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Ethnopharmacological properties of *Vernonia amygdalina* (Bitter Leave) medicinal plant

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Abstract
Vernonia amygdalina (bitter leave) plant is a medium sized shrub with abundant bitter principles in every part of it. All parts of the plant; roots, stems and leaves are pharmacologically useful. *Vernonia amygdalina* plant is used in phyto-medicine to treat fever, hiccups, kidney disease and stomach discomfort among others. Phytochemical, proximate and mineral compositions of the leaves, stems and roots of *Vernonia amygdalina* (bitter leave) plant was analyzed using the standard method of Association of Analytical Chemist (AOAC) and Atomic Absorption Spectrophotometric (AAS) method. The qualitative phytochemical result showed that the plant sample contain alkaloids, tannins, saponins, terpenoids, glycosides, phenols and flavonoids. Quantitatively, the result ranges from alkaloids 12.78 ± 11.41%, tannins 6.30 ± 3.44%, saponins 3.20 ± 1.40%, flavonoids 2.37 ± 0.68%, terpenoids 1.30 ± 1.18% and phenols 0.54 ± 0.36%. Proximate result ranges from (2.40 ± 2.24%) moisture, (0.50 ± 0.36%) ash, (5.77 ± 2.86%) protein, (0.55 ± 0.38%) lipid, (2.48 ± 1.62%) fiber, (97.76 ± 97.60%) dry matter and (94.30 ± 91.70%) nitrogen free element (NFE). Mineral results ranges from 145.36 ± 140.38ppm, 82.63 ± 73.73ppm, 86.61 ± 54.74ppm, 60.38 ± 53.98ppm, 5.03 ± 4.25ppm, 1.78 ± 0.86ppm, 0.62 ± 0.39ppm, 0.48 ± 0.31ppm, 0.70 ± 0.29ppm for Ca, Mg, Na, K, Fe, Mn, Cu, Zn and PO_4 respectively. The presence of these biomolecules in the plant explains the pharmacological potentials of *Vernonia amygdalina* roots, stems and leaves in therapeutic uses such as an antimalarial, antidiabetic, fertility agent, anti-cancer, anti-ulcer and cardio-protective agent. Also the leafy vegetables if consumed in sufficient amount would contribute greatly to the nutritional requirement for human health and to the food security of Nigerian population.

Keywords: *Vernonia amygdalina,* ethnopharmacological properties, medicinal plant

Introduction
Since time immemorial plants have been used ethnobotanically for food, shelter, medicine, clothing, hunting and in religious ceremonies, but their primary use has been for health care [1]. Different parts of medicinal plants have been used to cure specific ailments [2]. Recently, a gradual revival of interest in the use of medicinal plants in developing countries was rekindled because herbal medicines have been reported to be safe and without any adverse side effect especially when compared with synthetic drugs [3, 4]. The ongoing recognition of medicinal plants in recent times is due to various reasons; include increasing faith in herbal medicine [5]. On the top of that, an increasing dependence on the use of these medicinal plants in the industrialized organizations has been traced to the extraction of bioactive ingredients used in the development of drugs and chemotherapeutics from these plants as well as from conventionally used herbal remedies [6]. The therapeutic properties of plants could be based on the anti-oxidant, anti-microbial, antipyretic effects of the phytochemical constituents in them.[7] Phytochemicals are naturally occurring bioactive compounds in the leaves, stems, bark, fruits and roots of medicinal plants which have defense mechanism that protects the plants, animals and humans that feed on them from various diseases. Phytochemical constituents like terpenoids, tannin, saponin, alkaloids, phenolic compounds, etc exhibit various important pharmacological activities that is, anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities [8].
Vernonia amygdalina belongs to the family Compositae or Asteraceae, variously known as bitter leaf (English), *kiriolugbo* (Izon), *ewuro* (Yoruba), *shikawa* (Hausa), and *olubu* (Igbo), it is a tropical shrub, 1-3m in height with petiole leaf of about 6mm in diameter, and elliptic in shape [9]. The leaves are coloured dark green with a characteristic odour and a bitter taste. The species is indigenous to tropical Africa and is found wild or cultivated all over sub- Saharan Africa [10]. The leaves are eaten, after crushing and washing thoroughly to remove the

bitterness [11]. All parts of the plant are pharmacologically useful. Both the roots and leaves are used in phytomedicine to treat fever, hiccups, kidney disease and stomach discomfort among others [12]. The stem and root divested of the bark are used as chew-sticks in Nigeria. More importantly, the leaves are used to prepare the very popular bitter leaf soup in Nigeria, and are also reportedly consumed by goats in some parts of the country [13]. Antihelmitic, antimalarial and antitumourigenic properties have been properly reported for extracts from this plant [14, 15]. Other studies have demonstrated hypoglycaemic and hypolipidaemic effects of the leaf extract in experimental animals [16-18]. This study was conducted to determine the ethnopharmacological properties of the leaves, roots and stems of *Vernonia amygdalina* (bitter leave) medicinal plant by analyzing their phytochemical, proximate and mineral compositions.

Materials and Methods

Collection of plant materials

Vernonia amygdalina was collected from a vegetable garden in Yenagoa, Bayelsa State, Nigeria. The plant parts were washed, sundried, pulverized and stored in an airtight container for laboratory analysis.

Qualitative phytochemical screening

Phytochemical screening of the extracts was carried out by a procedure that was based on those earlier reports by [19 - 21].

Test for saponins

To 0.5g of extract, 5ml of distilled water was added in a test tube and the solution shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for terpenoids

0.5g of the extract was dissolved in 1ml of chloroform and 1ml acetic anhydride added, followed by the addition of 2ml of concentrated H_2SO_4. Terpenoids was indicated by formation of reddish violet colour.

Test for tannins

About 0.5g of the extract was boiled in 10ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and the solution observed for brownish green or a blue-black colouration.

Test for cardiac glycosides (keller-killiani test)

To 0.5g of extract dissolved in 5ml water was added 2ml of glacial acetic acid solution containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated H_2SO4. A brown ring at the interface indicated the presence of deoxysugar characteristics of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Test for flavonoids

Dilute ammonia (5ml) was added to a portion of an aqueous filtrate of the extract. Then, concentrated sulphuric acid (1ml) was added. A yellow colouration indicated the presence of flavonoids.

Test for alkaloids

Extract was dissolved in dilute HCl and filtered. Filtrates were treated with Mayer`s reagent (potassium mercuric iodide). Formation of a yellow coloured precipitate indicated the presence of alkaloids.

Quantitative phytochemical analysis

Depending on the above qualitative results the quantitative assay was carried out for Alkaloids, Tannins, Phenols, Saponin, Flavonoids and Terpenoids

Total tannins content determination

The tannins were determined by slightly modified Folin and Ciocalteu method. Briefly, 0.5 ml of sample extract was added with 3.75 ml of distilled water and 0.25 ml of Folin Phenol reagent, 0.5 ml of 35% sodium carbonate solution. The absorbance was measured at 725 nm. Tannic acid dilutions (0 - 0.5mg/ml) were used as standard solutions. The results of tannins were expressed in terms of tannic acid in mg/ml of extract.

Total phenol content determination

The phenols were determined by slightly modified Folin and Ciocalteu method. Briefly, to 200µl of the sample extract, 800µl of Folin Ciocalteu reagent mixture and 2 ml of 7.5% sodium carbonate added. The total content was diluted to 7 volumes with distilled water. The tubes were kept for 2 hours and allowed to incubate in the dark. The absorbance was measured at 765 nm. Gallic acid dilutions were used as standard solutions. The results of phenols are expressed in terms of Gallic acid in mg/ml of extract.

Total alkaloid content determination

40 ml of 10% acetic acid in ethanol was added to 1g of powdered sample, covered and allowed to stand for 4 hours. The filtrate was then concentrated on a water bath to get 1/4th of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and collected precipitate was washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed.

Total flavonoid content determination

The total flavonoids content of samples was determined by following the Aluminum chloride method. Plant concentrate was mixed with distilled H_2O and $NaNO_2$ solution. After 6 minutes, $AlCl_3$ solution was added and allowed to stand for 6 minutes. NaOH solution was added to the mixture. Immediately distilled H_2O was added to bring to the final volume and then the mixture was extensively mixed and allowed to stand for another 15 minutes. Optical density of the mixture was recorded at 510 nm. Rutin was used as a standard compound for the evaluation of total flavonoid. The total flavonoids were calculated using the standard curve, and expressed as rutin equivalent in mg/g of extracts.

Total saponin content determination

Test extract were dissolved in 80% methanol, 2ml of Vanillin in ethanol was added, mixed well and 2ml of 72% sulphuric acid solution was added, mixed well and heated on a water bath at 600^0C for 10minutes, absorbance was measured at 544nm against reagent blank. Diosgenin was used as a standard material and compared the assay with Diosgenin equivalents.

Total terpenoid content determination
The extract (1g) was macerated with 50ml of ethanol and filtered. To the filtrate, 2.5 ml of 5% aqueous phosphomolybdic acid solution was added and 2.5ml of concentrated H_2SO_4 was gradually added and mixed. The mixture was left to stand for 30minutes and then made up to 12.5ml with ethanol. The absorbance was measured at 700nm.

Methods for proximate analysis
The dry matter, moisture, ash, crude fat, crude protein (nitrogen x 6.25) and crude fibre contents were determined in powdered *Vernonia amygdalina* plants using the standard methods of the Association of Official Analytical Chemists [22] while Dry Matter and Nitrogen Free Element content was calculated based on the net difference between the other nutrients and the total percentage composition.

Estimation of ash
About 2g of the sample was weighed and taken in a vitreosil basin. The basin was heated in a low flame at the beginning till no fumes were given off by the charred mass. It was broken by a glass rod carefully and burnt in a muffle furnace at 550- 600°C for 4-5 hours. The muffle was allowed to cool to 150°C. The basin was then cooled in a desiccators and the ash content was then weighed. The total ash was calculated as follows:
% of total ash = weight of the ash ×100 / weight of the sample.

Estimation of moisture content
Fresh sample materials were taken in a flat bottom dish and kept overnight in a hot air oven at 100-110°C and weighed. The loss in weight was regarded as a measure of moisture content.

Estimation of crude protein (Micro-Kjeldahl Method)
Digestion: About 2gm of sample was taken in a Kjeldahl flask; 10gms of sodium sulphate and 0.5 gm of copper sulphate was added and mixed well. A few glass beads were added into the flask to prevent spurting while heating. Then 25 ml of concentrated H_2SO_4 was added and then heated at least for 15-20 minutes in inclined position. The solution was boiled until a greenish colour was obtained. It was allowed to cool.

Distillation
About 100 ml of distilled water was added to the Kjeldahl flask, shaken properly and transferred it into a 250 ml volumetric flask. Then the final volume was made up to 250 ml by adding distilled water. In a conical flask, 10-15 ml of 2% Boric acid was taken and the flask was placed below the condenser of the distillation apparatus. Thereafter, 5 ml of aliquot was transferred to the Micro Kjeldahl steam distillation apparatus and added 1 drop of phenolphthalein and 10-15 ml 40% NaOH. The distillation was carried out at least for 5-10 minutes until ammonia was free from aliquot. Titration: The distillation product was then titrated against N/10 H_2SO_4

Calculation is done as follows:

$$\% \text{ of Nitrogen} = \frac{\text{ml of N/10 } H_2SO_4 \text{ used up} \times 250 \times 0.0014 \times 100}{\text{Volume of aliquot} \times \text{gm of the substance taken}}$$

% of crude protein=% Nitrogen ×6.25

Estimation of crude lipid (Ether extract)
Five (5) gm of dry sample was weighed on a piece of glazed paper and transferred into an extraction thimble. The thimble was introduced into soxhlet extractor over a pad of cotton wool, so that top of the thimble is well above the top of the siphon. A clean dry flask was taken, weighed and was fitted with the extractor. Ether was poured along the side of the extractor until it begins to siphon off. Then another half of siphon full of ether was added. The equipment thus assembled with the flask was placed on a water bath at 60-80°C and the extractor was connected with the condenser. Cool water circulation was started in the condenser and allowed the extraction for 8 hours. Then the thimble with the material was removed from the extractor. The apparatus was assembled again and heated on a water bath to recover all the ether from the receiver flask. The receiver flask was disconnected and dried in a hot air oven at 100°C for 1hour, cooled and weighed.

$$\% \text{ of Ether extract} = \frac{(\text{Wt. of oil flask with ether extract} - \text{Wt. of the oil flask})}{\text{gm of the substance taken}} \times 100$$

Determination of crude fibre
About 2gm of moisture and fat free sample was weighed and transferred to the spout less one litre beaker. Thereafter, 200 ml of 1.25% H_2SO_4 was added. The beaker was placed on hot plate and allowed to reflux for 30 minutes, timed from onset of boiling. The content was shaken after every 5 minutes. The beaker was removed from the hot plate and filtered through a muslin cloth using suction. The residue was washed with hot water till it was free from acid. The material was transferred to the same beaker and added 200ml of 1.25% NaOH solution and refluxed for 30 minutes. Again filtered and the residue was washed with hot water till it was free from alkali. The total residue was transferred to a crucible and placed in hot air oven, allowed to dry to a constant weight at 80-110°C and weighed. The residue was ignited in muffle furnace at 550-600°C for 2-3 hours, cooled and weighed again. The loss of weight due to ignition was the weight of crude fiber.

$$\% \text{ of Crude Fiber} = \frac{(\text{Wt of the crucible with dry residue} - \text{Wt of crucible with ash})}{\text{gm of the substance taken}} \times 100$$

Procedure for mineral analysis
Estimation of Fe, Zn, Mg, Mn, Na, K and Cu
For this study, 0.5 gm of powdered dried sample was taken in a crucible and converted to ash in the muffle furnace at 580°C for 3 hours. After cooling in a desiccators, 10ml of concentrated Nitric acid, 4ml of Perchloric acid and 1ml of Sulphuric acid were added and digestion at high temperature was carried out until the content became clear, then the tube was cooled and the solution was transferred quantitatively to 50ml volumetric flask and the final volume was adjusted to 50ml by adding distilled water. The solution was used for determination of Fe, Zn, Mg, Mn, Na, K and Cu through atomic absorption spectrometry (AA203D). Calcium and Phosphorous estimation were done as per method described by Oyedele, 2001[23].

Results

Qualitative and Quantitative phytochemical analysis of bitter leaf plant

Qualitative phytochemical compositions of bitter leaf plant (Leaves, Stems and Roots) shows the presence of Alkaloid, Tannin, Saponin, Flavonoid, Terpenoid, Glycoside and Phenols as shown in table 1. While quantitative phytochemical composition of bitter leaf plant (Leaves, Stems and Roots) was highest in Alkaloids and Tannins and lowest in Phenols as shown in Fig. 1

Table 1: Qualitative Phytochemical Composition of Bitter Leaf Plant

Plant Samples	Phytochemical Properties						
	Alkaloid	Tannin	Saponin	Flavonoid	Terpenoid	Glycoside	Phenols
Leaves	+++	++	++	++	+	++	+
Stems	++	+	+	++	+	+	+
Roots	++	++	+	++	++	+++	++

(**Key**: +mild ++moderate +++abundance)

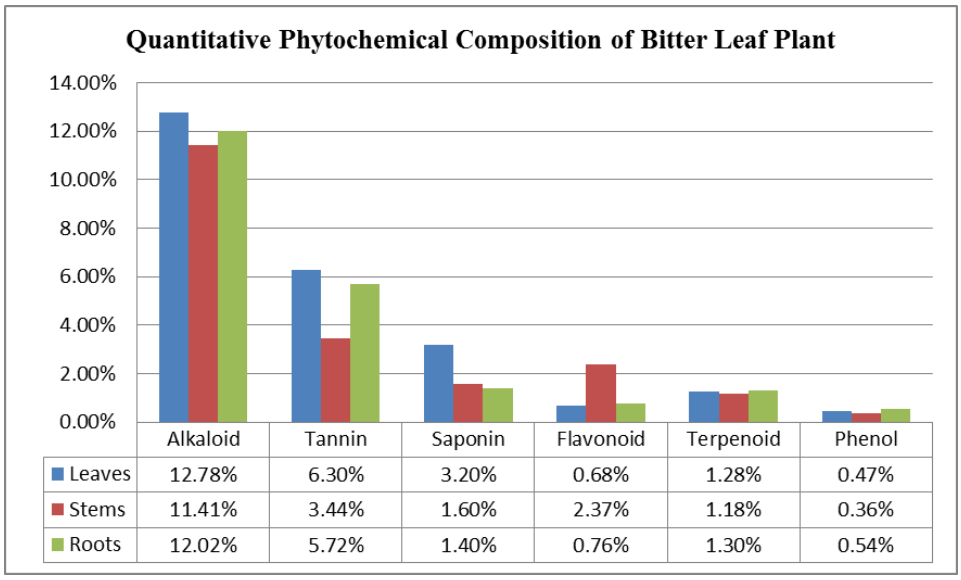

Fig 1: Quantitative Phytochemical Composition of Bitter leaf plant

Proximate analysis of bitter leaf plant

Proximate compositions of the leaves, stem and roots of bitter leaf plant was highest in protein and Moisture contents and lowest in Ash and Lipid contents as shown in Fig. 4.2.

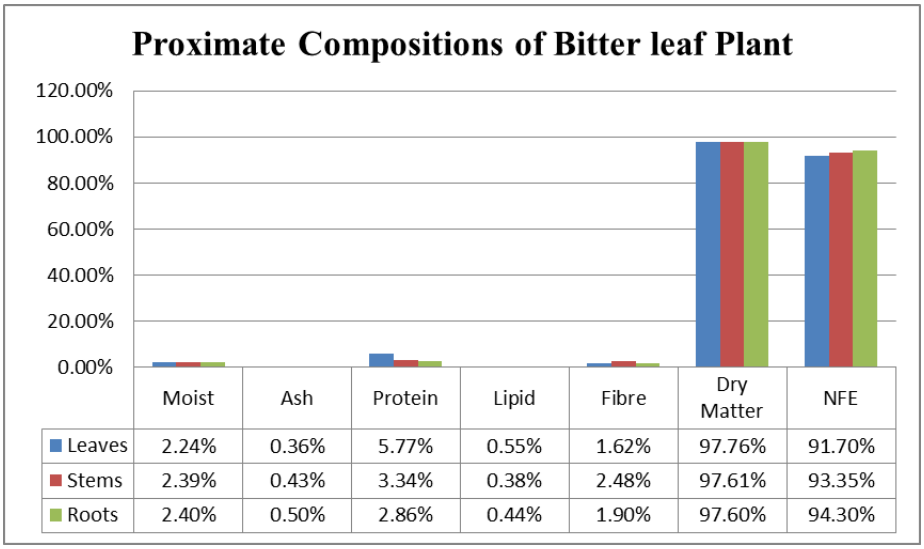

Fig 2: Proximate Compositions of Bitter leaf Plant

Mineral contents of bitter leaf plant

Mineral contents of Leaves, stems and roots of bitter leaf plants shows highest in Calcium, Magnesium and Sodium and Lowest in zinc and copper as shown in table 2.

Table 2: Mineral Content of Bitter Leaf Plant

Plant Samples	Minerals (ppm)								
	Ca	Mg	Na	K	Fe	Mn	Cu	Zn	PO_4
Leaves	142.84	74.55	86.61	53.98	5.03	1.78	0.62	0.42	0.70

Stems	140.38	73.73	65.48	58.27	4.25	1.48	0.39	0.31	0.29
Roots	145.36	82.63	54.74	60.38	4.67	0.86	0.50	0.48	0.60

Discussion

This study has revealed the presence of qualitative phytochemicals considered as active pharmaco-chemical constituents such as alkaloids, tannins, saponins, flavonoids, terpenoids, glycosides and phenols (Table 1). Quantitatively, the levels of alkaloids (12.78 ± 11.41%) were highest when compared with others in all the parts of *V. amygdalina* investigated. Alkaloids have been reported to uniformly invoke a bitter taste [24]. The use of the leaves for treatment of abdominal pain and painful uterus might be as a result of the analgesic property of alkaloids [25]. Generally, the plant has rich phytochemical constituents, of which attributed to several ethnopharmacological uses of different parts of *V. amygdalina* in Nigeria, presumably. The leaves are used for treatment of abdominal pain, cough, diabetes, diarrhoea, diuretic, fever, kidney and urinary problem, increase lactation, stomach ulcer, painful uterus, venereal disease and anti-aging [26]. In addition, the roots and leaves are used for infertility in women and weak joints; roots for aphrodisiac and amenorrhea; leaves and stem for oedema. Lipid lowering effects of both aqueous and methanol extracts of *V. amygdalina* leaves have been reported [27, 28]. The aforementioned pharmacological usefulness of the plant might be basically as a result of the flavonoid, glycoside, terpenoids, phenols, saponin and tannin contents of the plant.

The usefulness of the leaves as anti-aging agent might be as a result of the flavonoid content of the plants. Flavonoids act as free radical scavengers [29, 30]. The flavonoids content of the plant is 2.37 ± 0.68% (fig.1), this justifies its use as an anti-hypertensive agent since epidemiological and clinical studies have provided evidence of a potential role for flavonoids in lowering the risk of coronary heart diseases, cardiovascular, Alzheimer's, neurodegenerative diseases, as well as diabetes, osteoporosis and lung cancer [31] through anti-oxidative action and / or the modulation of several protein functions. Saponins content (3.20 ± 1.40%) suggests the usefulness of the vegetable as a potential fertility agent and also useful for the treatment of hypercholesterolemia. This suggested that saponins might be acting by interfering with intestinal absorption of cholesterol, thus producing antidiabetic effects [32, 33]. The utilization of the plant in treatment of diarrhoea can be attributed to the anti-diarrhoeal property of tannins. Tannins have been reported to possess anti-diarrhoeal ability [34, 35]. Tannins are known to be useful for the prevention of cancer as well as treatment of inflamed or ulcerated tissues [36, 37].

The high value of protein (5.77 ± 2.86%) in the plant presents them as excellent macronutrient sources for food. Proteins are building block units and the food protein is needed to make vital hormones, important brain chemicals, antibodies, digestive enzymes, and necessary elements for the manufacture of DNA. Some proteins are involved in structural support, while others are involved in bodily movement, or in defense against germs [38]. The crude fibre content of the plant is 2.48 ± 1.62%. Fibre cleanses the digestive tract by removing potential carcinogens from the body and prevents the absorption of excess cholesterol. Fibre also adds bulk to the diet and prevents the intake of excess starchy food [39] and may therefore guard against metabolic conditions such as hypercholesterolemia and diabetes mellitus [40]. Other findings [41] also showed evidence that a high intake of dietary fiber is associated with enhanced insulin sensitivity and therefore may have a role in the prevention and control of Type 2 diabetes. The substantial amount of fibre in *Vernonia amygdalina* plant shows that they can help in keeping the digestive system healthy and functioning properly. The moisture content was also high (2.40 ± 2.24%) indicating that the vegetable is susceptible to spoilage. The presence of ash content of the bitter leave plant suggests that it has a greater level of minerals.

The trace elements present in the medicinal plants will play significant roles in the formation of active constituents responsible for the curative properties. Moreover, some of these elements are very important in the human body [42]. The high concentration of potassium in the plants will be very important for enzyme activation, photosynthesis, water use efficiency, starch formation and protein synthesis. Potassium participates actively in the maintenance of the cardiac rhythm [43, 44]. Potassium levels 60.38 ± 53.98 ppm was high in the plant samples, and this is in agreement with many reports that potassium is the most abundant mineral in Nigerian agricultural products [45]. Calcium helps in the regulation of muscle contraction required by children, infants and foetuses for bones and teeth development [46]. Normal extracellular calcium concentration is necessary for blood coagulation and for the integrity of intracellular cement substance [47]. It also helps in the development of strong bone and teeth. Calcium levels ranges from 145.36 ± 140.38 ppm.

According to Perry [48], Mg and P have important roles in the metabolism of cholesterol as well as heart diseases. The presence of Mg in plants may be correlated with therapeutic properties against diabetes and cardiovascular diseases [49]. Phosphorus or phosphate is the component of more than 240 enzymes [50] and its deficiency in the organism is accompanied by multisystem dysfunction. Besides, PO_4 is responsible for sperm manufacture, fetus development and proper function of immune response [51-53]. The PO_4 concentration ranges from 0.70 ± 0.29 ppm. Iron is said to be an important element in the diet of pregnant women, nursing mothers, infants, convalescing patients and the elderly to prevent anaemia and other related diseases [54]. Copper level in the plant samples under study ranged from 0.20 - 0.12ppm. Copper is involved in the formation of red blood cells and synthesis of haemoglobin. It has a role in energy production, wound healing, skin and hair color as well. Copper is also involved in stimulating body defense system. Manganese regulates blood sugar levels, the production of energy and cell reproduction. Deficiency of manganese may result in birth defects if an expectant mother does not get enough of this important element [55].

Conclusion

The broad distribution of phytochemicals and nutrients in the *Vernonia amygdalina* plant studied support, as well as provide a basic rationale for its use in folk medicine. This study also indicates that *Vernonia amygdalina* leaves, besides serving as good source of pharmacologically active phytochemicals may also be useful as supplements in human and animal nutrition. The phytochemical profile shows the potential medicinal usefulness of the plant as an agent capable of ameliorating a myriad of diseases, including diabetes, malaria and cardiovascular problems. The mineral contents of the medicinal plant studied are a source of biologically important elements, which may play part in the observed

therapeutic properties of the plant. Further research work on the roots of the plant especially hydrogen cyanide and other toxic chemicals is highly recommended because of the poisonous nature of the roots as claimed by traditional healers.

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