± 0.03^{a}
Ash	3.6 ± 0.06^{a}	0.1 ± 0.00^{b}	0.00 ± 0.00^{b}
Crude Fibre	4.27 ± 0.03^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
Carbohydrates (By difference)	69.3 ± 0.15^{b}	4.7 ± 0.1^{c}	93.0 ± 0.1^{a}

All values represent mean ± standard error of mean of triplicate determinations. Means of the same row followed by different lettered superscripts differ significantly ($p<0.05$).

Elemental analysis of *Tapinanthus preussii* leaves

The elemental composition of the whole crude, aqueous and ethanolic extracts of *Tapinanthus preussii* leaves expressed as mg/100 g of dry matter are shown in Table 2. The results of the macro minerals showed that there was a significantly ($p<0.05$) higher concentration of all the available minerals in the whole crude compared to the aqueous and ethanolic extracts. It was also observed that the content of all the elements (minerals) present were significantly ($p<0.05$) higher in the aqueous extract compared to the ethanolic extract. The whole crude contained low levels of Zinc (0.33 ± 0.03 mg/100 g) and only trace amounts were present in the aqueous extract (0.01 ± 0.00 mg/100 g) whereas it was completely absent in the ethanolic extract. There were however trace amounts of lead (0.01 ± 0.00 mg/100 g) in the whole crude but it was not detected in both the aqueous and ethanolic extracts. There was complete absence of cadmium in the entire plant material and extracts.

Table 2: Elemental composition of the crude dry matter, aqueous and ethanolic extracts of *Tapinanthus preussii* leaves

Parameter	Content (mg/100 g)		
	Whole crude	Aqueous Extract	Ethanolic Extract
Fe^{2+}	9.4 ± 0.10^{a}	1.7 ± 0.08^{b}	0.01 ± 0.003^{c}
Ca^{2+}	281.7 ± 1.67^{a}	4.03 ± 0.08^{b}	0.5 ± 0.00^{c}
K^{+}	72.3 ± 1.45^{a}	0.6 ± 0.00^{b}	0.02 ± 0.003^{c}
Na^{+}	53.3 ± 1.67^{a}	6.2 ± 0.08^{b}	0.9 ± 0.003^{c}
Mg^{2+}	35.00 ± 0.00^{a}	0.5 ± 0.03^{b}	0.2 ± 0.00^{c}
PO_4^{---}	225.0 ± 0.00^{a}	0.8 ± 0.03^{b}	0.02 ± 0.00^{c}
Pb^{2+}	0.01 ± 0.00^{a}	ND	ND
Zn^{2+}	0.33 ± 0.03^{a}	0.01 ± 0.00^{b}	0.00^{b}
Cd^{2+}	ND	ND	ND

ND = not detected. All values represent mean ± standard error of mean (SEM) of triplicate determinations. Means of the same row followed by different lettered superscripts differ significantly ($p<0.05$).

Qualitative phytochemical constituents of *Tapinanthus preussii* leaves

The qualitative phytochemical composition of the whole crude leaf, aqueous and ethanolic extracts of *Tapinanthus preussii* determined are summarized in Table 3. It was generally observed that there were high levels of carotenoids whereas anthocyanins, cyanogenic glycosides and terpenoids reflected the absence of detectable quantities in the whole crude, aqueous and ethanolic extracts. The whole crude leaves also contained high levels of the following bioactive principles: alkaloids and flavonoids while anthraquinones, saponins, steroids and tannins were moderately detectable, whereas antioxidants, cardiac glycosides, phlobatannins and phenolics were only slightly detectable. In the aqueous extracts there were however high concentrations of flavonoids and saponins present but alkaloids, antioxidants,, phenolics and tannins were only moderately detectable meanwhile anthraquinones, cardiac glycosides and steroids were slightly detectable. Ethanolic extracts indicated that tannins were moderately detectable but alkaloids, antioxidants, cardiac glycosides, flavonoids, phlobatannins, saponins, steroids and phenols were only slightly detectable.

Table 3: Qualitative phytochemical composition of the crude dry matter, aqueous and ethanolic extracts of *Tapinanthus preussii* leaves

Parameter	Results		
	Whole Crude	Aqueous Extract	Ethanolic Extract
Alkaloids	+ + +	+ +	+
Anthocyanins	-	-	-
Anthraquinones	+ +	+	-
Antioxidants	+	+ +	+
Cardiac glycosides	+	+	+
Carotenoids	+ + + +	+ + +	+ + +
Cyanogenic glycosides	-	-	-
Flavonoids	+ + +	+ + +	+
Phlobatannins	+	-	+
Phenolics	+	+ +	+
Saponins	+ +	+ + +	+
Steroids	+ +	+	+
Tannins	+ +	+ +	+ +
Terpenoids	-	-	-

- = Absence of detectable quantity, + = slightly detectable, ++ = moderately detectable, ≥ +++ = High level.

Quantitative phytochemistry of *Tapinanthus preussii* leaves

The means of the quantitative phytochemical estimation of the whole crude, aqueous, and ethanolic extracts of *Tapinanthus preussii* leaves screened for secondary metabolites are summarized in Table 4. The whole crude, aqueous and ethanolic extracts showed the presence of all the tested phytochemicals in varied concentrations except cyanogenic glycosides that were not detected. Phytochemicals found in the aqueous extracts in decreasing order were as follows: alkaloids (770 ± 7.64 mg/100 g), carotenoids (235 ± 0.00 mg/100 g), flavonoids (178.33 ± 4.41 mg/100 g), anthraquinones (171.67 ± 6.01 mg/100 g), tannins (75 ± 0.00 mg/100 g), steroids (73.33 ±4.41 mg/100 g), antioxidants (44.77 ± 0.5 mg/100 g), saponins (38.33 ± 1.67 mg/100 g) and the rest were in trace concentrations. The levels were however different in the ethanolic extracts where it was observed that the phytochemicals in decreasing order were: carotenoids (541 ± 4.41 mg/100 g), flavonoids (323.33 ± 6.01 mg/100 g), tannins (130 ± 7.64 mg/100 g), steroids (116 ± 4.41 mg/100 g) while all others were present in minute concentrations. It can be concluded that of the phytochemicals present in the extracts, alkaloids, anthraquinones and saponins were significantly (p<0.05) higher in the aqueous extract compared to the concentrations in the ethanolic extract whereas carotenoids, flavonoids, tannins, steroids, antioxidants, phlobatannins and terpenoids were significantly (p<0.05) higher in the ethanolic extract than in the aqueous extract. No significant (p>0.05) differences were observed in all other components that were present in trace amounts i.e. cardiac glycosides and phenolics in both extracts.

Table 4: Quantitative phytochemical screening of the crude dry matter, aqueous and ethanolic extracts of *Tapinanthus preussii* leaves

Parameter	Whole Crude (mg/100g)	Aqueous Extract (mg/100g)	Ethanolic Extract (mg/100g)
Alkaloids	1338.0 ± 25.22^{a}	770.0 ± 7.64^{b}	05.3 ± 1.67^{C}
Anthocyanins	65.00 ± 50.00^{a}	16.67 ± 1.67^{b}	11.67 ± 1.67^{C}
Anthraquinones	230.0 ± 5.00^{a}	171.67 ± 6.01^{b}	58.33 ± 4.41^{C}
Antioxidants (DPPH% inhibition)	67.17 ± 0.20^{a}	44.77 ± 0,50^{c}	47.60 ± 0.40^{b}
Cardiac glycosides	12.00 ± 0.00^{a}	3.17 ± 0.17^{b}	3.17 ± 0.17^{b}
Carotenoids	1857.0 ± 4.41^{a}	235.0 ± 0.00^{c}	541 ± 4.41^{b}
Cyanogenic glycosides	ND	ND	ND
Flavonoids	855.00 ± 7.64^{a}	178.33 ± 4.41^{c}	323.33 ± 6.01^{b}
Phlobatannins	24.00 ± 1.00^{a}	13.33 ± 1.67^{b}	23.33 ± 2.89^{a}
Phenolics	18.33 ± 0.60^{a}	6.0 ± 0.00^{b}	5.33 ± 0.24^{b}
Saponins	43.33 ± 1.67^{a}	38.33 ± 1.67^{a}	26.67 ± 1.67^{b}
Steroids	230.00 ± 7.64^{a}	73.33 ± 4.41^{c}	116.00 ± 4.41^{b}
Tannins	173.33 ± 4.41^{a}	75.00 ± 0.00^{c}	130.0 ± 7.64^{b}
Terpenoids	35.00 ± 0.00^{a}	11.65 ± 1.67^{b}	38.33 1.67^{a}

ND = not detected. All values represent mean ± standard error of mean of triplicate determinations. Means of the same row followed by different lettered superscripts differ significantly (p<0.05).

Discussion

Although *Tapinanthus preussii* has been advocated as a traditional plant treatment in folkloric medicine in parts of Southern Nigeria, Gabon and Cameroon, scientific studies to evaluate its efficacy are lacking. It is a plant parasitizing on *Theobroma cacao* and belongs to the family Loranthaceae commonly referred to as the African mistletoe. A wide range of biological activities, have been ascribed to this group of plants including: antidiabetic, anti-inflammatory, antimicrobial, antioxidant and anticancer potentials.

The percentage yield obtained for *Tapinanthus preussii* leaves was 20% aqueous and 12.06% ethanolic extracts. This implies that in this study water was a better extracting solvent relative to ethanol. The result of this study was in agreement with that reported by Doughari *et al*. (2008) [30] who obtained a relatively higher yield of 52% aqueous extract for *Sena obtusifolia* whereas El- Mahmood (2009) [31] obtained 3.9% for *E. hirta* which was relatively lower. On the other hand, Kantalak *et al*. (2004) [32] obtained a relatively higher yield of ethanolic extracts for the following plants: 20.8% *H. suaveolens*, 24.4% *M.cordifolia piz ex fresen*, 22.7% *O. basilicam* L. and 28.2% *Forma citratum* bark.

The result of this study reveals that, different solvents of extraction as well as methods of extraction affect changes in

the content of the extracts. The type of solvents used are of great importance here, because, the polarity of some metabolites like most essential oil components are not soluble in aqueous solvents. Other factors that could have influenced the percentage yield of the plant used in this study compared to other plants include the plant species, geographical location, age of the plant, period of the year (season) the plant was collected, drying temperature, the extraction method used as well as plant part used. Other factors like the extraction temperature would have helped to increase the percentage yield by immobilizing the cells in the plant tissue membranes (flip flop movement of the fluid membrane) opening the pores for metabolites to diffuse through easily. Tissue processing by grinding to powder also increases the surface area for the solvents to penetrate all the tissues (Dick and Herry, 1996) [33]. The length of time used in the extraction process would have also played a great role since some volatile secondary metabolites like tannins require just mild conditions with increasing extraction time (Ajaiyeoba *et al*., 2005) [12]. The mode of preparation and administration of herbal remedies is fundamental to determining the efficacy for pharmacological evaluations (Mensor *et al*., 2001) [34]. In this part of the world, herbal remedies are prepared with either water or alcohol, which informed the choice of solvents in this study.

Proximate analysis is a scientific inquiry done to partition the approximate amount of both nutrients and non-nutrients (hazardous substances) within an organic material into categories based on common chemical properties. Reports by Moyosore *et al*. (2013) [35] stated a relatively lower level (67.2 ± 0.1%) of the moisture content in the aqueous extract of *Tapinanthus bangwensis.* On the other hand, reports by Fagbohun *et al*. (2013) [36] established that the moisture content in the ethanol extract of *Loranthus micranthus* (6.49%) agreed favorably with the result obtained in this study. Due to the high values of the moisture content in the aqueous extract of *Tapinanthus preussii* (94.6%), those of *Ipomoea batattas* (82.21%) was relatively low (Antia *et al*., 2006) [37]. High moisture content promotes susceptibility to microbial growth and enzyme activity (Adejumo and Awosanya, 2005) [38]. This may have probably accounted for the great difficulty in preserving the aqueous extract of *Tapinanthus preussii*. On the other hand low moisture content indicates less chances of microbial degradation of drugs during storage and the general requirement for moisture content in a crude drug is not more than 14% (British Pharmacopoeia, 1980) [39], thus the values obtained from this study was within the accepted range for the whole crude and ethanolic extracts but not for the aqueous extract and this is in agreement with Musa *et al.* (2005) [40] and Schuna (2010) [[41].

The composition of crude protein 14.7 ± 0.29% present in the whole crude compared favorably with *Tapinanthus bangwensis* (15.32 ± 0.44%) and in most cases surpassed those for most medicinal plants (Odoemena and Ekpo, 2005; Abolaji *et al*., 2007) [42, 43]. This is indicative of the potential benefit of *Tapinanthus preussii* since proteins are essential for the synthesis of body tissues and regulatory substances such as enzymes and hormones (Vaughan and Judd, 2003) [44]. The crude protein present in the aqueous extract of this study was much lower (0.5%) than that obtained in the aqueous extract of *Tapinanthus bangwensis* (6.8 ± 0.4%) implying that the whole crude plant material in *Tapinanthus preussii* is a better source of protein (14.7 ± 0.29%). Protein which is the major compound containing nitrogen in any food sample is used as an index of "protein termed crude protein" as distinct from true protein (Okon, 2005) [45]. Variation in the content of components of the same class may be related to genetic origin, geographical location, source, handling, solvent of extraction, extraction time and cultivation conditions. In addition, analytical techniques employed may also be responsible for the slight variations in the final results obtained (Okwuonu *et al*., 2017) [46].

The crude fat (0.2±0.003%) in this study was low in the whole crude compared to that of *T. bangwensis* (1.74%), *Talinum triangulare* (5.90%), *Baseila alba* (8.71%), *Amaranthus hybridus* (4.80%), *Calchorus africanum* (4.20%) (Akindhunsi and Salawu 2005) [47]. Low (0.4%) crude fat was detected in the ethanolic extract of *Tapinanthus preussii* whereas 9.65 ± 0.3% was observed in that of *Tapinanthus bangwensis* (Moyosore *et al*., 2013) [35]. Therefore comparing the value of crude fat obtained from other leaf extracts showed that *Tapinanthus preussii* leaves have low fat content. Dietary fat functions in increased palatability of food by absorbing and retaining flavors (Antia *et al*., 2006) [37]. A diet providing 1.2% of its caloric energy as fat is said to be sufficient to humans since excess fat consumption is implicated in certain cardiovascular disorders such as atherosclerosis, cancer and aging (Kris Etherton *et al*., 2002) [48].

The total ash content which is the measurement of the amount of residual inorganic substances not volatilized after an organic matter has been ignited by heat and burnt away. Physiological ash may be derived from the plant tissue itself or from the extraneous matter (non-physiological ash), especially sand and soil adhering to the leaf surface, and these are determined together (African Pharmacopoeia, 1986) [49]. In this study the total ash content of 3.6 ± 0.06% was found to be reasonably low, indicating that there was low mineral content preserved in the leaves of the plant or a consequence to volatilization due to the high temperature applied. A high ash value is indicative of substitution, adulteration or improper handling in preparing the extract (Chandel *et al*., 2011) [50]. This is similar to an earlier study by Moyosore *et al*. (2013) [35] who reported a value of 3.5±0.1% for total ash content of *Tapinanthus bangwensis* but in contrast with reports of Akindhunsi and Salawu (2005) [47] who reported the ash content of some leafy vegetables commonly consumed in Nigeria such as *Talinum triangulare* (20.05%), *Occinum graticinum* (8.0%) and *Herbiscus esculentus* (8.0%).

The crude fibre content of 4.27 ± 0.03% for *Tapinanthus preussii* leaves was low when compared to 12.33% for *Tapinanthus bangwensis*, 6.20% for *Talinum triangulare*, 6.40% for *Piper guineeses*, 7.0% for *Corchorus olitorius*, 6.5% for *Vernonia amygdalina*, 19.54% for *Loranthus micranthus* and 22% for *Aspillia africana* (Fagbohun *et al*., 2013; Uduak and Ikoedem, 2013 and Akindhunsi and Salawu, 2005) [36, 51, 47] indicating that *Tapinanthus preussii* leaves contain less fibrous components compared to all the above listed plants. Non-starchy vegetables are the sources of dietary fibre (Agoston *et al*., 1995) [52] and are employed in the treatment of diseases such as obesity, diabetes, cancer and gastrointestinal disorders (Saldanha, 1995) [53]. Crude fibre is that portion of plant material left after sequential hydrolysis by dilute acid and dilute alkali. On the average, crude fibre contains 50-80% cellulose, 20% hemicelluloses and 10-50%of lignin (Trowell, 1974) [54].

The total carbohydrate content by difference obtained from this study (69.3 ± 0.15%) for the whole crude was high when compared to *Loranthus micranthus* leaves (49.76%), *Aspilia africana* petals (59.69%) and *Tapinanthus bangwensis* leaves (12.72 ± 0.1%) but however low when compared to the petals

of *Tithonia diversifolia* (79.94%) (Fagbohun *et al*., 2013; Moyosore *et al*., 2013; Uduak and Ikoedem, 2013) [36, 35, 51]. The caloric value of *Tapinanthus preussii* makes it a good source of energy for humans and livestock compared to some vegetables such as pumpkin leaves, taro leaves, mushrooms and tomatoes (FAO, 2006) [55]. However the carbohydrate content in the ethanolic extract was significantly higher (93.0 ± 0.1%) compared to the aqueous extract (4.7 ± 0.1) and therefore can serve as a better source of energy relative to pumpkin leaves and tomatoes.

The elemental content of plants play crucial roles in enhancing the activities of plants against different diseases due to a definite correlation between mineral content in the human body with some disease conditions (Ceyik *et al.*, 2003) [56]. More than 27% of known enzymes require minerals for their activity as may be seen with antioxidant enzymes (Abdulkadir *et al.*, 2011) [57]. This result is in contrast with that of Moyosore *et al*. (2013) [35] where a different trend was observed in *Tapinanthus bangwensis* with much lower values for some minerals like Calcium and phosphorus which were 90.70 ± 0.025 mg/100g and 62 ± 0.025 mg/100g respectively. This may be attributed to the species difference of the plants. Very low values of all the minerals were reported in the work by Bassey (2012) [58] where *Tapinanthus globiferus* parasitic on both *Pentaclethra macrophylla* (African bean seed) and *Cola acuminate* further confirming relationship at species and host level. This may also be attributed to the time of collection of plant material and geographical location which may influence the accumulation of minerals

These inorganic elements play an important role in physiological processes involved in human health. Calcium as an essential mineral was high in the leaves of *Tapinanthus preussii*. This suggests that this leaf sample can produce a significant proportion of calcium and other essential minerals if consumed appropriately. Calcium is important to humans because of its contribution in blood clotting, muscle contraction, bone and teeth formation/repairs and in some enzymatic metabolic processes (National Research Council, (NRC), 1989) [59]. These elements act as inorganic cofactors in metabolic processes hence their absence can lead to impaired metabolism (Iheanacho and Udebuani, 2009) [60]. Since calcium helps in bone formation and blood coagulation, calcium and phosphorus deficiency may contribute to bone loss and other symptoms associated with rickets such as bowl and knock knees, curvature of the spine, pelvic and thoracic deformities. Potassium is an intracellular cation and with sodium it controls the electric potential of the body's nerve pressure (Adeyeye and Aye, 2005) [61]. Sodium plays a major role in regulating the amount of water in the body; also, the passage of sodium in and out of cells is necessary for many body functions, like transmitting electrical signals in the brain and in the muscles. Distorted enzymatic activity and poor electrolyte balance of blood plasma are related to inadequate Na^+, K^+ and $Mg^{2+,}$ as they are the most required elements in living cells (Alli, 2009) [62]. Although magnesium was present in a relatively small concentration in *Tapinanthus preussii*, it is also known to prevent cardiomyopathy, muscle degeneration, growth retardation, alopecia, dermatitis, immunologic dysfunction, gonadal dystrophy, impaired spermatogenesis, congenital malformations and bleeding disorders.

Iron is the key element in the metabolism of almost all the living organisms. It is an essential component in hundreds of proteins and enzymes. As an essential trace element/metal, it plays numerous biochemical roles in the body including oxygen binding in haemoglobin and acting as important catalytic center in many enzymes. *Tapinanthus preussii* can therefore be recommended for inclusion in the diets of patients with iron deficiency anaemia. The mineral composition values obtained in this study probably reflects the physiological state of the leaves when they were harvested (Udoessien and Ifon, 1984) [63].

The qualitative phytochemical analysis obtained in this study agrees partially with the works of Osadebe and Uzochukwu (2006) [64] who reported that the dried leaves of *Loranthus micranthus* Linn contained alkaloids, saponins, flavonoids, tannins as well as cyanogenic glycosides that was absent in both extracts of *Tapinanthus preussii*.

The crude aqueous and ethanolic extracts were also subjected to quantitative phytochemical screening with a view to widening the scope of determining the possible secondary metabolite in the plant materials. Alkaloids, tannins, anthraquinones, steroids, flavonoids, phenols, saponins, carotenoids and cardiac glycosides were present in both the aqueous and ethanolic extracts of the plant in varied proportions. These results suggest that the ethanolic extract contained more bioactive components which were in consonance with previous results on *Viscum album*, the European mistletoe (Pietrzak *et al*., 2014) [65]. However, Fagbohun *et al*. (2013) [36] reported that the ethanolic extract of *Loranthus micranthus* contained varied amounts of all the phytochemicals found in the ethanolic extract in *Tapinanthus preussii*, with the exception of saponins, steroids and flavonoids. This variation might be due to differences in the mistletoe species, host plants and geographical location as suggested by Osadebe *et al*. (2004) [66]. It was also observed that the solubility and reactions of the phytochemicals might have been solvent dependent due to their differences in polarity. This plant was found to be rich in alkaloids, antioxidants, phenolics, tannins, flavonoids, saponins and carotenoids. These identified phytochemicals are known to exhibit medicinal as well as physiological activity (Sofowora, 1993) [22] and therefore may be good for therapeutic preparations in the treatment of various infections and metabolic disorders (Fagbohun *et al*., 2013) [36]. Alkaloids are organic compounds that contain nitrogen, known to exhibit marked physiological activity when administered to animals (Okwu, 2004) [67]. Pure isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents for central nervous system stimulant, topical analgesic, ophthalmologic, antispasmodic and bactericidal effects (Osuagwu *et al*., 2007; Samali *et al*., 2012) [68, 69]. They are also used in relieving pains, anxiety and depression (Jisika *et al.*, 1992) [70]. They are however toxic due to their stimulatory effects, leading to excitation of cells and neurological dysfunction (Obochi, 2006; Ekam and Ebong, 2007) [71, 72].

Anthraquinones were also present in the aqueous and ethanolic extracts. Their derivatives known collectively as anthracenediones include many important drugs e.g. laxatives like imodium; antimalarials like rufigallol; antineoplastics used in the treatment of cancer like anthracymin which are naturally occurring phenolic compounds based on the 9, 10-anthraquinone skeleton. The health benefits may stem from anthocyanins alone or from their synergistic interactions with other phenolic compounds.

Cardiac glycosides were quite minimal in both the aqueous and ethanolic extracts. They are important in medicine because of their action on the heart and are used in cardiac insufficiency. Thus cardiac glycosides are drugs that can be used in the treatment of congestive heart failure and cardiac

arrhythmia. They work by inhibiting the Na^+/k^+ pump resulting in an increase in the levels of sodium ions in the myocytes, which then leads to a rise in the level of calcium ions. This inhibition increases the amount of Ca^{2+} ions available for contraction of the heart muscle, improves cardiac output and reduces distention of the heart (Zhang *et al*., 2012) [73].

Carotenoids (tetraterpenoid i.e. 8 isoprene units) act as antioxidants i.e. efficient free radical scavengers and also enhance the vertebrate immune system. Antioxidants hasten the healing of wounds and inflamed mucous membrane (Osuagwu *et al*., 2007 and Samali *et al*., 2012) [68, 69]. Epidemiological studies have shown that flavonoids and carotenoids are inversely related to mortality from coronary heart diseases (Donald and Cristobal, 2006) [74].

Flavonoids are water-soluble plant phenolics that have been shown to have antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, antithrombotic and vasodilatory activity (Ekam and Ebong, 2007). The potent antioxidant activity of flavonoids and their ability to scavenge hydroxyl radicals, superoxide anions and lipid peroxy radicals may be their most important function (Allan and Miller, 1996) [75].

Saponins are capable of neutralizing some enzymes in the intestine that can become harmful, building the immune system and promoting wound healing. They also prevent excessive absorption of cholesterol and reduce the risk of cardiovascular diseases (Akinpelu and Onakoya, 2006; Olaleye, 2007) [76, 77]; strengthen the contractions of cardiac muscles (Aja *et al.*, 2010; Schneider and Woliling, 2004) [78, 79]; exhibit cytotoxic effects and growth inhibition against a variety of cells making them have anti-inflammatory and anticancer properties (Akinmoladun *et al., 2010*) [80].

Tannins usually enable the plants medicinal attributes of treating dysentery and urinary tract infection. They have also been found to posses astringent properties as well as a wide range of usage, ranging from antiviral (Brune *et al*., 1989) [81], antibacterial (Akiyama *et al.*, 2001) [82], antiparasitic (Herbert and Albrecht, 2005) [83], hasten the healing of wounds and inflamed mucous membranes (Okwu, 2004) [67] as well as inhibition of HIV replication in infected H9 lymphocytes with little toxicity as in epigallitannins.

Conclusion

This study has provided a scientific justification for the use of water and locally distilled alcohol as solvents in the herbal preparations of crude leaf extracts of *Tapinanthus preussii* since they contained clinically relevant phytoconstituents (like flavonoids, carotenoids, anthraquinones and saponins), as well as nutritionally relevant inorganic minerals (like Fe^{2+}, Ca^{2+}, K^+, Na^+, Mg^{2+}, and PO_4^{2-}) and proximate constituents (like carbohydrates, lipids and proteins) in varied and significant amounts.

Further studies however needs to be carried out in order to isolate and identify the active principle(s) in the extract responsible for ameliorating the numerous disorders that these extracts have been implicated in their remedy as well as characterize and elucidate its mode of action and toxicological effect. It will also be necessary to standardize, formulate and commercialize this Phyto medicines to health care providers and consumers.

Acknowledgement

The Authors wish to acknowledge the technical support received from the entire technical team of the Kappa Biotechnology laboratory, Ibadan, Oyo State Nigeria and from the Energy Centre, University of Benin, Benin City Nigeria.

References

1. Gill FB. The Machiavellian Frontiers 1973; 38(2):2-5.
2. Judd WS, Campbell CS, Kellogg EA, Stevens PF, Donaghue MJ. Plant systematics: a phylogenetic approach. Sinauer Associates, Inc. Sunder land Massachusetts, USA. 2002, 20-42.
3. Griggs P. Mistletoe, myth, magic and medicine. The Biochemist 1991; 13:3-4.
4. Murray NB. African Indigenous medicine. Research Functions, Nairobi. 1998; 613-615.
5. Owolabi OJ, Omogbai EK Obasuyi O. Antifungal and antibacterial activities of the ethanolic and aqueous extract of *Kigella africana* (Bignoniaceae) stem bark. African Journal of Biotechnology 2007; 6:1677-1680.
6. Iwu MM. Antidiabetic properties of Bridelia ferruginea leaves. Planta Medica. 1980; 39:247-252.
7. Alarcon-Aguilara FJ, Roman-Ramos R, Perez-Gutierrez S. Aguilar- Contreras, A, Contreras-Weber CC Flores-Saenz JL. Study of the anti-hyperglycemic effect of plants used as antidiabetics. Journal of Ethnopharmacology. 1998; 61(2):101-110.
8. Dwuma-Badu D, Withers SF, Ampofo SA, El-Azizi MM, Slatkin DJ, Schiff Jr. PL Knapp JE. Isolation of the alkaloid from natural sources in 1978. Journal of Natural Products. 1986; 49(5):854-858.
9. Ray SD, Patel N, Shah N, Nagori A, Naqvi A. Stohs SJ. Pre-exposure to a novel nutritional mixture containing a series of phytochemicals prevents acetaminophen induced programmed and unprogrammed cell deaths by enhancing BCL-X_L expression and minimizing oxidative stress in the liver. Molecular and cellular biochemistry. 2006; 293(1-2):119-136.
10. Gill LS, Akinwumi C. Nigerian folk medicine: practices and beliefs of the Ondo people. J Ethnopharmacol. 1986; I18:257-266.
11. Honork LL. Cultivation and processing of medicinal plants. John Willey and Sons Ltd. Baffin Slane United Kingdom. 1992; 3(7):19-22.
12. Ajaiyeoba E, Ashidi J, Abiodun O, Okpako L, Ogbole O, Akinboye D, *et al*. Antimalarial ethnobotany: *In vitro* antiplasmodial activity of seven plants identified in the Nigerian middle belt. Pharmaceutical biology. 2005; 42(8):588-591.
13. EL-Mahmood A, Doughari J, Ladan N. Antimicrobial screening of stem bark extracts of Vitellaria paradixa against some enteric pathogenic microorganisms. Afr. J Pharm. 2008; 2(5):89-94.
14. Doughari JH, El-Mahmood AM, Tyoyina I. Antimicrobial activity of leaf extracts of *Senna obtusifolia* (L). Afr. J Pharm. Pharmacol. 2008; 2(1):7-13.
15. Hark L, Deen D. The whole grain diet miracle. Dorling Kimbesley Ltd UK, 2006, 213-220.
16. Farnsworth NR. Biological and phytochemical screening of plants. J Pharm. Sci. 1989; 55:225-276.
17. Kalemba D, Kunicka A. Antibacterial and antifungal properties of essential oils. Curr. Med. Chem. 2003; 10:813-822.
18. Cline E. Anti-bacterial properties of plants used medicinally in Gloucester village, Sierra Leone. Thesis, Department of Botany, University of Sierra Leone, Fourah Bay College, Freetown, Sierra Leone, 1985.

19. Brown KM, Arthur JR. Selenium, selenoproteins and human health: A review. Public Health Nutr. 2001; 4:593-599.
20. Papp LV, Lu J, Holmgren A, Khanna KK. From selenium to selenoproteins: Synthesis, identity and their role in human health. Antioxidant Redox Signaling. 2007; 9:775-806.
21. Harborne JB. Phytochemical Methods. A guide to modern techniques of plant analysis, 3rd Ed. New Delhi: Springer (INDIA) Pvt. Ltd, 1998, 124-126.
22. Sofowora A. Screening plants for bioactive agents. In: Medicinal Plants and Traditional Medicinal in Africa, second ed., Spectrum Books Ltd., Sunshine House, Ibadan, Nigeria, 1993, 134-158.
23. Williams GO, Omoh LE. Mitotic effects of the aqueous leaf extract of Cymbopogon citratus in Allium cepa root tips. Cytobios. 1996; 87(350):161-168.
24. Association of official Analytical Chemist (AOAC). Official methods of analysis. 16th Edn. of the Association of Analytical Chemists, Washington DC, 1996, 6-10.
25. Isaac RA, Kerber JD. Atomic absorption and flame photometry: Techniques and uses in soil, plant and water analysis. In: Instrumental Methods for analysis of soil and Plant Tissue. Walsh, L.M. (ed.), Soil Science Society of America, Medison, Wisconsin, 1972, 17-38.
26. Perkin-Elmer Corporation. Analytical procedure for atomic absorption spectrometry. In: Selected Methods for Soil and Plants Analysis Manual Series No. 1. IITA, Oyo Rd. Ibadan, Nigeria, 1997, 21-22.
27. Trease GE. Trease and Evans' Pharmacognosy: A Physician's Guide to Herbal Medicine. Bailliere Tindall London. Tsinghua Sci. Technol. 1989; 13:460-465.
28. Harborne JB. Phytochemicals Methods. A Guide to Modern Techniques of Plant Analysis, 2nd ed. Chapman and Hall Ltd., London, 1984, 49-282.
29. IBM Corp. IBM SPSS Statistics for Windows, version 22.0. Armonk, NY: IBM Corp, 2013.
30. Doughari JH EL-Mahmood AM Tyoyina I. Antimicrobial activity of leaf extracts of *Senna obtusifolia* (L). Afr. J. Pharm. Pharmacol. 2008; 2(1):7-13.
31. EL-Mahmood MA. Efficacy of crude extracts of garlic (*Allium sativum* Linn.) against nosocomial Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniea and Pseudomonas aeruginosa. J Med. Plants Res. 2009; 3(4):179-185.
32. Kantalak P, Griangsak E, Benjamart C Pinchaya N. The study of antbacterial activity of some medicinal plants in Lamiaceae family, Suranaceae. J Sci. Technol. 2004; 11:52-59.
33. Dick AJS, Herry HN. Extraction of secondary metabolites from plant material: A review. Trends in Food Science and Technology. 1996; 71:19-22.
34. Mensor LL, Menezes FS, Leitao GG, Reis AS, Dos Santos TC, Coube CS, *et al*. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res. 2001; 15:127-130.
35. Moyosore O, Nwoke SC, Imaga NA, Magbagdeola AO, Ebuehi, AO, Okafor OA. Preliminary Investigation of The Nutritional Qualities, Phytochemical Properties and Antioxidant Activities of Aqueous Extract of Mistletoe Leaves (*Tapinanthus bangwensis*) Grown on Orange Trees. The FASEB Journal. 2013; 27:794-816.
36. Fagbohun A, Adedirin O, Akanji F, Afolayan M, Adebiyi A, Pius I, *et al*. Chemical characterization and antimicrobial activity of Loranthus micranthus Linn leaves. J Med. Plants Res. 2013; 7(45):3332-3336.
37. Antia BS, Akpan EJ, Okon PA, Umoren IU. Nutritive and anti-nutritive evaluation of sweet potatoes (Ipomoea batatas) leaves. Pakistan Journal of Nutrition. 2006; 5(2):166-168.
38. Adejumo TO, Awosanya OB. Proximate and mineral composition of four edible mushroom species from South Western Nigeria. African Journal of Biotechnology. 2005; 4(10):1084-1088.
39. British Pharmacopoeia. Ash value, Acid insoluble, water soluble extractive and Alcohol extractive. Appendix XII Majesty Stationery office London. 1980; 2:1276-1279.
40. Musa KT, Katasayl UA, Abdrahman EM, Ashok MB, Abubakar MS. Pharmacognostic studies on the leaves of *Dyschoriste perrottetii* Nees. (Acanthaceae), Afr. J Pharm. Res. Develop. 2005; 2(1):79-83.
41. Schuna C. Importance of Moisture Content in Foods. Ehow Contributor. Sciences. 2010; 78(5):431-441.
42. Odoemena CSI, Ekpo BAJ. Phytotherapeutic potentials and biochemical study of Nypa Fructicans (wurmb). J pharm. Bio Resource. 2005; 2:89-92.
43. Abolaji AO, Adebayo HA, Odesanmi OS. Nutritional qualities of three medicinal plant parts (*Xylopia aethiopica*, *Blighia sapida* and *Parinari polyandra*) commonly used by pregnant women in the western part of Nigeria. Pakistan Journal of Nutrition. 2007; 6(6):665-668.
44. Vaughan JG, Juddk PA. The Oxford Book of Health Foods: A Comprehensive Guide to Natural Remedies. 1st Ed., Oxford University Press, New York, USA, 2003, 17-22.
45. Okon EU. Handbook of basic food and beverage analysis. Etovin Public. AKS. Nigeria, 2005, 53-70.
46. Okwuonu UC, Dorothea BG, Njoya H, Iyemene PT. Phytochemical, proximate and elemental constituents of Aspilia africana (*Wild sunflower*) flowers. Journal of Medicinal Plants Studies. 2017; 5(4):22-27.
47. Akindhunsi AA, Salawu SO. Phytochemical screening and nutrient-antinutrient composition of selected tropical green leafy vegetables. African journal of biotechnology 2005; 4:497-501.
48. Kris-Etherton, PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, *et al*. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am. J Med. 2002; 113:71S-88S.
49. African Pharmacopoeia. 1st ed. OAU/STRC Publications. 1986; 2:128-144.
50. Chandel HS, Pathak AK, Tailang M. Standardization of some herbal antidiabetic drugs in polyherbal formulation. Pharm. Res. 2011; 3(1):49-56.
51. Uduak E, Ikoedem U. Comparative phytochemical and physic-chemical properties of Aspiliia Africana (Pers) C.D. Adams and Tithonia diiversifolia (Hemsl) A. Gray petals as a scientific backing to their Trado medical Potentials. Med. 2013; 3(2):88-100.
52. Agoston DV, Palkovits CG, Fitzgerald SF, Brenneman DE. Developmental changes in the inducibility of fos-like immunoreactivity in primary embryonic spinal cord cultures. Developmental brain research. 1995; 89(2):173-186.
53. Saldanha LG. Fiber in the diet of US children: results of national surveys. Pediatrics. 1995; 96(5):994-997.
54. Trowell H. Diabetes mellitus death-rates in England and Wales 1920-1970 and food supplies. The Lancet. 1974; 304(7887):998-1002.

55. FAO. Global forest resources assessment 2005: progress towards sustainable forest management. Forestry Paper 147. United Nations Food and Agriculture Organization (FAO), Rome. 2006. Available from, 2014: http:// www.fao.org.
56. Ceyik U, Ergen E, Budak G, Karabulut A, Tiraolu E, Apaydin G, *et al*. Elemental Analysis of Akçaabat Tobacco and its Ash by EDXRF Spectrometry. Journal of Quantitative Spectroscopy and Radiative Transfer. 2003; 78:409-415.
57. Abdulkadir IE, Aliyu AB, Ibrahim MA, Audu SBD, Oyewale AO. Antioxidant activity and mineral elements profiles of Isoberlinia doka leaves from Nigeria. Australian Journal of Basic Applied Science. 2011; 5(12):2507-2512.
58. Bassey ME. Phytochemical Investigations of Tapinanthus Globiferus (*Loranthaceae*) from Two Hosts and the Taxonomic Implications. International Journal of Chemical, Environmental and Pharmaceutical Research. 2012; 3(2):174-177.
59. National Research Council (NRC). Recommended Dietary Allowances 8th edn. Food and Nutrition Board. National Academy of Science, Nigeria. 1989, 20-40.
60. Iheanacho K, Udebuani AC. Nutritional composition of some leafy vegetable consumed in Imo-State, Nigeria. J Appl. Sci. Environ. Manag. 2009; 13(3):35-38.
61. Adeyeye EI, Aye PA. Chemical composition and the effect of salts on the food properties of Triticum durum whole meal flour. Pakistan Journal of Nutrition. 2005; 4(3):187-196.
62. Alli Smith YR. Determination of chemical composition of Senna-siamea (Cassia Leaves). Pakistan Journal of Nutrition. 2009; 8:119-121.
63. Udoessien EI, Ifon ET. Chemical studies on the unripe and ripe fruits of Dennettia tripatela (pepper fruit). Food chemistry. 1984; 13(4):257-263.
64. Osadebe PO, Uzochukwu LC. Chromatographic and antimotility studies on the extracts of Loranthus micranthus. J of Pharrna. And Allied Sci. 2006; 3(1):263-268.
65. Pietrzak W, Nowak R, Olech M. Effect of extraction method on phenolic content and antioxidant activity of mistletoe extracts from Viscum album subsp. abietis. Chemical Papers. 2014; 68(7):976-982.
66. Osadebe PO, Okide GB, Akabogu LC. Study on anti-diabetic activities of crude methanolic extracts of *Loranthus rnicranthus* (Linn) sourced from five different host trees. Journal of Ethnopharmacology. 2004; 95:133-138.
67. Okwu DE. Phytochemical and vitamin content of endogenous spices of south Eastern Nigeria. J Sus. Agric. Environ. 2004; 6:30-37.
68. Osuagwu GGC, Okwulelie IC, Emenike JO. Phytochemical and mineral component of the leaves four Nigerian Pterocarpus (JACQ) species. Int. J Mol. Med. Adv. Sci. 2007; 3(1):6-11.
69. Samali A, Florence DT, Odeniran OA, Cordelia ON. Evaluation of chemical constituents of Phyllanthus naruri. Afr J Pharm and Pharmacol. 2012; 6(3):125-128.
70. Jisika M, Ohigashi H, Nogaka H, Tada T, Hirota M. Bitter steroid glycosides, Vernon sides A1, A2, and A3 and related B1 from the possible medicinal plant vernonia amygdalina used by wild Chimp. Tetrahedron. 1992; 625-630.
71. Obochi GO. Effect of alcohol-kolanut interaction on biochemical indices of neuronal function and gene expression in Wistar albino rats. A PhD Thesis submitted to the Graduate School, University of Calabar-Nigeria, 2006.
72. Ekam VS, Ebong PE. Serum protein and enzymes levels in rats following administration of antioxidant vitamins during caffeinated and non-caffeinated paracetamol induced hepatotoxicity. Nigeria J Physiol. Sci. 2007; 22(1):65-68.
73. Zhang D, Ciciriello F, Anjos SM, Carissimo A, Liao J, Carlile GW, *et al*. Ouabain mimics low temperature rescue of F508del-CFTR in cystic fibrosis epithelial cells. Frontiers in pharmacology, 2012, 3.
74. Donald RB, Cristobal M. Antioxidant activities of flavonoids, J Agric. 2006; 52:125-757.
75. Alan L, Miller ND. Antioxidant flavonoids: structure, function and clinical usage. Alternative Medicine Review. 1996; 1:103-111.
76. Akinpelu DA, Onakoya TM. Antimicrobial activities of medicinal plants used in folklore remedies in south western Nigeria. African journal of biotechnology. 2006; 5:1078-1081.
77. Olaleye MT. Cytotoxic and antibacterial activities of methanolic extract of Hibiscus sabdariffa. J Med. Plant Res. 2007; 1(1):9-13.
78. Aja PM, Okaka ANC, Onu PN, Ibiam U, Urako AJ. Phytochemical constituents of Talinum triangulare (water leaf) leaves. Pakistan Journal of Nutrition. 2010; 9(6):527-530.
79. Schneider G, Wolfling J. Synthetic cardenolides and related compounds. Current organic chemistry. 2004; 8(14):1381-1403.
80. Akinmoladun AG, Obuotor EM, Farombi EO. Evaluation of antioxidant and free radical scavenging capacities of some Nigerian indigenous medicinal plants. Journal of Medicinal Food. 2010; 13(2):444-451.
81. Brune M, Rossander L, Hallberg L. Iron absorption and phenolic compounds: importance of different phenolic structures. European Journal of Clinical Nutrition. 1989; 43:547-558.
82. Akiyama H, Fujii K, Yamasaki O, Oono T, Iwatsuki K. Antibacterial action of several tannins against Staphylococcus aureus. Journal of antimicrobial chemotherapy. 2001; 48(4):487-491.
83. Herbert K, Albrecht FK. Antileishmanial activity and immune modulatory effects of Tannins and related compounds on leishmania parasitized RAW 264.7 cells. Phytochmistry. 2005; 66(17):2056-2071.