European Journal of Biological Sciences 8 (3): 116-123, 2016
ISSN 2079-2085

DOI: 10.5829/idosi.ejbs.2016.116.123

Composition and *in vitro* Antioxidant Capacity of Alomo Bitters

[1]*J.C. Anionye and* [2]*E.C. Onyeneke*

[1]Department of Medical Biochemistry, College of Medical Sciences, University of Benin, Benin City, Nigeria
[2]Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

Abstract: Modern pharmacology had its origin in medicinal plants and till date, some drugs are products of active components from plants. The term "bitters" as it is used presently, is a beverage, often alcoholic, flavoured with herbal essences that gives it a bitter or bitter-sweet flavour. Bitters are produced from herb and root extracts, from the narcotic components of (primarily) tropical and subtropical plants and spices. Bitters are marketed as a "cure-all" patent medicine in Nigeria and a lot of them have never been subjected to scientific scrutiny. Hence, this study was aimed at ascertaining the composition and in vitro antioxidant capacity of Alomo bitters, one of such bitters, with the hope that this will help give some credence to its broad pharmacological claims. The results showed that Alomo bitters have high moisture content of 94.12%. The results also indicate that Alomo bitters have high alcohol contents of 43%. The aqueous and ethanolic extracts of Alomo bitters indicate that they contain varying degrees of amino acids, proteins, saponins, tannins, alkaloids, glycosides/reducing sugar, terpenoids, flavonoids, phytosterols and phlobatannins. Alomo bitters also contain significant amounts of Na, K, P, Ca, Mg, Zn, Mn, Fe and Cu, while Pb, Cr and Se were not detected. Alomo bitters have IC_{50} values for 50% ABTS inhibition with the value of 25.46mg/ml indicating that the bitters have a relatively high antioxidant capacity as claimed by the producers of the bitters.

Key words: Alomo bitters · Antioxidant capacity · Pharmacological agents and medicinal plants

INTRODUCTION

Medicinal plants have been defined and associated with gods and spiritual invocations. However with the advent of science, civilisation and Christianity, there has been a drastic decline in this practice. Modern pharmacology had its origin in these medicinal plants and till date some drugs are products of active components from plants. Medicinal plants have been defined as those plants which contain in one or more of their organs, substances that can be used for the synthesis of useful drugs [1 and 2].

The term "bitters" as it is used presently, is a beverage, often alcoholic, flavoured with herbal essences that gives it a bitter or bittersweet flavour. The generic term applies to all bitter liquors and herbal bitters. Bitters are produced from herb and root extracts, from the narcotic components of (primarily) tropical and subtropical plants and spices. They are usually dark in colour and valued for their ability to promote appetite and digestion hence their use as patent medicine and as aid in digestion and as flavouring in cocktails. Bitters are made up of numerous groups of chemical compounds extracted from the herbs and roots (medicinal plants) that have the common characteristic of a bitter taste and act to increase the vital energy centres in the body [3 and 4].

Unorthodox traditional medicine practice which employ the use of herbs (medicinal plants) have in recent times been gaining much publicity and recognition, for their solution to ailments seemingly elusive to the system of orthodox medical practice. Modern science may have widened for some time the differences in terms of medication between orthodox and unorthodox/traditional medicine, this gap seems to be closing fast as the current trend is that they are both adopting practices from each other [1 and 2]. This has led to the resurgence of an ancient remedy for digestive problems in the repackaging of "herbal bitters" and products like it in an "orthodox way".

Corresponding Author: J.C. Anionye, Department of Medical Biochemistry,
College of Medical Sciences, University of Benin, Benin City, Nigeria.

The bitter of interest here is Alomo Bitters, a Ghanaian product (composed of seven (7) herbal constituents). "Bitters" in summary are generally claimed to be effective in curing all allergic, metabolic and immunological conditions where the diagnosis points to a fault in the digestive process, improves immunity, help in anaemia, wound healing and blood clotting by increasing the population in tissues, of red blood cells, white blood cells and platelets, help with inflammatory conditions of the gastrointestinal tract (Colitis, Crohn's disease, nonspecific inflammation). In addition to the action of bitters on digestive secretions aiding good digestion, they also strengthen the tone of tissues throughout the digestive tract, as well as aid in the healing of damaged mucous membranes. They are generally said to regenerate and heal mucosal lining of the G.I.T especially duodenal and gastric ulcers. This helps resolve conditions ranging from gastroesophageal reflux to ulcers to leaky gut syndrome.

Bitters have been claimed to help heal piles/haemorrhoids and improve sexual function. Enhance blood circulation, purification of blood by the kidneys, blood pressure regulation through arterial dilatation and prevent formation of kidney stones, cleanse the colon of impurities and have also been said to possess anti-tumour properties and especially protects against, colo-rectal cancers. They are also said to have anti-inflammatory, antibiotic and antifungal properties.

Bitters have also been said to ensure good digestion of fats and oils and proper functioning of the liver as regards to excretion, reduce accumulated fat (triglycerides) and cholesterol levels thereby confering on it hypolipidaemic properties. They are said to reduce excess body fat and promote healthy weight loss, act as a liver tonic and body detoxifier; being hepatoprotective and enhancing its functions generally and helping in body detoxification. Bitters act on the pancreas and liver, help in cell division and growth of the pancreas thereby helping to normalizing blood sugar and promote the production and release of pancreatic enzymes. Some are even said to have hypoglycaemic properties.

In modern herbal medicine, "bitter principles" occupy a central place in herbal therapeutics, bearing the acrid constituents. Most people consuming herbal medicines complain about the bitterness of the medicines prescribed. This is the only defining attribute of herbal medicine and the only feature to set it apart from other therapies [2 and 4]. In times past our traditional diets were not devoid of bitter foods as is presently the case in most modern diets, hence Green 1991 [5] desires that we see the medicinal side of bitters in an entirely different light in other that we use it to prevent what he termed the "Bitter Deficiency Syndrome" of our era, which in his opinion is the predisposing factor to many ailments of our time [4 and 5]. All these make the study of the constituent and pharmacological effects of present day bitters desirable.

MATERIALS AND METHODS

Material: Alomo bitters were purchased from reputable pharmaceutical stores opposite the University of Benin Teaching Hospital (UBTH), Ugbowo Lagos Road, Benin City, Edo State, Nigeria. The bitters were bought as liquid formulations and stored at room temperature throughout the period of the study.

Proximate Analysis: The moisture content of the herbal bitters was determined using the gravimetric method [6]. The ash content was estimated using the method of AOAC [7]. The fat content was determined using the method of AOAC [8]. The crude fibre content was determined by the difference in weight after calcination, following the digestion of the sample in sulphuric acid and sodium hydroxide solutions and the residue being calcined [9]. The protein content of the bitters was determined from the organic nitrogen content by Kjeldahl method [9] The carbohydrate content of the bitters was determined by the difference method by adding moisture, fat, protein and ash content and the value deducted from 100 [9]. The reducing sugar content of the bitters was determined using the dinitrosalicylic acid method [10]. The glucose content was determined using the glucose oxidase method [11]. Triplicate measurements were performed and the mean computed.

Alcohol Composition Analysis: The determination of the alcohol content was done using the AOAC [7] method.

Extraction of Bitters for Qualitative Phytochemical Analysis: Because there may be differential solubility in the constituent of the bitters relative to the polarity of the solvent used the bitters were further extracted in distilled water and ethanol. Aqueous Extraction: 10ml of bitters sample was added to 90ml distilled water and boiled on slow heat for 2hours. It was then filtered using a Whatmann No. 42 filter paper (125mm); the filtrate was collected and further concentrated to make the final volume one-fourth of the original volume and stored at 4oC in an air tight container [12]. Preparation of ethanolic

extract: 10ml of the bitters sample was taken and put into 50ml of absolute ethanol in a flat bottom flask. The flask was plugged with cotton wool and then kept on a rotary shaker at 190-220rpm for 24hrs. After 24 hours the sample-solvent mix was then filtered using a Whatmann No. 42 filter paper (125mm); the filtrate was collected and the solvent evaporated to make the final volume one-fourth of the original volume and stored at 4oC in an air tight container [12]. Qualitative Phytochemical Analysis

The test for phytosterol/steroids, amino acids, protein, saponins, tannins, alkaloid, cardiac glycoside, terpenoid, flavonoids, phlobatannins, was determined using the method described in Santhi and colleagues [12]. The test for glycosides/reducing sugars was carried out using the method described by Onyeike and Osuji [9]. The Borntrager's test was used for the detection of anthraquinones [13].

Quantitative Phytochemical Analysis: The determination of total phenols was by spectrophotometric method [14], while alkaloids was as described by Harborne [16] and Edeoga and colleagues [14]. The determination of tannin was by the Van – Burden and Robinson method [14], while the determination of saponin was as described by Edeoga and colleagues [14]. The determination of flavonoids was by the method of Boham and Kocipai-Abyazan [15], while that of cyanogenic glycosides was by the AOAC [17] method. Elemental Analysis: Extraction and determination of calcium and magnesium in the bitters sample were determined by the titrimetric method using ethylenediaminetetraacetic acid (EDTA), [17]. The sodium and potassium concentrations were determined using the flame photometer (JENWAY PFP 7 model) [17], while phosphorus was determined by Olsen and Sommers [18] and the AOAC [17] methods. The determination of the trace metals iron, zinc, manganese, copper, lead, chromium and selenium were done using the bulk scientific VGP210 atomic absorption spectroscopy/spectrophotometry, [17].

Determination of the Total Antioxidant Capacity: The ability of the herbal bitters to scavenge 2,2'-azinobis-(3ethylbenzothiazoline-6-sulphonic acid) radical cation (ABTS+) which gives an idea of the total antioxidant capacity, was determined by the improved spectrophotometric version described by Re and colleagues [19].

ABTS Radical Cation Decolourisation Assay: In this improved version, ABTS+-the oxidant, will be pregenerated by persulfate oxidation of 2,2,-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) – (ABTS2-). Triplicate measurements were performed and the mean computed. DPPH free radical scavenging activity: DPPH (2,2-diphenyl-1-picrylhydrazyl radical) scavenging activity of the herbal bitters was measured by the spectrophotometric method described by Jain and colleagues [20]. Triplicate measurements were performed and the mean computed. Hydroxyl radical scavenging activity: This was assayed as described by Kunchandy and Rao [21] with a slight modification [22]. The assay is based on quantification of the degradation product of 2-deoxyribose by condensation with TBA. Triplicate measurements were performed and the mean computed.

Statistical Analysis: The results are expressed in Mean±SEM. Students t-test was used to compare the means. $P<0.05$ was considered significant.

RESULTS

Table 1 shows that Alomo bitters has a high moisture and alcohol content, no fibre and a higher ash content compared to other food nutrients.

Table 2 shows that considering both the aqueous and ethanolic extracts of the bitters, the bitters used in this research contains amino acids, proteins, saponins, tannins, alkaloids, phlobatannins, plant sterols (steroids), terpenoids and flavonoids but did not contain cardiac glycosides, glycosides/reducing sugar and anthraquinones. As the key indicates, these phytochemicals were present in varying degrees of slight, moderate and high. The quantitative phytochemical composition of the bitters, indicate saponins as the highest constituent and tannins the least among those determined.

Table 3 summarizes the means of the quantitative elemental composition of the bitters, with magnesium being the highest constituent and manganese the lowest. The results show that the bitters contain significant amounts of Na, K, P, Ca, Mg, Zn, Mn, Fe and Cu, while Pb, Cr and Se where not detected.

Table 4 shows that Paxherbal bitters has the ability to inhibit the ABTS radical, scavenge DPPH and the hydroxyl radical, hence it has antioxidant capacity. Statistical evaluation however shows that the concentration required for 50% inhibition of ABTS and DPPH by ascorbic acid (IC50) and 50% inhibition of the OH radical by mannitol (IC50) is significantly ($P<0.05$) higher than that of yoyo bitters.

Table 1: Proximate and Alcohol composition of the Alomo Bitters

Proximate composition	Ash	Moisture	Fibre	Fat & oil	Protein	Carbohydrates	Glucose	Reducing sugar	Alcohol
Quantity in Alomo Bitters (%)	1.50±0.09	94.12±1.04	0.00±0.00	1.39±0.07	0.78±0.05	2.21±0.62	0.05±0.00	0.55±0.01	42.90±0.66

Table 2: Qualitative and Quantitative Phytochemical Composition of Alomo Bitters

	Qualitative composition of Alomo Bitters		
Parameters	Aqueous extract	Ethanol extract	Quantitative composition of Alomo bitters (%)
Phytosterol	-	-	ND
Amino acids	+	+	ND
Proteins	+	+	ND
Saponins	+	+	1.20±0.06
Tannins	+	+	0.04±0.01
Alkaloids	+	+	0.19±0.01
Cardiac glycosides	-	-	ND
Glycosides/reducing sugar	-	++	ND
Cyanogenic glycosides	ND	ND	0.09±0.00
Terpenoids	+++	++	ND
Flavonoids	+	+	0.03±0.01
Phlobatannins	++	+++	ND
Anthraquinones	-	-	ND
Total phenols	ND	ND	0.11±0.02

Key: +++ = Highly Present;++ = Moderately Present;+ = Slightly Present;-= Absent; ND = Not Determined

Table 3: Quantitative Mineral Composition of the Alomo Bitters

Mineral composition	Ca	Mg	Na	K	P	Fe	Zn	Mn	Cu	Pb	Cr	Se
Quantity in Alomo bitters (mg/100ml)	20.20±0.86	218.74±4.36	3.80±0.26	172.00±1.53	29.77±1.22	8.00±0.29	2.20±0.35	0.25±0.01	0.50±0.03	<DL	<DL	<DL

Where <DL means less than detection limit. Triplicate measurements were performed and the mean computed. The values are expressed as Mean±SEM.

Table 4: Antioxidant Capacity by IC_{50} Values of the Control and Test Bitters using various methods.

Groups	IC_{50} (mg/ml) by Total antioxidant capacity	IC_{50}(mg/ml) by DPPH Scavenging ability	IC_{50}(mg/ml) by Hydroxyl Radical scavenging ability
Standard	0.08±0.00	0.05±0.01	204.64±4.68
Alomo bitters	25.46±0.25	16.02±0.30	456.36±20.79

Values are expressed as Mean±SEM. Values in the same column with different superscript letters differ statistically significantly ($P<0.05$) from one another.
*Standard for total antioxidant capacity and DPPH scavenging ability was ascorbic acid while that of hydroxyl radical scavenging ability was mannitol.

DISCUSSION

It is often summarized that bitters stimulate digestive secretions and the metabolism as a whole and in so doing increase appetite, relieve constipation and generally ease the heavy glumness of sluggish digestion. But, this is really too simple and cursory a summation and a deeper look into the actions of bitters is not only theoretically insightful but practically invaluable, especially as some plant products have been known to be toxic to the human system [3 and 4]. Their composition as they are presently constituted have never been ascertained neither has their numerous pharmacological claims being subjected to proper scientific scruitiny yet the use of bitters is getting popular.

The results showed that Alomo bitters have the high moisture content of 94.12%. The results shown in table 1 of this study indicate that Alomo bitters have very high alcohol contents of 43%. The high alcohol content (43%) of the Alomo bitters and the final presentation/nature of the product and its packaging may be the reason for it being prone to abuse as reported by Salaam and Brown [23].

The aqueous and ethanolic extracts of Alomo bitters indicate that generally they contain in varying degrees amino acids, proteins, saponins, tannins, alkaloids, glycosides/reducing sugar, terpenoids, flavonoids, phytosterols and phlobatannins. It is however of note that the terpenoids where present not just in both extracts of Alomo bitters but in relatively higher amounts when compared to other phytochemical constituents. Alomo bitters was additionally higher in phlobatannins.

Other phytochemical constituents of bitter plants include glycosides, flavonoids, tannins, saponins and phenols which not only contribute bitter principles but also other active principles that contribute the other pharmacological effects associated with bitters as we will see in our further discussion on their quantitative phytochemical composition. Alomo bitters however have appreciable amounts of alkaloids, tannins, flavonoids, total phenols, saponins and cyanogenic glycosides. Compared to other phytochemical constituents alomo bitters have saponins as their highest constituent. This study is in agreement with that of Awa and James (2013), which showed that bitters contain appreciable amounts of the phytochemicals especially tannin and phenol. Tannins as a group of compounds have received a great deal of attention in recent years, since it was suggested that the consumption of tannin-containing beverages, especially green teas and red wines, can cure or prevent a variety of ailments [25]. Many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumour activity and a wide range of anti-infective actions, have been assigned to tannins [26 and 27]. Tannins are complex phenolic polymers, which can bind to proteins and carbohydrates resulting in reduction in digestibility of these macromolecules and thus inhibition of microbial growth [28 and 29]. Tannins from the bark, roots and other parts of many plants especially Euphorbiaceae are used to treat cells that have gone neoplastic [30]. Tannins are reported to have astringent properties on mucous membranes [31]. So these same claims associated with herbal bitters may be traced to its tannin content. Flavonoids are regarded as having anti-inflammatory (inhibit inflammatory metabolites and granulation tissue formation), anti-allergic (inhibit histamine release) and anti-oxidant effects. They are well known for strengthening and protective effect on fragile capillary and venous structures. Flavonoid often exhibit their therapeutic function through an ability to inhibit enzyme system (eg lipooxygenase, cyclo-oxygenase, elastase and aldose reductase) as well as free radical scavenging and co-factor activity for the anti-oxidant, vitamin C. Other actions demonstrated by different flavonoids include hepatoprotective, anti-spasmodic, hypo-cholesterolaemic, diuretic, anti-viral and anti-bacterial effects. Examples of flavonoids include quercitrin, quercetin, kaempferol [32 and 33]. So these same claims associated with herbal bitters may be traced to its flavonoid content. Saponins when present in high concentration in the plants as compared to other phytochemicals, will likely indicate that extracts from these plants are used in wound healing and bleeding treatment. Saponins have properties of precipitating and coagulating red blood cells and they also have cholesterol binding properties (hence their hypolipidaemic/hypocholesterolaemic property), formation of foams in aqueous solutions and haemolytic activity [34 and 35]. Some of these same claims associated with herbal bitters may therefore be traced to their saponin content.

Cyanogenic glycosides: the main worry about this constituent is its toxicity because in large doses hydrogen cyanide (hydrocyanic acid) which is released from its aglycone part inactivates respiratory enzymes, shuts down the CNS and leads to death, but for this level of toxicity to be experienced, large doses of the herbal/plant material will have to be consumed. Luckily, our bodies can neutralize cyanides and eliminate them through urine. Cyanogenic glycosides consumed in smaller doses are broken down slowly and are easily detoxified by the body. One needs to consume about 500mg three times a day to produce a toxic effect. In a bitter almond there are between 4mg to 9 mg of hydrogen cyanide, so you will need to consume about 150 bitter almonds a day before you will experience any toxic effect [36, 37 and 38].

This research shows that the cyanogenic glycoside content of the herbal bitters especially in Alomo bitters of 0.09%, as shown in Table 2 is safe and too low to cause any toxicity in the prescribed dosage the bitters are normally consumed; instead the range is enough to harness any positive effect known in the medicinal use of cyanogenic glycosides.

Alomo bitters contain significant amounts of Na, K, P, Ca, Mg, Zn, Mn, Fe and Cu, while Pb, Cr and Se were not detected in them. Recent promotion of herbs as health foods commonly includes reference to their mineral contents. Unfortunately, little consideration is generally given to the fact that only five mineral elements are considered essential for metabolism in substantial amounts (calcium, magnesium, potassium, phosphorus and sodium), while ten others (chromium, cobalt, copper, fluorine, iodine, iron, manganese, molybdenium, selenium and zinc) are important in trace amounts only; of these,

probably only selenium, molybdenuim, manganese, chromium and fluorine are essential [39]. Controversy exists over metabolism need versus optimal intake. Toxic levels are often very near the required dosages for "normal diets" and minerals like lead (Pb), arsenic (As), mercury (Hg), silver (Ag) and cyanide (CN^-) are toxic and of no significant use in the human body [40]. In case of the Pb concentration, the suggested concentration in plant species that is "safe" is 0.2 to 0.6 mg/100ml, however, WHO recommendations for Pb level in humans, is that it should not exceed 10 ppm [41]. It is noteworthy that the bitters of this study are not contaminated with lead (Pb) so there is no danger of Pb toxicity from them. There has been some speculation that mild deficiencies of minerals may be beneficial. Dietary deficiencies are common with iron, calcium, iodine and fluorine [39]. So the bitters used in this study can be of help in mineral deficiencies especially in cases of Na, K, P, Ca, Mg, Zn, Mn, Fe and Cu deficiencies.Sodium (Na) and Potassium (K) play significant roles in acid-base balance, fluid balance, nerve function and proper neuromuscular and cardiac activity/function, [40], their presence in bitters may as well explain these same roles said to be played by bitters. Contribution of Phosphates to overall health by its involvement in energy transfer, phosphorylation/ dephosphorylation reactions, lipid metabolism by being a constituent of lipoproteins and acid-base balance and enzyme action [40] may as well explain the claim of bitters contribution to overall health and lipid metabolism. Normal extracellular calcium concentrations are necessary for blood coagulation and for the integrity of intracellular cement substances and integrity and proper functioning of nerves, skeletal muscle and heart/smooth muscle [42], so the presence of calcium in the bitters may well explain these same claims about bitters. Concentration on which Zn affects human health ranges from 100 to 500 mg/l (10-50mg/100ml) [43]. The presence of zinc in the plants could mean that the plants can play valuable roles in the management of diabetes, which result from insulin malfunction [42], so apart from the claim of bitters playing a role in glucose metabolism, the presence of Zinc may well contribute to the claim that bitters play a role in free radical scavenging activity, improved growth, sexual activity and wound healing.

That Alomo bitters have IC_{50} values for 50% ABTS inhibition with the value of 25.46mg/ml indicate that the bitters can be said to have a relatively high antioxidant capacity as claimed by the producers of the bitters. Also, it has DPPH and OH scavenging activity. Herbal products have been said to have phytochemical constituents that confer on them antioxidant properties [35 and 44], this is in agreement with this research as the fact that these bitters can inhibit the ABTS, DPPH and OH radical confirms the antioxidant capacity of the herbal bitters. Primarily, antioxidant effect is due to phenolic compounds such as phenolic acid, flavonoids and phenolic diterpenes and their mode of action as antioxidant compounds is due to their redox reaction properties which can absorb and neutralize free radicals by quenching singlet and triplet oxygen (Krauss and Howard, 2000; Hussain *et al.,* 2011).

CONCLUSION

The biochemical assay results of this study with inferences derived from some of the already established effects of some of the phytochemical and mineral constituents of these bitters, gave some evidence that the herbal bitters of this study may be said to have the potential or possibility of having the following pharmacological properties - hypolipidaemic, hypoglycaemic, anti-anaemic and anti-inflammatory, stimulant and immunity-boosting, choleretic/ hepatoprotective and antihepatotoxic, *invivo* and *invitro* antioxidant capacity and by extension anticarcinogenic as well as antihypertensive properties and the ability to protect against cardiovascular diseases generally.

REFERENCES

1. Soforowa, A.E., 1982. Medicinal plants and traditional medicine in Africa (2nd edn.). John Wiley and Sons, Chichester, New York. USA. pp: 1-14.
2. Hoffmann, D. (ed.) 2003. Medical Herbalism: The Science and Practice of Herbal Medicine. (1st edn). Healing Arts Press, Vermont. pp: 1-672.
3. Hoffmann, D. (ed.) 2000. Healthy Digestion: A Natural Approach to Relieving Indigestion, Gas, Heartburn, Constipation, Colitis & More, (1st edn). Storey Publishing, LLC, Massachusetts. pp: 1- 123.
4. Mcdonald, J., 2014. Blessed bitters. Pp 141-154. http:// www.herbcraft.org/bitters.pdf. Accessed Feb. 20th 2014.
5. Green, J., (ed.) 1991. The Male Herbal: Health Care for Men and Boys. (1st edn). The Crossing Press, California, USA. pp: 1-340.
6. FAO: Compositional Analysis Methods. In: Manuals of food quality control 7. Food analysis: general techniques, additives, contaminants and composition. Food and Agricultural Organisation of the United Nations Food and Nutrition, Paper 14(7):203-232. 1986.

7. AOAC: Official Method of Analysis of the Association of Official Analytical Chemists, (14th edn.). Williams S.W. (ed.). AOAC, incorporated, Washington DC. pp: 1-2000. 1984.
8. AOAC: Official Method of Analysis of the Association of Official Analytical Communities. (17th edn). Horwitz. W. (Ed.). AOAC International, Gaithersburg, Maryland, USA. pp: 1-2200. 2000.
9. Onyeike, E.N. and J.O. Osuji, (eds). 2003. Research Techniques in Biological and Chemical Sciences. (1st edn). Springfield Publishers Ltd, Owerri, Nigeria. pp: 1-403..
10. Miller, G.L., 1972. Estimation of reducing sugar by dinitosalicylic acid method. Analytical Chemistry, 31: 426-428.
11. Malik, C.P. and M.B. Singh, (eds.). 1980. Plant Enzymology and Histoenzymology (1st edn.). Kalyani Publishers, New Delhi, India. pp: 1-286.
12. Santhi, R., G. Lakshmi, A.M. Priyadharshini and L. Anandaraj, 2011. Phytochemical screening of Nerium oleander leaves and Momordica charantia leaves. International Research Journal of Pharmacy, 2(1): 131-135.
13. Trease, G.E. and W.C. Evans, 1989. A Textbook of Pharmacognosy, (13th edn), Bailliere Tindall Ltd., London. pp: 176-180.
14. Edeoga, H.O., D.E. Okwu and B.O. Mbaebie, 2005. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology, 4(7): 685-688.
15. Boham, B.A. and R. Kocipal-Abyazan, 1974. Flavonoids and condensed tannins from leaves of Haiwaiian Vaccinium vaticulatum and V. calycinium. Pacific Science, 48: 458-463.
16. Harborne, J.B., 1973. Phytochemical methods. In: A guide to modern techniques of plant analysis. JB Harborne (ed.) Chapman and Hall, London. UK. pp: 49-279.
17. Olsen, S.R. and L.E. Sommers, 1982. Phophorus, In: Page, A.L., Miller, R. H. And Keeney, D.R, (eds). Methods of Soil Analysis, Part 2. Chemical and Microbial Properties. Madison, WI: American Society of Agronomy and Soil Science Society of America, USA. pp: 403-430.
18. Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans, 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, 26: 1231-1237.
19. Jain, A., M. Soni, L. Deb, A. Jain, S. Rout, V. Gupta and K. Krishna, Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of Momordica dioica leaves. Journal of Ethnopharmacology, 115: 61-66.
20. Kunchandy, E. and M.N.A. Rao, 1990. Oxygen radical scavenging activity of curcumin. International Journal of Pharmacology, 58: 237-240.
21. Hazra, B., S. Biswas and N. Mandal, 2008. Antioxidant and free radical scavenging activity of Spondias pinnata. BMC Complementary Alternative Medicine, 8: 62-63.
22. Salaam, A.O. and J. Brown, 2012. Lagos “area boys”, substance usage and potential risk factors. International Journal of Mental Health and Addiction, 10(1): 83-96.
23. Awa, I.N. and V.O. James, 2013. Analyses of the effect of Swedish Bitters on selected metabolic profiles. International Research Journal of Pharmacy, 4(8): 120-127.
24. Serafini, M., A. Ghiselli and A. Ferro-Luzzi, 1994. Red wine, tea and anti-oxidants. Lancet, 344: 626-627.
25. Haslam, E., 1996. Natural polyphenols (vegetable tannins) as drugs: Possible modes of action. Journal of Natural Products, 59: 205-215.
26. Okwu, D.E. and M.E. Okwu, 2004. Chemical composition of *Spondias mombin* linn plant parts. Journal of Sustainable Agriculture and the Environment, 6(2): 140-147.
27. Bulter, L.G., 1989. Effects of Condensed Tannins on Animal Nutrition. In: ‘‘Chemistry and Significance of Condensed Tannins’’. Hemmingway, R.W. and Kachey, J.J. (Eds). Plenum Press, New York. pp: 391-402.
28. Nwogu, L.A., C.U. Igwe and A.A. Emejulu, 2008. Effects of leaf extract on the liver function profile and haemoglobin concentration of albino rats. African Journal of Biotechnology, 2(12): 240-242.
29. Duke, J.A. and K.K. Wain, 1981. Medicinal Plants of the World. Computer index with more than 85,000 entries. 3: 1-1654.
30. Egunyomi, A., J.O. Moody and O.M. Eletu, 2009. Antisickling activities of two ethnomedicinal plant recipes used for the management of sickle cell anaemia in Ibadan, Nigeria. African Journal of Biotechnology, 8(1): 20-25.
31. Trease, G.E. and W.C. Evans, 2005. Pharmacognosy, (15th edn.) An Imprint of Elsevier, New Delhi, India. pp: 246-417.
32. Kokate, C.K., A.P. Purohit and S.B. Gokhale, (eds) 2007. “Pharmacognosy” (37th edn). Nirali Prakashan, Pune, India. pp: 244-368.

33. Sodipo, O.A., J.A. Akiniyi and J.U. Ogunbamosu, 2000. Studies on certain characteristics of extracts of bark of *Pansinystalia macruceras* (K schemp) pierre exbeille. Global Journal of Pure and Applied Sciences, 6: 83-87.
34. Hussain, I., R. Ullah, R. Ullah, M. Khurram, N. Ullah, A. Baseer, F.A. Khan, M.R. Khattak, M. Zahoor, J. Khan and N. Khan, 2011. Phytochemical analysis of selected medicinal plants. African Journal of Biotechnology, 10(38): 7487-7492.
35. Wade, N., 1977. Laetrile at Sloan-Kettering: A Question of Ambiguity. Science, 198(4323):1231-1234.
36. Stock, C.C., G.S. Tarnowski, F.A. Schmid, D.J. Hutchison and M.N. Teller, 1978a. Antitumor tests of amygdalin in transplantable animal tumor systems. Journal of Surgical Oncology, 10(2): 81-88.
37. Stock, C.C., D.S. Martin and K. Sugiura, 1978b. "Antitumor tests of amygdalin in spontaneous animal tumour systems. Journal of Surgical Oncology, 10(2): 89-123.
38. Nightingale, S.L., 1984. "Laetrile: the regulatory challenge of an unproven remedy". Public Health Report, 99(4): 333-8.
39. Crellin, J.K., A.L. Jane-philpot and T. Bass, (Eds) 1997. Herbal medicine Past and Present: A reference guide to medicinal plants (1st Edn). Duke University Press. USA. pp: 1-560.
40. Chatterjea, M.N. and R. Shinde, 2007. Textbook of Medical Biochemistry, (7th Edn). JayPee Brothers Medical Publishers LTD, New Delhi, India. pp: 1-255.
41. WHO 2007. Guidelines for assessing quality of herbal medicine. World Health Organization, Geneva, Switzerland. pp: 1-105.
42. Okaka, J.C. and A.N.O. Okaka, (eds) 2001. Food Composition, Spoilage and Shelf Life Extension. (1st edn.) Ocjarco Academic Publishers, Enugu, Nigeria. pp: 54-56.
43. Macnicol, R.D. and P.H.T. Beckett, 1985. Critical tissue concentrations of potentially toxic elements. Plant Soil, 85: 107.
44. Loliger, J., 1991. The Use of Antioxidants in Food. In: O. I. Aruoma, & B.Halliwell (Eds.), Free Radicals and Food Additives. pp: 129-150.