Evaluation of the Phytochemical composition, Micronutrient status and Antioxidant Properties of *Aloe vera*, *Piper nigrum*, *Telfairia occidentalis*, and *Vernonia amygdalina* Found in South-Eastern Nigeria

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ABSTRACT

Vegetables occupy prominent positions in the diets of several Nigerian ethnic groups. This study compared the phytochemical, antioxidants and micronutrients composition of four varieties of medicinal plants. The aqueous extracts of freshly plucked leaves were obtained using standard procedures. The phytochemical, antioxidant and micronutrient content of the vegetables were quantified using standard analytical methods. The result shows a significant variation ($P < 0.05$) in the concentration of alkaloid in *Aloe vera* compared to *Piper nigrum*, *Vernonia amygdalina* and *Telfairia occidentalis* leaf with a concentration of 11.28 mg/100g. The flavonoids contents of *Piper nigrum* significantly varied ($P < 0.05$) compared to other medicinal plants with a concentration of 9.30 mg/100g while *T. occidentalis* has the least flavonoid with a concentration of 2.7 mg/100g. *A. vera* has a significantly high levels of glycoside and tannin compared to other phytochemical with a concentration of 13.60 and 89.50 mg/100g respectively, while *P. nigrum* had a higher concentration of steroids compared to other medicinal plants, with a composition of 9.28 mg/100g. The result of vitamin analysis shows a higher concentration of vitamin A, E and and C in *V. amygdalina* compared to other medicinal plants. The result of ferric reducing and ferric chelating properties shows that *V. amygdalina* has the ability to degrade iron easily compared to other medicinal plants with a composition of 4.93 umol/l compared to *T. occidentalis* with a composition of 7.82 umol/l and was able to chelate iron compared to other medicinal plants. The scavenging effects of *A. vera*, *P. nigrum*, *V. amygdalina*, *T. occidentalis* extracts on DPPH radicals were excellent, especially in the case of *V. amygdalina* compare to vitamin C but *T. occidentalis* revealed a low value of antioxidant activity. In all, *V. amygdalina* leaf revealed the best antioxidant properties. From the results, the generated data could help to draw attention to the benefits accruable from consumption of these vegetables and hence recommend their sustained inclusion in human diet

Keywords: Phytochemicals, micronutrients, antioxidants, *Aloe vera*, *Piper nigrum*, *Telfairia occidentalis* and *Vernonia amygdalina*

INTRODUCTION

The practice of traditional medicine is as old as the origin of man [1]. The use of plants in traditional medicine referred to as herbalism or botanical medicine falls outside the mainstream of the Western or Orthodox medicine [2]. It has been estimated that about two third of the

world's population (mainly in the developing countries) rely on traditional medicine as their primary form of health care [3]. The use of traditional medicine in the treatment and management of diseases in the African continent cannot fade away and this could be attributed to the socio-cultural, socio-economic, lack of basic health care and qualified personnel [4].

Arising from their biodiversity and perhaps the rich complement of phytochemicals and secondary metabolites, plants have from antiquity been used as sources of medicament against various ailments [5]. In rural areas where access to modern health facilities is limited by the level of development, plants/herbs remain the mainstay of the health care system. Additionally, current research in medicinal plants is beginning to lend credence to their efficacy and potency and in most instances over and above the existing conventional and chemotherapeutic options particularly as it concerns degenerative disease complexes including diabetes mellitus.

Plants are endowed with various phytochemical molecules such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity [6]. Studies have shown that many of these antioxidant compounds possess anti-inflammatory, anti-atherosclerotic, anti-tumor, anti-mutagenic, anti-carcinogenic, anti-bacterial, and anti-viral activities. The ingestion of natural anti-oxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing. In recent years, there has been a worldwide trend towards the use of the natural phytochemicals present in berry crops, teas, herbs, oilseeds, beans, fruits and vegetables.

The action of phytochemicals varies by colour and type of the food. They may act as anti-oxidants or nutrient or protection or to prevent carcinogens (cancer causing agents) from forming. Some phytochemicals with physiological properties may be elements rather than complex organic molecules. For example, selenium which is abundant in many fruits and vegetables is involved with major metabolic pathways, including thyroid hormone metabolism and immune function. There are currently many phytochemicals in trials for a variety of diseases. Lycopene has been tested in human studies for cardiovascular diseases and prostrate cancer. The contributions of Polyphenols to the prevention of cardio-vascular diseases, cancer, osteoporosis and anti-oxidant character with potential health benefits have been reported.

Vegetables vary considerably in their nutrient contents and are good sources of vitamins, essential amino acids, proteins, as well as minerals and antioxidants [7]. They are included in meals mainly for their nutritional value although some are reserved for the sick due to their medicinal properties [8]. Generally, the active principles found in vegetables can be extracted and used in different forms which include infusions, syrups, concoctions, decoctions, infusion oils, essential oils, ointments and creams in the treatment/management and prevention of some diseases [9].

Telfairia occidentalis, *Aloe vera* and *Vernonia amygdalina* leaves, and *Piper nigrum* used as vegetables in various countries of the world have been employed as an effective cure in treating several diseases, ranging from diabetes, malaria, laxative, dysentery, flu, kidney stone elimination etc. This study evaluated their phytochemical, antioxidant and micronutrients compositions.

MATERIALS AND METHODS

Collection of Plant Materials

Telfairia occidentalis, *Aloe vera* and *Vernonia amygdalina* leaves, and *Piper nigrum* were obtained from Uli, Ihiala LGA, Anambra State, Nigeria and were identified and authenticated from standard sources. The collected samples were dried in shade, crushed to coarse powder and stored appropriately for further studies.

Preparation of Plant Extracts

Known weights (400g) of fresh leaves of *T. occidentalis*, *A. vera*, *V. amygdalina*, and *P. nigrum* separated from the stem, were washed with clean water to remove dirt and sand, drained, and chopped. They were macerated in 500 ml of tap water and then filtered to obtain homogenous aqueous extracts.

Phytochemical screening

The phytochemical analysis of the leaves of *T. occidentalis*, *A. vera*, *V. amygdalina* leaves, and *P. nigrum* were carried out according to the method of [10] and [11].

Quantitative phytochemical analysis

Alkaloids Determination

Five grams (5g) of the sample was weighed into a 250 ml beaker and 200 ml of 20% acetic acid in ethanol was added and covered and allowed to stand for 4 hours at 25°C. This was filtered with filter paper No. 42 and the filtrate was concentrated using a water bath (Memmert) to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH_4OH (1% ammonia solution). Then, filter with pre-weighed filter paper. The residue on the filter paper is the alkaloid, which is dried in the oven (precision electrothermal model BNP 9052 England) at 80°C. The alkaloid content was calculated and expressed as a percentage of the weight of the sample analyzed [10]; [11].

Calculation:

$$\% \, Alkaloids = \frac{\text{weight of filter paper with residue} - \text{weight of filter paper}}{\text{Weight of sample analyzed}} \, x \, 100$$

Flavonoids Determination

Ten (10)g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight (Boham and Kocipai, 1994).

Calculation:

$$\% \, Flavonoids = \frac{(\text{weight of crucible} + \text{residue}) - (\text{weight of crucible})}{\text{Weight of sample analyzed}} \, x \, 100$$

Determination of Saponin

Exactly 5g of the sample was put into 20% acetic acid in ethanol and allowed to stand in a waterbath at 50°C for 24hours. This was filtered and the extract was concentrated using a waterbath to one-quarter of the original volume. Concentrated NH_4OH was added drop-wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed. The saponin content was weighed and calculated in percentage.

Calculation:

$$\% \, Saponins = \frac{weight \, of \, filter \, paper \, with \, residue \, - \, weight \, of \, filter \, paper}{Weight \, of \, sample \, analyzed} \, x \, 100$$

Cardiac Glycosides Determination

To 1ml of extract was added 1ml of 2% solution of 3,5-DNS (Dinitro Salicylic acid) in methanol and 1ml of 5% aqueous NaOH. It was boiled for 2 minutes (until brick-red precipitate was observed) and the boiled sample was filtered. The weight of the filter paper was weighed before filtration. The filter paper with the absorbed residue was dried in an oven at 50°C till dryness and weight of the filter paper with residue was noted [12]

The cardiac glycoside was calculated in %:

Calculation:

$$\% \, Cardiac \, glycosides = \frac{weight \, of \, filter \, paper \, with \, residue \, - \, weight \, of \, filter \, paper}{Weight \, of \, sample \, analyzed} \, x \, 100$$

Tannin Determination by Titration

To 20g of the crushed sample in a conical flask was added 100mls of petroleum ether and covered for 24 hours. The sample was then filtered and allowed to stand for 15 minutes allowing petroleum ether to evaporate. It was then re-extracted by soaking in 100ml of 10% acetic acid in ethanol for 4hrs. The sample was then filtered and the filterate collected.

Then, 25ml of NH_4OH were added to the filterate to precipitate the alkaloids. The alkaloids were heated with electric hot plate to remove some of the NH_4OH still in solution. The remaining volume was measured to be 33ml. Then 5ml of this was taken and 20ml of ethanol was added to it. It was titrated with 0.1M Na0H using phenolphthalein as indicator until a pink end point is reached. Tannin content was then calculated in % ($C_1V_1 = C_2V_2$) molarity.

Calculation

C_1 = conc. of Tannic Acid; acid; V_2 = Volume of Base

C_2 = conc. of Base; V_1 = Volume of Tannic

Therefore, $C_1 = \frac{C_2V_2}{V_1}$

$$\% \, Tannic \, acid \, contents = \frac{C_1 \, x \, 100}{Weight \, of \, sample \, analyzed}$$

Estimation of some micronutrients

Estimation of Vitamin A (Beta-Carotene)

Method: Vitamin A was estimated by the method of [13].

Principle: The assay is based on the spectrophotometeric estimation of the colour produced by vitamin A acetate or palmitate with TCA.

Procedure: All procedures were carried out in the dark to avoid the interference of light. Then 1.0g of extract was mixed with 1.0 ml of saponification mixture and refluxed for 20 minutes at 60°C in the dark. The tubes were cooled and 20ml of water was added and mixed well. Vitamin A was extracted twice with 10ml of (40°-60°C) petroleum ether. The two extracts were pooled and washed thoroughly with water. Anhydrous sodium sulphate was added to remove excess moisture. An aliquot of the extract (1.0ml) was taken and evaporated to dryness at 60°C. The residue was dissolved in 1.0ml chloroform. Standards (vitamin A palmitate) of concentrations ranging from 0-7.5 μg were pipetted out into a series of test tubes. The volume in all the tubes was made up to 1.0 ml with chloroform. TCA reagent (2.0 ml) was added rapidly, mixed and the absorbance was read immediately at 620nm in a spectrophotometer (Genesys 10UV). The same procedure was repeated for the sample tubes also. Vitamin A content was expressed as ug/g.

Determination of Vitamin C

Methods: Ascorbic acid was analysed by the spectrophotometric method described by Roe and Keuther (1943)

Principle: Ascorbate is converted into dehydroascorbate on treatment with activated charcoal, which reacts with 2,4-dinitrophenyl hydrazine to form osazones. These osazones produce an orange coloured solution when dissolved in sulphuric acid, whose absorbance can be measured spectrophotometrically at 540nm.

Procedure: Ascorbate was extracted from 1g of the plant sample using 4% TCA and the volume was made up to 10ml with the same. The supernatant obtained after centrifugation at 2000rpm for 10 minutes was treated with a pinch of activated charcoal, shaken vigorously using a cyclomixer and kept for 5 minutes. Standard ascorbate ranging between 0.2-1.0ml and 0.5ml and 1.0ml of the supernatant were taken. The volume was made up to 2.0ml with 4% TCA. DNPH reagent (0.5ml) was added to all the tubes, followed by 2 drops of 10% thiourea solution. The contents were mixed and incubated at 37°C for 3 hours resulting in the formation of osazone crystals. The crystals were dissolved in 2.5ml of 85% sulphuric acid, in cold. To the blank alone, DNPH reagent and thiourea were added after the addition of sulphuric acid. The tubes were cooled in ice and the absorbance was read at 540nm in a spectrophotometer (Genesys 10-S, USA). A standard graph was constructed using an electronic calculator set to the linear regression mode. The concentration of ascorbate in the samples were calculated and expressed in terms of mg/g of sample. One milliliter of 0.05M iodine solution liberates 0.00886g of Vitamin C.

Determination of Vitamin E

Methods: Tocopherol was estimated in the plant samples by the Emmerie-Engel reaction as reported by [14]

Principle: The Emmerie-Engel reaction is based on the reduction of ferric to ferrous ions by tocopherols, which, with 2,2'-dipyridyl, forms a red colour. Tocopherols and carotenes are first extracted with xylene and read at 460nm to measure carotenes. A correction is made for these after adding ferric chloride and read at 520nm.

Procedure: The plant sample (2.5g) was homogenized in 50ml of 0.1N sulphuric acid and allowed to stand overnight. The contents of the flask were shaken vigorously and filtered through Whatman No.1 filter paper. Aliquots of the filtrate were used for the estimation. This was determined by the futter – mayercolometric method with association of vitamin chemist's [15]. 1g of the sample was mixed with 10ml of ethanoic sulphuric acid and boiled gently under reflux for 30mins. It was transferred to a separating funnel and treated with 3x30ml diethyl ether and recovering ether layer each time, the ether extract was transferred to a dessicator and dried under for 30mins and later evaporated to dryness at room temperature. The dried extract dissolved in 10ml of pure ethanol. 1ml of the dissolved extract and equal volume of standard vitamin E were transferred to separate tubes. After continuous addition of 5mls of absolute alcohol and 1ml of concentrated nitric acid solution, the mixtures were allowed to stand for 5mins and the respective absorbance measured in a spectrophotometer at 410nm with blank reagent at zero.

Determination of minerals

Mineral analysis was conducted using Varian AA240 Atomic Absorption Spectrophometer according to the method of APHA 1995 (American Public Health Association)

Working principle: Atomic absorption spectrometer's working principle is based on the sample being aspirated into the flame and atomized when the AAS's light beam is directed through the flame into the monochromator, and onto the detector that measures the amount of light absorbed by the atomized element in the flame. Since metals have their own characteristic absorption wavelength, a source lamp composed of that element is used, making the method relatively free from spectral or radiational interferences. The amount of energy of the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample.

Procedure: The sample is thoroughly mixed by shaking, and 100ml of it is transferred into a glass beaker of 250ml volume, to which 5ml of conc. nitric acid is added and heated to boil till the volume is reduced to about 15-20ml, by adding conc. nitric acid in increments of 5ml till all the residue is completely dissolved. The mixture is cooled, transferred and made up to 100ml using metal free distilled water. The sample is aspirated into the oxidising air-acetylene flame. When the aqueous sample is aspirated, the sensitivity for 1% absorption is observed.

In vitro Antioxidant assay

DPPH free radical scavenging activity

Method: The rapid screening assay was performed by the method proposed by [16].

Principle: DPPH radical reacts with an antioxidant compound that can donate hydrogen, and gets reduced. DPPH, when acted upon by an antioxidant, is converted into diphenylpicryl hydrazine. This can be identified by the conversion of purple to light yellow colour.

The DPPH radical is employed as a substrate to evaluate antioxidant activity. Reduction in DPPH radical is measured by decrease in absorbance induced by antioxidants present in the sample.

Procedure: The leaf extracts (20µl) were added to 0.5ml of methanolic solution of DPPH and 0.48ml of methanol. The mixture was allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol, without the leaf extracts, served as the positive control. After 30 minutes of incubation, the discolouration of the purple colour was measured at 518nm in a spectrophotometer (Genesys 10-S, USA). The radical scavenging activity was calculated as follows:

$$\% \, Scavenging \, activity = 100 - \frac{A518 \, (Sample) - A518 \, (Blank)}{A518 \, (Blank)} \, x \, 100$$

Total Antioxidant Capacity (TAC) assay

Method: The Total Antioxidant Capacity (TAC) was determined by the phosphomolybdate method according to [17].

Principle: This assay is based on the inhibition of the production of nitroblue tetrazolium formation of the superoxide ion by the plant extracts and is measured spectrophotometrically at 560nm.

Procedure: An aliquot (30 mL) of different concentrations (20, 40, 60, 80 and 100 mg/l) of the test extracts will be mixed with 3 mL of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate) taken in test tubes. The tubes will be capped with aluminium foil and incubated in a boiling water bath at 95°C for 90 min. The reaction mixture will be allowed to cool to room temperature and the absorbance of the solution was measured at 695 nm against a blank containing 3 mL of reagent solution and the appropriate volume of the dissolving solvents. The blank will be incubated under the same conditions as the test samples.

Ascorbic acid and gallic acid will be used as standard reference compounds to compare the activities of the extracts.

Ferric Reducing Property

Method: The reducing property of the extracts will be determined as described by [18].

Principle: The principle of the assay is the quantification of ferric degradation product, by its condensation with the extract

Procedure: 0.25 ml of the extracts will be mixed with 0.25 ml of 200 mMSodium phosphate buffer pH 6.6 and 0.25 ml of 1% Potassium ferrocyanide. The mixture will be incubated at 50 °C for 20 min, thereafter 0.25 ml of 10% trichloroacetic acid will be added and centrifuge at 2000 rpm for 10 min, 1 ml of the supernatant will be mixed with 1 ml of distilled water and 0.2 ml of ferric chloride and the absorbance was measured at 700 nm.

Fe²⁺ Chelating assay

Method: The ability of the extracts to chelate Fe²⁺ was determined using a modified method described [19] **Principle:** The principle is based on the ability of the extract to chelate iron (II).

Procedure: Briefly, 150 mM FeSO$_4$ was added to a reaction mixture containing 168µl of 0.1M Tris-HCl pH 7.4, 218ml saline and extract and the volume will be made up to 1 ml with distilled water. The reaction mixture was incubated for 5 min, before the addition of 13 µl of 1, 10-phenantroline. The absorbance was read at 510nm. The Fe (II) chelating ability was subsequently calculated with respect to the reference which contains all the reagents without extracts.

Statistical Analysis

The results were expressed as mean ± SEM for three to four independent experiments performed in triplicate and were analyzed by one way analysis of variance, followed by Duncan's multiple-range test. Differences between groups were considered significant when $p<0.05$.

RESULTS

Result of Qualitative Phytochemistry

The result of the qualitative phytochemicals were present in Table 1. The result revealed a high presence of alkaloid in *A. vera* compared to *P. nigrum*, *V. amygdalina* and *T. occidentalis*. The concentration of flavonoid tends to be high compared to the other medicinal plants. Glycosides was high in *A. vera*, *P. nigrum* and *V. amygdalina* while *T. occidentalis* shows a low level. The result of protein shows that protein was present in the four different medicinal plants while reducing sugar was absent in *P. nigrum* and *V. amygdalina*. Saponin, tannin and terpenoids were high in all the samples while steroids were predominant in *A. vera* and *P. nigrum*

Table 1:   Qualitative phytochemicals of medicinal plants.

Parameters	*Aloe vera*	*P. nigrum*	*V. amygdalina*	*T. occidentalis*
Alkaloid	+++	++	+	+
Flavonoid	++	+++	++	++
Glycoside	+++	+++	+++	+
Protein	++	++	++	++
Carbohydrate	++	++	++	+
Reducing Sugar	+	-	-	+
Saponin	+++	++	++	+++
Tannin	+++	+++	+++	+++
Resins	+	+	+	++
Terpenoid	+++	+++	+++	+++
Steroid	+++	+++	+	+

Key: + = Present; ++ = Moderately present; +++ = Highly present; - = Absent.

Result of Quantitative Analysis

The result of the quantitative phytochemical analysis shows a significant variation (*P<0.05*) in the concentration of alkaloid in *A. vera* compared to *P. nigrum*, *V. amygdalina* and *T. occidentalis* with a concentration of 11.28 mg/100g and *V. amygdalina* has the least concentration of 4.10 mg/100g (Fig. 1). The flavonoids contents of *P. nigrum* significantly varied (P<0.05) when compared to other medicinal plants with a concentration of 9.30 mg/100g while *T. occidentalis* has the least flavonoid with a concentration of 2.70 mg/100g. *A. vera* has a significantly high glycoside and tannin levels compared to other phytochemical with a concentration of 13.60 and 89.50 mg/100g respectively, while *P. nigrum* had a higher concentration of steroids compared to other medicinal plants, with a composition of 9.28 mg/100g.
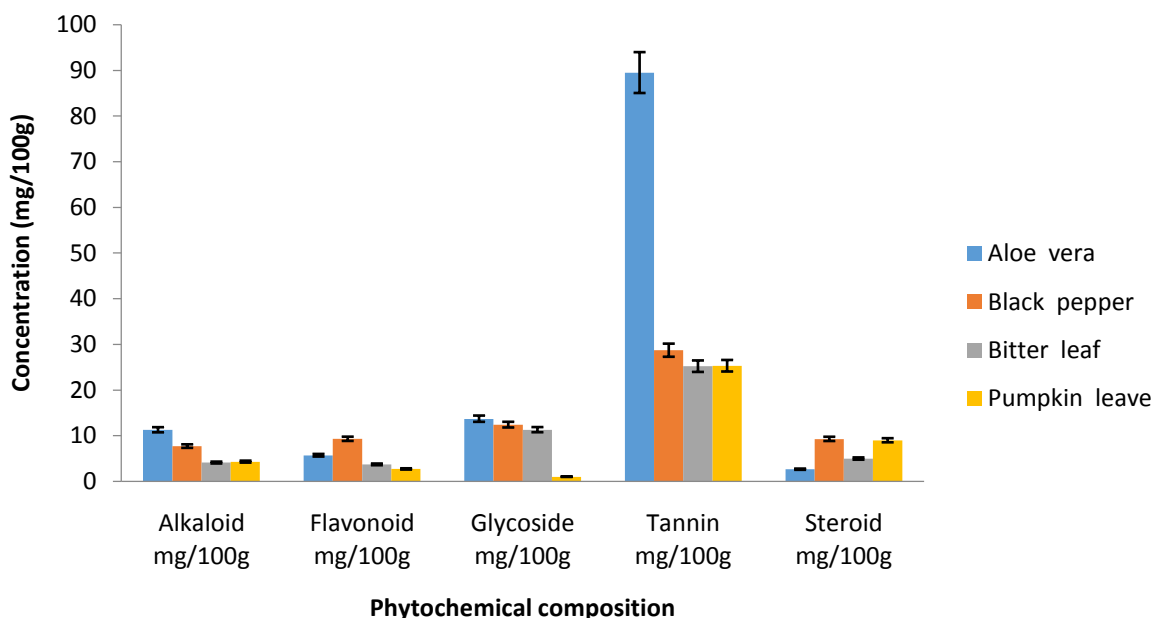
Figure 1: Phytochemical composition of medicinal plants.

Result of Micronutrient Analysis

The result of vitamin analysis (Table 2) shows a higher concentration of vitamin A in *V. amygdalina* compared to other medicinal plants with a composition of 140 mg/kg while *P. nigrum* had the least concentration of vitamin A the result was significantly varied ($p<0.05$). The composition of vitamin C in *A. vera* significantly varied with other medicinal plants ($P<0.05$) compared to other medicinal plants with a composition of 245.48±0.19 mg/kg while *Piper nigrum* has the least composition of vitamin C with a composition of 79.20±0.22 mg/kg. Vitamin E significantly varied in *A. vera* compared to other medicinal plants with a composition of 2.035±0.12 mg/kg.

The results obtained from the anti-oxidant mineral analysis (Fig. 2), revealed that *V. amygdalina* and *T. occidentalis* leaf had a higher concentration of iron compared to other medicinal plants. Similarly, *V. amygdalina* had the highest selenium and magnesium contents compared to the rest of the plants. This was followed closely by *T. occidentalis.*

Table 2: Micronutrient analysis of the four different medicinal plants (mg/kg).

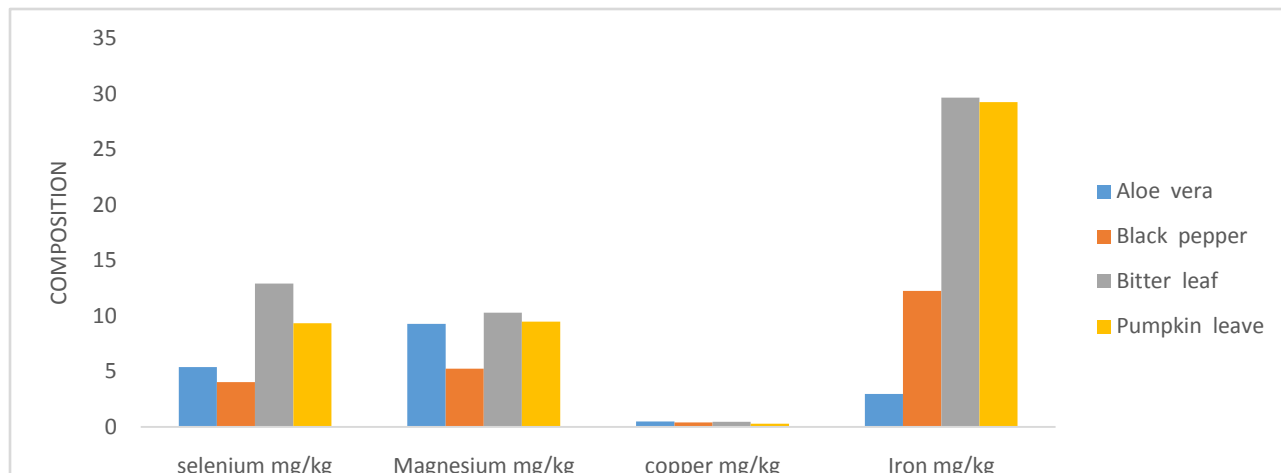| Parameters	Aloe vera	P. nigrum	V. amygdalina	T. occidentalis
Vitamin A	48.48±1.18	34.12±0.32	140.80±0.20	74.11±0.11
Vitamin C	245.48±0.19	79.20±0.22	118.70±0.22	210.38±0.28
Vitamin E	2.035±0.12	0.992±0.19	0.8025±0.18	1.125±0.18

Figure 2: Mineral composition of the medicinal plants.

Effect of DPPH Scavenging Effect of the Medicinal Plants

The result obtained from the DPPH radical scavenging assay (Table 3) shows a decrease in DPPH scavenging activity with increase in the concentration of plant extracts however the result of figure 3 shows that *V. amygdalina* show a more potent DPPH scavenging activity compared to other medicinal plant with a reduction in the scavenging activity compared to other medicinal plants.

The IC_{50} results obtained from Table 3 were presented in Table 4. The results revealed that *V. amygdalina* has a better DPPH radical scavenging ability because the lower the IC_{50} compared to vitamin C is an indication of better anti-oxidant ability.

The DPPH radical scavenging activity results are shown in Table 5 as comparable with known antioxidant Vitamin C. From the analysis we could conclude that the scavenging effects of *A. vera*, *P. nigrum*, *V. amygdalina*, *T. occidentalis* extracts on DPPH radicals were excellent, especially in the case of V. amygdalina compare to vitamin C but pumpkin leaf revealed a low value of antioxidant activity. Overall, V. amygdalina revealed the best antioxidant properties.

Table 3: DPPH radical scavenging ability of different percentage concentration (%) of the plant extracts.

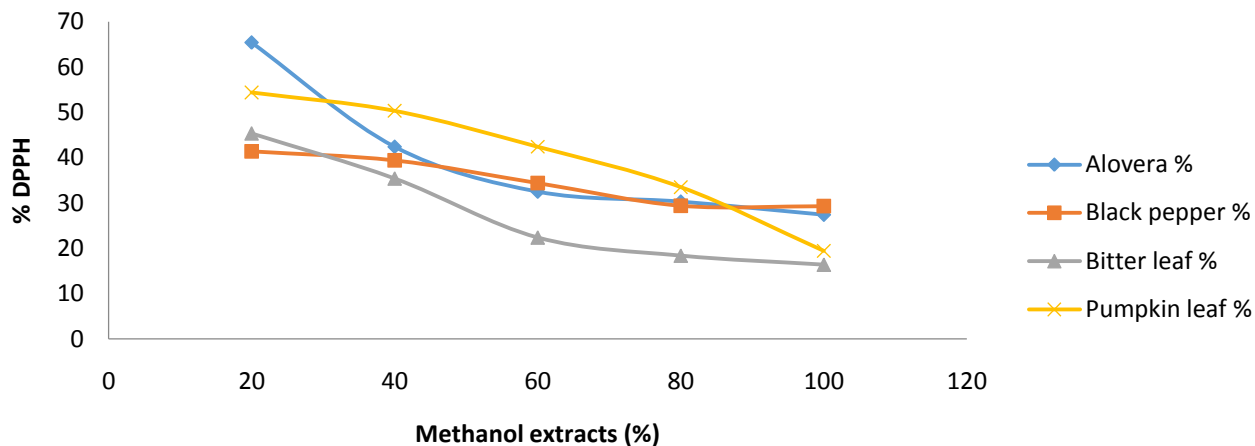| Methanol extract %	A. vera	P. nigrum	V. amygdalina	T. occidentalis
20	65.38	41.39	45.35	54.39
40	42.38	39.39	35.40	50.33
60	32.483	34.31	22.38	42.39
80	30.28	29.32	18.12	33.48
100	27.39	29.29	16.39	19.32

Figure 3: DPPH scavenging activity of the four medicinal plants

Table 4: The IC_{50} of the various extracts compared to vitamin C (%)

Plant Extracts	Mean ± SD
Aloe vera	39.58±1.18
Piper nigrum	34.74±0.98
V. amygdalina	27.53±0.21
T. occidentalis	39.98±0.12
Vitamin C	12.88±0.34

Effect of Total Antioxidant Capacity of Medicinal Plants

The result obtained from the analysis of the total antioxidant capacity of the plant extracts (Table 5 and Fig. 6) shows an increase in total antioxidant capacity of *aloe vera* compared with other medicinal plants, this is followed by *V. amygdalina* which has higher total antioxidant capacity compared to *P. nigrum* and *T. occidentalis*.

The IC_{50} of the total antioxidant capacity activity of the various extracts are shown in Table 6 as comparable with known antioxidant Vitamin C. From the analysis, it can be concluded that the antioxidant capacity of the four medicinal plant were significantly varied ($p<0.05$) when compared with vitamin C. However, *A. vera* (56.48 %) was slightly closer to vitamin C (79.32 %) compared to other varieties.

Table 5: The total antioxidant capacity of the plant extracts (%).

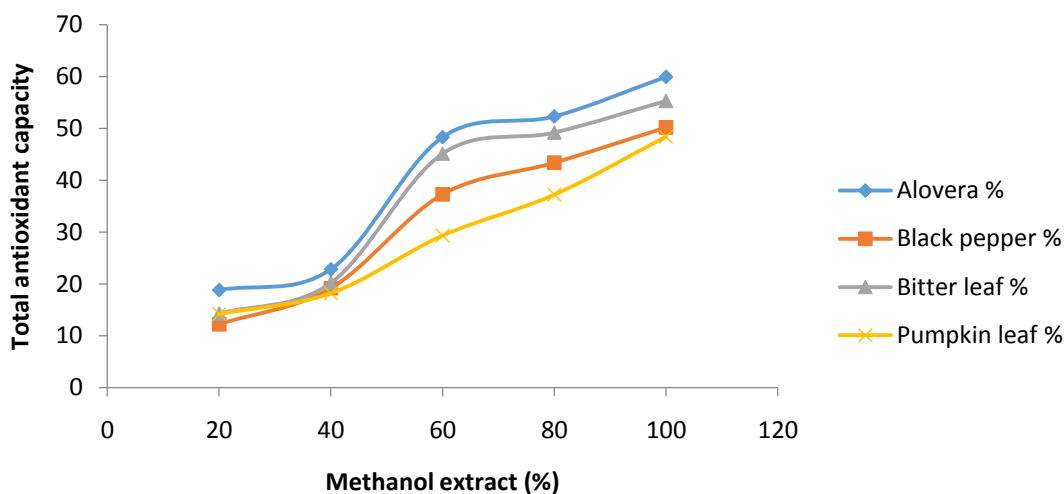| Methanol extract %	*A. vera*	*P. nigrum*	*V. amygdalina*	*T. occidentalis*
20	18.83	12.24	14.29	14.18
40	22.83	19.18	20.20	18.19
60	48.28	37.28	45.13	29.30
80	52.28	43.39	49.18	37.20
100	59.90	50.19	55.29	48.40

Figure 4: Total Antioxidant capacity of Medicinal plants.

Table 6: The IC$_{50}$ value of the total antioxidant capacity compared to vitamin C (%).

Samples	Mean ± SD
A. vera	56.48
P. nigrum	49.48
V. amygdalina	53.29
Pumpkin leaf	44.38
Vitamin C	79.32

Effect of Ferric Reducing Power and Iron Chelating Properties

The results of the ferric reducing power and the iron chelating effects of the extracts were presented in figure 5. The result shows that *T. occidentalis* exhibited a higher reducing power, 7.83±0.08 umol/l which is followed by *V. amygdalina* (7.19±0.01 umol/l) compared to other medicinal plants *P. nigrum* with the least composition of 4.93±0.03 umol/l. *A. vera* however exhibited 5.38±0.01 u/mol.

Nonetheless, *V. amygdalina* was able to chelate iron more (11.92±0.03 umol/l) compared to other medicinal plants at P<0.05. This is an indication that *V. amygdalina* may be a more potent antioxidant. This was followed by *T. occidentalis* (10.28±0.05 umol/l), *A. vera* (8.49±0.02 umol/l) and *P. nigrum* (7.38±0.02 umol/l).
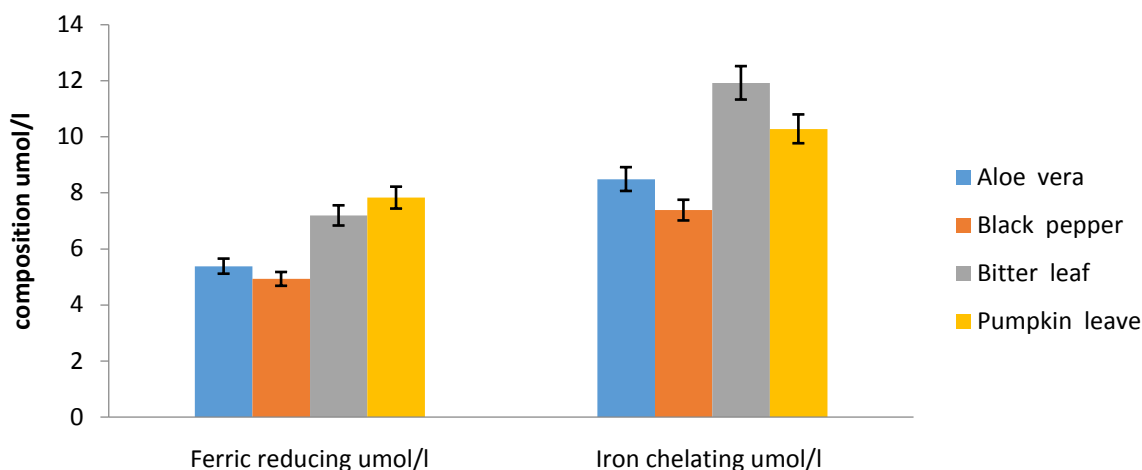
Figure 5: Ferric reducing and Ferric chelating properties of medicinal plants.

DISCUSSION

Phytochemicals and antioxidants are tremendously important substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. The phytochemical and antioxidant potential of four medicinal plants were investigated in the search for new bioactive compounds from natural resources.

The findings of this phytochemical studies agree with that of several researchers that demonstrated that these medicinal plants have an antibacterial activity against several species of bacteria and even fungi. Therefore, the efficacy of these extracts as reported by [20] may be due to the age of the plant, solvent extraction, extraction method and the period of harvest of plant materials. The statistical analysis shows a significant variation (P<0.05) in the concentration of alkaloid in *A. vera* compared to *P. nigrum*, *V. amygdalina* and pumpkin leave with a concentration of 11.28 mg/100g and *V. amygdalina* has the least concentration of 4.10 mg/100g. *P. nigrum* significantly varied (P<0.05) in flavonoid compared to other medicinal plants with a concentration of 9.30 mg/100g while pumpkin has the least flavonoid with a concentration of 2.7 mg/100g. *A. vera*

was significantly high in glycoside and tannin compared to other phytochemical with a concentration of 13.60 and 89.50 respectively, while *P. nigrum* had a higher concentration of steroids compared to other medicinal plants, with a composition of 9.28 mg/100g.

Vegetables are considered as important components of any diet. Fruits and vegetables are known to contain vitamins and minerals necessary for maintenance of good health. The good health benefits of vegetables are also ascribed to their inherent phytochemical compounds which help in counteracting the deleterious effects of free radicals and highly reactive species *in vivo* [21]. Nigeria is blessed with a rich array of green leafy vegetables that serve as vital ingredients in soups and medicine.

V. amygdalina and *T. occidentalis* are one of the most popular vegetables used in cooking in Southern Nigeria. These vegetables, though popular as cooking ingredients, are also known to possess several medicinal values [22].The result of vitamin analysis as shown in figure 2 shows a higher concentration of vitamin A in *V. amygdalina* compare to other medicinal plants with a composition of 140mg/kg while *P. nigrum* had the least

concentration of vitamin A, the result was significantly varied (p<0.05). The composition of vitamin C in *A. vera* significantly varied with other medicinal plants with a composition of 245mg/kg while *P. nigrum* has the least composition of vitamin C with a composition of 79.20mg/kg. Vitamin E significantly varied in *A. vera* compared to other medicinal plants with a composition of 2.035±0.12 mg/kg while pumpkin leaf had a higher concentration of iron compared to other medicinal plants. The therapeutic effects of several medicinal plants are usually attributed to their antioxidant properties [23]. Tocopherol constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators. Flavonoids are the most widespread group of natural compounds and probably the most important natural phenolics as a result of their health–promoting benefits [22];[21]. *A. vera* had a higher vitamin E concentration compared to others.

DPPH free radical scavenging ability is one of the most popular methods utilized in screening for anti-oxidative activity. The DPPH radical is employed as a substrate to evaluate antioxidant activity. Reduction in DPPH radical is measured by decrease in absorbance induced by

antioxidants present in the sample. An important variable estimated from DPPH free radical scavenging test is termed IC_{50}. IC_{50} expresses the amount of antioxidant required to decrease the DPPH radical concentration by 50% [23]. The result from figure 4 shows decrease in DPPH scavenging activity with increase in the concentration of plant extracts however the result of figure 4 shows that *V. amygdalina* show a more potent DPPH scavenging activity compared to other medicinal plant.

The reducing capacity of an extract may be an important indicator of its potential anti-oxidative activity *[23]*. In this study, we assessed the reducing capacity of our extracts by utilizing two (2) assay methods; ferric reducing antioxidant potential (FRAP) and reducing power. The principle of FRAP method is based on the reduction of a ferric-2,4,6-tri(2-pyridyl)-*s*triazine [Fe (III)-TPTZ] complex to its ferrous 2,4,6-tri (2-pyridyl) -*s*-triazine [Fe(II)-TPTZ] complex coloured form in the presence of antioxidants. This complex has an intense blue colour that can be monitored at 593nm [24]. On the other hand, the reducing power of an antioxidant measures its ability to donate electrons. Both assays showed higher reducing capacity for *V. amygdalina* compared to the other extracts.

CONCLUSION

The results follow this trend as *V. amygdalina* and *T. occidentalis* extracts revealed higher amount of all groups of antioxidants (including total phenol, flavonoids) as well as a higher anti-oxidative capacity (in terms of the DPPH free radical scavenging capacity, FRAP and reducing power) when compared with

other extract. These data do not in any way prejudice against the consumption of other extracts.However the generated data were used to draw attention to the benefits accruable from consumption of these vegetables and recommend their sustained inclusion in human diet.

REFERENCES

1. Doughari, J. H., Human, S.I., Bennade, S. and Ndakidemi, P.A. (2009). Phytochemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin producing bacteria. *Journal of Medicinal Plants Research*, **3**(11): 839- 848.

2. Evans, W.C. (2002) Pharmacognosy 15th ed. W.B Saunders, Edinburgh; p. 585.

3. Sumner, J. (2000). The Natural History of Medicinal Plants. 1st ed. Timber Press. Portland; P. 235.

4. Elujoba, A.A., Odeleye, O.M., Ogunyemi, C.M. (2005). Traditional medicine development for medical and dental primary health care delivery system in Africa. *African Journal of Traditional, Complementary and Alternative Medicines*, **2**(1): 46-61.

5. Farombi, E. O., and Owoeye, O. (2011). Antioxidative and Chemopreventive Properties of *Vernonia amygdalina*and Garcinia biflavonoid. *International Journal of Environmental Research and Public Health,* **8**: 2533-2555.http://dx.doi.org/10.3390/ijerph8062533.

6. Zheng, W. and Wang, S.Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *J. Agric Food Chem.* **49**(11): 5165-70.

7. Liao, Z., Chen, M., Tan, F., Sun, X. and Tang, K. (2004). Microprogagation of endangered Chinese aloe. *Plant Cell, Tissue and Organ Culture.* **76** *(1): 83–86*

8. Okafor, J.C. (1983). Horticulturally promising indigenous wild plant species of the Nigerian forest zone Acts. *Horticulture*, **123**: 165-177.

9. Sofowora, A. (1996). Medicinal Plant and Traditional Medicine in Africa. 2nd edition. Spectrum Books, Ibadan, Nigeria, p. 112.

10. Harborne, J.B. (1973). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, Chapman and Hall, London, UK. pp. 49-188.

11. Trease, G.E. and Evans, W.C. (1989). *A Textbook of Pharmacognosy*. 13th Edition. Bailliese, Trindall., London, 397-453.

12. Obdoni, B. and Ochuko, P. (2001). Phytochemical studies and comparative efficacy of the crude extracts of some homostatic plants in Edo and Delta States of Nigeria. *Global J. Pure Appl. Sci.*, **8**: 203–208.

13. Bao J.Y., Cai M., Sun G., Wang and Corke, H. (2005): Anthocyanins, Flavonoid and Free Radical Scavenging Activity of thines Baybery (Myrialrubia) extracts and their colour properties and stability. *Journal of Agric Food Chem.,* **53**: 2327-2332.

14. Kherallah, M., Delgado, C., Gabre-Madhin, E., Minot, N. and Johnson, M. (2002). Reforming Agricultural Markets in Africa. IFPRI. The Johns Hopkins University Press, Baltimore: USA. Pg. 2.

15. Jaramillo, M. and Alejandra, M. (2001). Phylogeny and Patterns of Floral Diversity in the Genus Piper (Piperaceae). American Journal of Botany. **88**(4): 706–16. doi:10.2307/2657072. PMID 11302858.

16. Pumpkin (2007). Varieties of pumpkins. Available: http://www.pumpkinnook.com. Retrieved: 27/12/2017)

17. Ogundare, A.O. (2011). Antibacterial properties of the leaf extracts of Vernonia amygdalina, Ocimum gratissmum Corchorois olitorius and Manihot palmate *J. Microbiol. Antimicrob*. **3**(4): 77-86.

18. Ola, S.S., Catia, G., Marzia, I., Francesco, V.F., Afolabi, A.A. and Nadia, M. (2009). HPLC/DAD/MS characterisation and analysis of flavonoids and cynnamoil derivatives in four Nigerian green-leafy vegetables. *Pakistan Journal of Nutrition*. **3**: 2-3.

19. Iweala, E.E.J. and Obidoa, O. (2010). Studies on some biochemical and histological changes associated with long term consumption of leaves of *Ocimum gratissimum* L. in male rats. *American Journal of Food Technology,***5**(6): 376-384. http://dx.doi.org/10.3923/ajft.2010.376.384

20. Conde Nast. 2014. Retrieved 25 October 2017.

21. Padmanabhan, P. and Jangle, S. N. (2012). Evaluation of DPPH radical scavenging activity and reducing power of four selected medicinal

plants and their combinations. *International Journal of Pharmaceutical Science andDrug Research,* **4(**2), 143-146.

22. Gil, M. ., Tomas-Barberan, F. A., Hess-Pierce, B. and Kader, A. A. (2002). Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. *Journal of Agricultural and Food Chemistry,* **50**: 4976-4982. http://dx.doi.org/10.1021/jf02013 6b

23. Chanda, S., Dave, R., & Kaneria, M. (2011). In vitro antioxidant property of some Indian medicinal plants. *Research Journal of Medicinal Plant,* **5**(2), 169 179.http://dx.doi.org/10.3923/rjm p.2011.169.179

24. Yim, H. S., Chye, F. Y., Tan, C. T., Ng, Y. C., & Ho, C. W. (2010). Antioxidant Activities and Total Phenolic Content of Aqueous Extract of *Pleurotus ostreatus* (Cultivated Oyster Mushroom). *Malaysian Journal of Nutrition,* **16**(2), 281-291.