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Quantitative Analysis of Phytochemical Constituents and Invitro Antioxidant Potentials of Poly-Herbal Formulation

Oshomoh, E. O., Uwaya, D. O. and Uzama-Avenbuan, O.
Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin. Edo State. Nigeria.
Corresponding Author: Oshomoh, E. O. E-mail: emmananuel.oshomoh@uniben.edu; Tel: 08055452141

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Abstract

Plants of medicinal value have contributed to the well-being of many people, individually and to the world at large. Vernonia amygdalina, Greenwayodendron suaveolens, Euphorbia heterophylla and Xylopia aethiopica are all known to have medicinal values varying in their effect. The quantitative analysis of phytochemical constituents and some invitro antioxidant properties of Greenwayodendron suaveolens, Vernonia amygdalina, euphorbia heterophylla and Xylopia aethiopica leaves were determined. Phenols, flavonoids, tannins, saponins and tannins were the phytochemicals detected from the plants. The determination of the macro and micro elements such as iron, manganese, potassium, calcium, magnesium, copper, lead, cadmium, chromium, and nickel was made directly on each final solution using flame photometer and a Bulk Scientific 210 VGP, atomic absorption spectroscopy (AAS). The 1, 1-Diphenyl-2-picrylhydrazyl (DPPH), 2, 2-azino di-(3-ethylbenzothiazoline- 6-sulfonic acid) (ABTS), hydroxyl free radical (OH-) and ferric reducing antioxidant power (FRAP) were investigated using one way analysis of variance (ANOVA), $P<0.05$ was obtained. In DPPH, ABTS, FRAP and OH- scavenging activities, Vernonia amygdalina, Greenwayodendron suaveolens and Euphorbia heterophylla was found to have scavenging activities which increased as their concentrations increase. The medicinal potential of these plants could be as a result of their phytochemicals, micro and macro elements and their free radical scavenging abilities. More investigation is necessary to be carried out to further prove their anti-oxidative potentials.

1. Introduction

Plants of medicinal value have contributed to the well-being of many people throughout the world due to the presence of biologically active ingredients such as flavonoids, alkaloids, tannins, and phenolic compounds. Countless number of these indigenous medicinal plants are used as herbs and food in order to achieve specific physiological effects in the individual body. Pregnant and lactating women sometimes eat it because of its medicinal properties [1]. The four plants used to formulate the poly-herbal drugs are *Greenwayodendron suaveolens, Xylopia aethiopica, Vernonia amygdalina* and *Euphorbia heterophylla.*

Plate1: *Greenwayodendron suaveolens* plant

Plate 2: *Xylopia aethiopica*

Plate 3: *Vernonia amygdalina* plant

Plate 4: *Euphorbia heterophylla* plant

Greenwayodendron suaveolens is a species of plant in the genus *Greenwayodendron*, and a member of the Annonacea family. It is a monophyletic tree genus from tropical Africa. It is widely distributed in Western and Central Africa from Ivory Coast to Angola [2]. It is deciduous, medium sized to fairly large tree up to 35-45 meters tall, has bole branches for up to 25 m, straight, cylindrical and sometimes grooved at the base [3].

The plant is either used alone mix with other plants for the treatment of numerous diseases, which include parasitic diseases like uncomplicated malaria and Helminthiasis [4]. Generally, this plant is either used as a water extract or as an enema for back pain, sexual weakness, malaria, headaches, loss of appetite, snake pain, pelvic pain, hepatitis, insanity, rheumatism, epilepsy, constipation, abdominal pain, pain gear and many more. [5]. Despite its bitter taste, the leaves are sometimes eaten by several species of monkeys, as reported by some Nkundo hunters. In some areas in the Central African Republic and in Cameroon, the leaves of this tree, also named "ancient tobacco" or "forest tobacco", are used and evaluated as cigarettes [6].

Xylopia aethiopica belongs to the kingdom Plantae, Division Tracheophyta (vascular plant), Class Magnoliopsida, Family Annonaceae and Genus Xylopia. It is fragrant tree with a height of more than 20 m and a diameter of 60 to 75 cm. The fruit is cylindrical pods which are attached with a width of 2 to 3 mm. It is indigenous to the lowland rainforest and moist fringe forests in the savanna zones of Africa. Its common names are Ethiopian pepper, negro-pepper and spice tree [7].

Xylopia aethiopica a plant of the Tropical Africa, in forest areas and particularly in rivers and in arid regions [8].

A number of investigations have established the anti-inflammatory and antipyretic properties of this herb. The seeds of *Xylopia aethiopica* are known for their many medicinal characteritics. In Africa, it is used as a spice and local medicine to treat diarrhea, colic, snake disease, cardiovascular disease, and diabetes; and in southern Nigeria for the treatment of infections transmitted sexually [9]. In

Nigeria, fruit extracts and bark extracts are used to treat bronchitis, bile and dysentery. Fruits are used to neutralize karmic pain and laxatives, and are believed to increase fertility and provide assistance [10].
Vernonia amygdalina belongs to the kingdom Plantae, phylum tracheophytes, Class Angiosperm, Order Asterales, Family Asteraceae and Genus *Vernonia*. It is a shrub plant that is grown in tropical Africa. It has a height of about 2-5 m. It is generally known as bitter leaf in English due to its bitter nature. Other names it is called include oriwo (Edo), onugbo (Igbo), chusar-doki (Hausa), ewuro (Yoruba), etidot (Ibibo), ityuna (tiv), ndoleh (Cameroon) and awonwono (Akan) [11].
V. amygdalina is a native plant that is widely used for nutritional and therapeutic purposes in Nigeria. It produces large amounts of food and is resistant to drought [12]. The presence of nutrient inhibitors which include tannins, alkaloids, glycosides and saponins [13].

The macerated plant leaves are consumed as herbs and vegetables and, whereas water extracts serve as a tonic to prevent several diseases. In Tanzania, it has been reported that *V. amygdalina* is used by wild chimpanzees to treat parasitic diseases. [14]. It has also been reported that the effects of antiplasmodia of the lactone and steroid sesquiterpene components of the plant were effective against *Plasmodium falciparium* invitro. [15] reported that *V. amygdalina* (Bitter leaf tea) is used all over West Africa to treat diabetes and metabolic infections related to the liver. This plant has gained exceptional importance because it has been shown to be active in the treatment of humans against HIV/AIDS and has strong anthelmintic and antimalarial properties [16] and antitumorigenic properties [17]. In Ghana, young leaves rather than old ones have been proven with animal models to have antidiabetic and anti-inflammatory activities [17, 18]. From the pharmacological studies, shows that extracts from the leaves have hypolipidemic and hypoglycemic characteristics in experimental animals and can thus be useful to treat diabetes [19]. The antidiabetic effect of water extracts from the *Vernonia amygdalina* leaves was reported [20].

Euphorbia heterophylla belongs to the kingdom: Plantae, phylum: tracheophytes, Class: Angiosperm, Class: Eudicots, Order: Malpighiales, Family: Euphorbieae, Genus: *Euphorbia* [21]. Some of the common names are Mexican fireplant, Japanese poinsettia, painted spurge, milkweed, fire on the mountain and kaliko plant. It has a height of about 30 to 100 cm with hollow stems which may be branched or with simple angular ribs [22].
In general, Euphorbia with around 1,600 species, have adhesives such as latex milk [23]; some are carcinogenic, very irritating to the skin and toxic to livestock and humans [23]. In tropical and temperate regions of the world, the plants are widely dispersed in nature, ranging from plants and shrubs to trees [24].
Euphorbia heterophylla leaf is used as a laxative, anti-gonorrhoea, migraine, and wart in traditional medicinal practice. The lattices of the plant have been used as fish poisons, insecticides, and poisons [24]. The leaves are used as an anticonvulsant and cough medicine in some parts of Kogi, Nigeria. It has been reported that *E. heterophylla* leaves contain quercetin [25] and diterpenoids in the roots [26]. *E. heterophylla* leaves have also been reported to have skin irritation, antitumor/anticancer which stimulates tumors and, more recently, anti-HIV euphorbia activity [27]. *E. heterophylla* is a medicinal herb commonly referred to as "weed bush". It grows in semi-humid places, particularly in cassava, grape and soybean plantations. However, chemical research reports about *E. heterophylla* are rare. It is known that the leaves have an antibacterial effect [24].

The aim of this current study is to evaluate the antioxidant properties of *Greenwayodendron suaveolens, Vernonia amygdalina,* and *Euphorbia heterophylla,* elemental analyses and phytochemical studies on *Greenwayodendron suaveolens, Vernonia amygdalina, Euphorbia heterophylla and Xylopia aethiopica*.

2. Methodology

2.1 Sample Collection and Identification

Greenwayodendron suaveolens plant was obtained from the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City. *Vernonia amygdalina* plant was collected from Ikpoba Hill in Ikpoba Okha Local Government Area, Edo State. *Xylopia aethiopica* plant was collected from Uselu market in Egor Local Government Area, Edo State. *Euphorbia heterophylla* plant was collected from Afeyeh Okpameri, Akoko-Edo Local government Area, Edo State, and were all identified by a taxonomist in the Department of Plant Biology and Biotechnology, University of Benin, Benin City.

2.2 Plant Sample Treatment

The plant sample was washed thoroughly in clean water, the fruits of *Xylopia aethiopica* and the leaves of *Vernonia amygdalina, Greenwayodendron suaveolens,* and *Euphorbia heterophylla* plant were destalked and air-dried at room temperature for two weeks to achieve a sum total drying of the leaves and then pulverized into fine powder and weighed with standard weighing balance..

2.3 Method of Extraction

A total of 1000 g of powdered leaves of *Greenwayodendron suaveolens,* 1000 g of *Vernonia amygdalina,* 250 g of *Euphorbia heterophylla* and 310 g of *Xylopia aethiopica* were dissolved in 10 litres, 10 litres, 2.5 litres and 3.1 litres of distilled water respectively. The mixtures were left to stand for four (4) days with continuous stirring, after which they were filtered and the filtrates of each was concentrated and stored in the fridge.

2.4 Determination of antioxidant properties.

The antioxidant property was carried out on the aqueous extracts of *Vernonia amygdalina, Greenwayodendron suaveolens,* and *Euphorbia heterophylla* only.

2.5 DPPH (1, 1-Diphenyl-2-picrylhydrazyl) SCAVENGING ACTIVITY.

The method described by [27] was used to measure the scavenging ability of the natural antioxidants of the plants extracts towards the stable free radical of DPPH.

2.6 ABTS [2, 2-azino di-(3-ethylbenzothiazoline- 6-sulfonic acid)] SCAVENGING ACTIVITY.

The method described by [28] was used to measure the ABTS. The stock solutions included 7.4 mM $ABTS^{+}$ solution, 2.6 mM potassium persulphate solution and 2, 2-azino di-(3-ethylbenzothiazoline-6-sulfonic acid). The working solution of ABTS was obtained by the stock in methanol to give absorbance of 0.70 at 734 nm.

2.7 OH^{-} FREE RADICAL

OH^{-} free radical scavenging method was described by [28], modified by [29].

2.8 FRAP (Ferric Reducing Antioxidant Power)

[30] described the method used to measure FRAP.

2.9 Quantitative Phytochemical Composition of Aqueous Extracts of *Greenwayodendron suaveolens, Vernonia amygdalina, Euphorbia heterophylla and Xylopia aethiopica*.

2.10 Total Phenolic Content Determination

The quantity of total phenolics in the plant extract was determined by the method of [31] with slight variation using tannic acid as a standard.

2.11 Alkaloids Content Determination

The total alkaloid content was measured using the method described by [32].

2.12 Flavonoid Content Determination

The amount of flavonoid was determined on triplicate aliquots of the homogenous cabbage extract (1.5 g) [33]. Thirty-microliter aliquots of the extract of methanol were used for the determination flavonoid. Samples of the extract were diluted with 90 µl methanol, 6 µl of 10 % Aluminum chloride ($AlCl_3$), 6 µl of 1mol/l Sodium acetate (CH_3CO_2Na) were added and lastly, 170 µL of methanol was added. The absorbance was determined at 415 nm after 30 min. Quercetin was used as a standard for determinig the amount of flavonoid (Ug Qe/g).

2.13 Total Saponins Content Estimation

A method used to estimate the total content of saponins based on vanillin-sulphuric acid colorimetric reaction with some variations was described by [34].

2.14 Tannins Content Determination

About 0.20 mL of the plant sample was poured into 20 mL of 50% methanol and was positioned in a water bath at 77 ^{0}C – 80 ^{0}C for 1 hour and shaken. Quantitative filtration of the extract using a double layered Whatman No.1 filter paper was carried out. About 20 mL of distilled water, 2.5 mL Folin-Denis reagent and 10 mL 17% Na_2CO_3 were added and stirred together and the entire mixture was allowed to stand for 20 min. A series of standard tannic acids solutions were prepared in methanol and their absorbance as well as samples was read after colour development on a UV/ Visible spectrophotometer at a wavelength of 760 nm. Total tannin content was calculated from calibration curve.

2.15 Determination of Total Nitrogen

A modified method of micro-Kjeldahl as described by [35] was used for crude protein determination. Three grams each of the defatted samples were separately weighed on pre-weighed into micro-Kjeldahl digestion flask together with few anti-bumping granules. Two grams of catalyst mixture ($CuSO_4$: Na_2SO_4: SeO_2, 5:1:0_2 w/w) was added to each flask and then 10 mL nitrogen free concentrated H_2SO_4 also added to each flask. The flasks were placed in inclined position on a heating mantle in a fume cupboard. Digestion was started at temperature of 30 ^{0}C until frothing ceased and then heating was increased to 50 ^{0}C for another 30 mins and finally at full heating (100 ^{0}C) until a clear solution was obtained. Simmering was continued below boiling point for another 30 min to ensure complete digestion and conversion of nitrogen to ammonium sulphate. After digestion was completed, samples were allowed to cool and then transferred quantitatively to 100 mL volumetric flasks with washing and cooling to room temperature. Volumes were made up to mark with distilled water.

1. 5 ml of the filtrate from the digest was transferred with the aid of a 10 ml pipette into a 25 ml standard flask. 2.5 ml of the Alkaline Phenate was added and the solution shaken to mix properly. Then 1 ml of Sodium Potassium Tartarate was added, shaken properly followed by the addition 2.5 ml of sodium hypochlorite. The solution was made up to the 25 ml mark with distilled water and the absorbance of the resultant solution measured at 630 nm with use of UV/Visible Jenway 6715 Spectrophotometer,. The Nitrogen standards were treated the same way with the sample.

CALCULATION

$$N\ (mg/kg) = \frac{\text{Instrument Reading } \mathbf{X} \text{ Slope Recip } \mathbf{X} \text{ Color Vol. } \mathbf{X} \text{ Digest Vol.}}{\text{Weight of Sample } \mathbf{X} \text{ Aliquot Taken}} \quad (1)$$

2.16 Digestion of Samples

The digestions of the samples were done in accordance with the method described by [35] with little modification.

2.17 Metals Analysis

The metals of interest in this study are Iron, Manganese, potassium, calcium, magnesium, copper, lead, cadmium, chromium, and nickel. The determination of these metals in the samples was made directly on each final solution using Flame Photometer and a Bulk Scientific 210 VGP, and Atomic Absorption Spectroscopy (AAS).

2.18 Statistical Analysis

The data were expressed as mean ± standard error of mean (SEM) and 'n' represents the number of replicates. One way Analysis of Variance (ANOVA) was performed with Newman Keul's post hoc test. All data were analyzed using graph Pad Prism (UK) software version 6. $P<0.05$ indicates significant difference between compared data.

3. Results and Discussion

Table 1: Quantitative analysis of Phytosterols present in *Greenwayodendron suaveolens, Vernonia amygdalina, Euphorbia heterophylla* and *Xylopia aethiopica*.

Samples	Total tannin (ug/ml)	Total flavonoid (ug/ml)	Total phenols (ug/ml)	Total saponin (ppm)	Total alkaloid (%)
G. suaveolens	6.575	20.323	20.330	2.259	1.600
V. amygdalina	8.493	44.839	25.275	2.657	0.800
E. heterophylla	8.356	33.065	17.033	1.904	1.200
X. aethiopica	12.468	41.935	24.835	3.096	0.600

Table 2: Elemental analysis of Macro and Micro nutrients of the aqueous extract of *Greenwayodendron suaveolens, Vernonia amygdalina, Euphorbia heterophylla* and *Xylopia aethiopica*.

S/N	Parameters	A	B	C	D
1	Calcium (Mg/Kg)	75.00	48.00	56.00	15.00
2	Copper (Mg/Kg)	0.20	0.24	0.21	0.28
3	Iron (Mg/Kg)	4.80	3.40	2.90	10.50
4	Magnesium (Mg/Kg)	70.10	63.00	112.00	83.01
5	Manganese (Mg/Kg)	0.73	0.66	1.19	0.86
6	Cadmium (Mg/Kg)	0.10	BDL	BDL	0.10

7	Chromium (Mg/Kg)	0.05	0.09	0.01	0.03
8	Lead (Mg/Kg)	BDL	BDL	BDL	BDL
9	Potassium (Mg/Kg)	136.20	177.10	80.20	74.10
10	Nickel (Mg/Kg)	BDL	BDL	BDL	0.10
11	Nitrogen (%)	0.5690	0.3456	0.2950	0.2571
12	Phophorus (Mg/Kg)	54.404	56.411	18.135	54.404

Keys: A= *Greenwayodendron suaveolens;* B= *Vernonia amygdalina*; C= *Euphorbia heterophylla*; D= *Xylopia aethiopica;* BDL= Below Detection Limit.

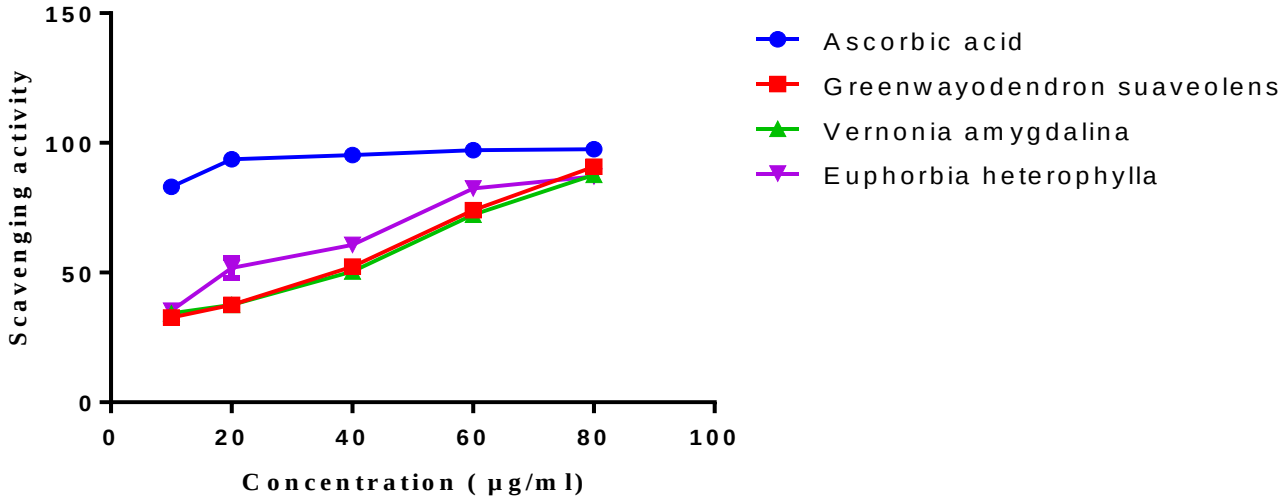

Figure 1: DPPH scavenging activity of Ascorbic acid, *Greenwayodendron suaveolens, Vernonia amygdalina and Euphorbia heterophylla*.

Greenwayodendron suaveolens, Vernonia amygdalina and Euphorbia heterophylla shows DPPH scavenging activities. The scavenging power of the plants increases as the concentration increases. At 80µg/ml, the scavenging activities of the plants are comparable to that of ascorbic acid.

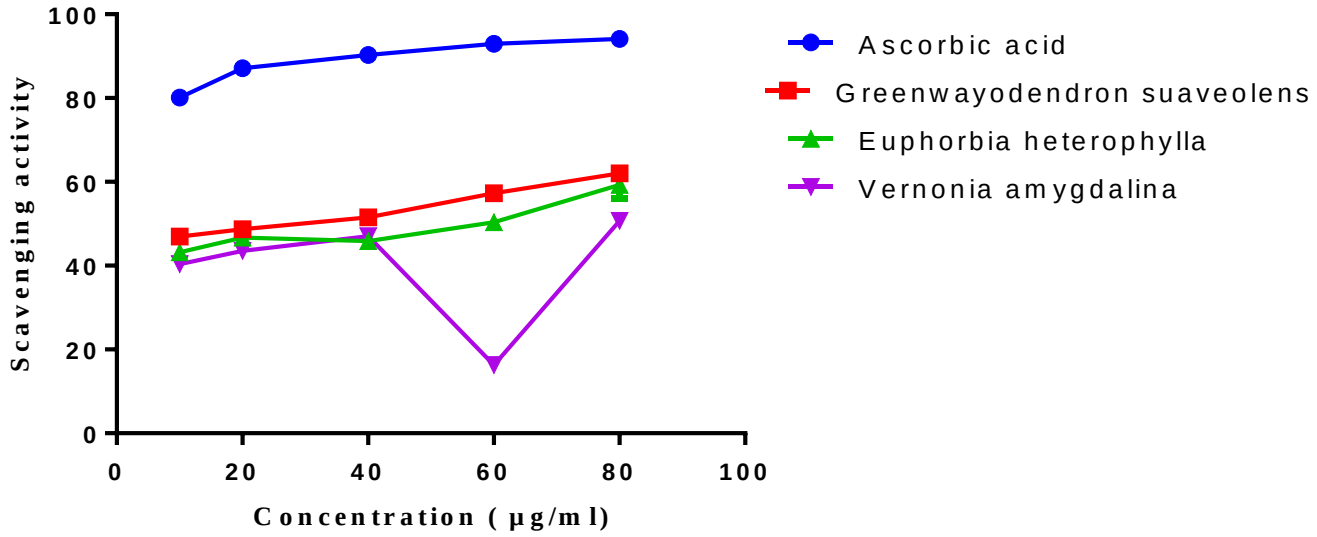

Figure 2: ABTS scavenging activity of Ascorbic acid, *Greenwayodendron suaveolens, Vernonia amygdalina and Euphorbia heterophylla*.

Greenwayodendron suaveolens, Vernonia amygdalina and Euphorbia heterophylla shows ABTS radical scavenging activities. The scavenging power of the plants increases as the concentration increases. However, the scavenging activities of the plants are not comparable to that of ascorbic acid.

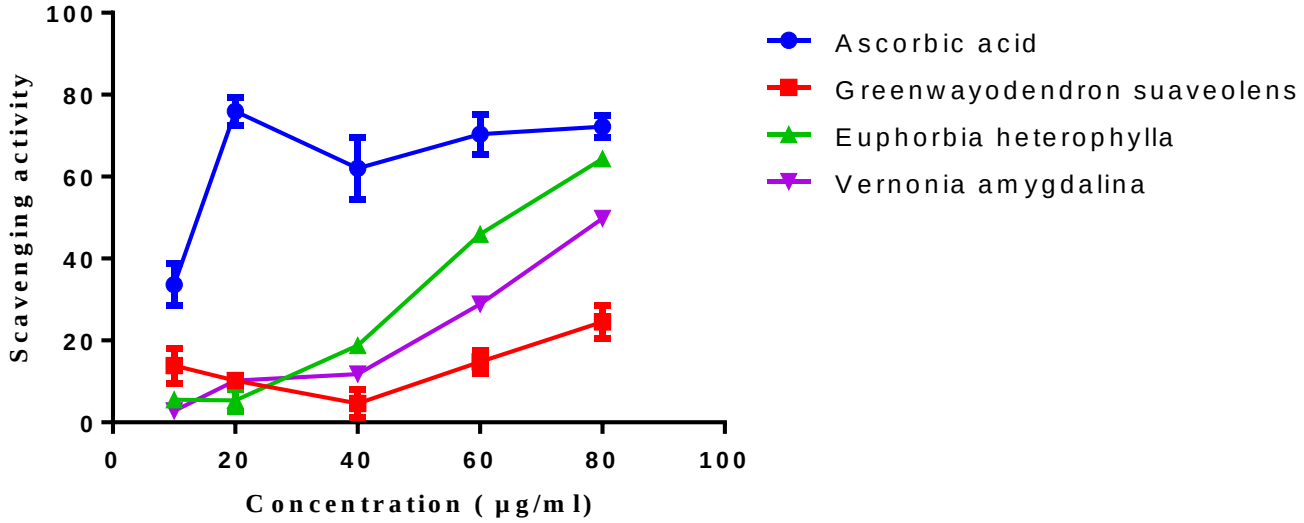

Figure 3: OH^- free radical scavenging activity of Ascorbic acid, *Greenwayodendron suaveolens, Vernonia amygdalina and Euphorbia heterophylla*.

Greenwayodendron suaveolens, Vernonia amygdalina and Euphorbia heterophylla shows OH^- free radical scavenging activities. The scavenging power of the plants increases as the concentration increases. However, the scavenging activities of the plants are not comparable to that of ascorbic acid.

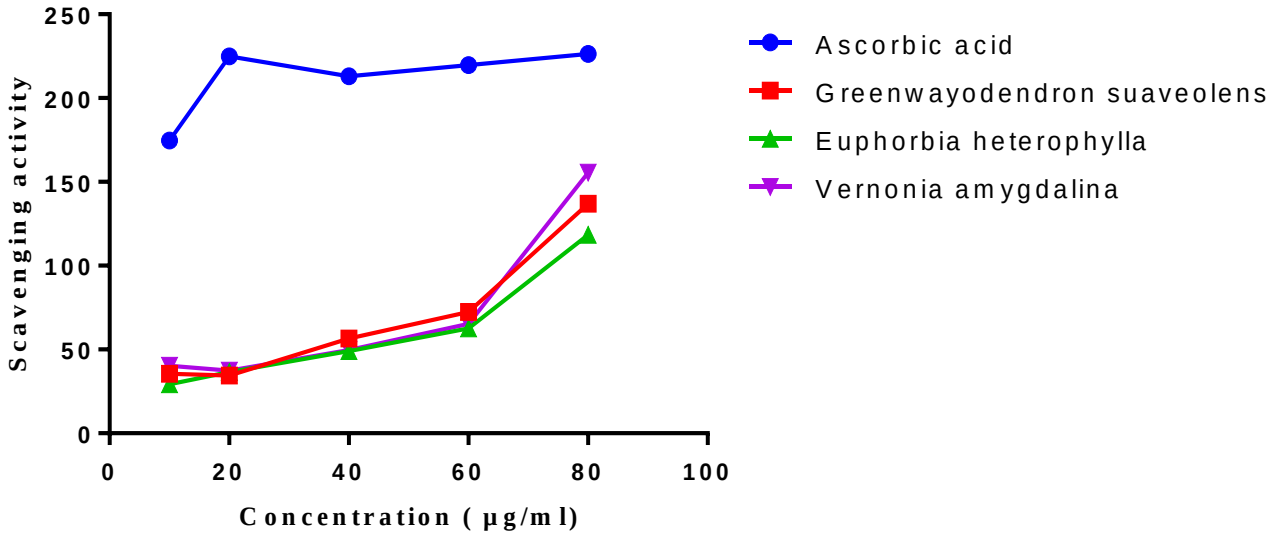

Figure 4: FRAP scavenging activity of Ascorbic acid, *Greenwayodendron suaveolens, Vernonia amygdalina and Euphorbia heterophylla.*

Greenwayodendron suaveolens, Vernonia amygdalina and Euphorbia heterophylla shows FRAP free radical scavenging activities. The scavenging power of the plants increases as the concentration increases. However, the scavenging activities of the plants are not comparable to that of ascorbic acid.

3.1. Discussion

Plants have been used for centuries in the treatment of various ailments. Research has shown that the medicinal effects of plants are based on their phytochemicals and non-phytochemical constituents [36]. This study shows the quantitative phytochemical and non- phytochemical constituents of *Greenwayodendron suaveolens, Vernonia amygdalina, Euphorbia heterophylla and Xylopia aethiopica* (Tables 1 and 2). Total tannins composition *in G. suaveolens, V. amygdalina, E. heterophylla and X. aethiopica* were 6.575, 8.493, 8.356 and 12.468 respectively. However, the composition of tannins was highest in *X. aethiopica* with a concentration of 12.468 ug/ml. Tannins is used in medicine, especially in the treatment of Asian (Japanese and Chinese) naturopathy. Herbal extracts containing tannins are used as a diuretic strengthening agent, against gastric and duodenal tumours [37, 38] and as an anti-inflammatory, antiseptic and hemostatic drug [38]. Total flavonoids composition in *G. suaveolens, V. amygdalina, E. heterophylla and X. aethiopica* were 20.232, 44.839, 33.065 and 41.935 respectively (table 1). The composition of flavonoids was highest in *V. amygdalina* with a concentration of 44.839 ug/ml. Flavonoids are intended to function as insect repellents, natural fungicides (phytoalexins) and potential regulators of the indole acetic acid plant hormones [32]. Phenols composition in *G. suaveolens, V. amygdalina, E. heterophylla and X. aethiopica* were 20.330, 25.275, 17.033 and 24.835 respectively. The composition of phenols was highest in *V. amygdalina* with a concentration of 25.275 ug/ml. The presence of phenols and flavonoids in the extract can help prevent oxidative stress by scavenging free radicals and bioactivation of carcinogens for excretion in the liver [39]. Total saponins composition in *G. suaveolens, V. amygdalina, E. heterophylla and X. aethiopica* were 2.259, 2.657, 1.904 and 3.096 respectively. However, the composition of saponnins was highest in *X. aethiopica* with a value of 3.096 ppm. Saponins are used in preparation and development of vaccines [40]. Total alkaloids composition in *G. suaveolens, V. amygdalina, E. heterophylla and X. aethiopica* were 1.600, 0.800, 1.200 and 0.600 respectively. The composition of alkaloids was highest in *G. suaveolens* with a percentage of 1.600 % Alkaloids have a broad spectrum of pharmacological activities, including

antimalarial, anti-asthmatic, antiarrhythmic, analgesic, antihyperglycemic and antibiotic agents [40].

G. suaveolens, V. amygdalina, E. heterophylla and X. aethiopica showed that they contained both macro and micro elements including calcium, copper, iron, magnesium, manganese, chromium, potassium, nitrogen and phosphorus (Table 2).

Cadmium is present in *G. suaveolens* and *X. aethiopica* in the same concentration of 0.1 Mg/Kg and absent in both *V. amygdalina* and *E. heterophylla*. According to [41], cadmium has no known biological functions in higher organisms, but according to [42], administration of cadmium to cells causes oxidative stress and increases the levels of antioxidants to protect the cells against macro molecular damage. Nickel is absent in *G. suaveolens, V. amygdalina, E. heterophylla* and present only in *X. aethiopica* with a concentration of 0.1 mg/kg. Lead is absent in all four plants.

Calcium composition in *G. suaveolens, V. amygdalina, E. heterophylla and X. aethiopica* were 75.00, 48.00, 56.00 and 15.00 mg/kg and it was highest in *G. suaveolens* with a concentration of 75.00 mg/kg. From the result the plants can supply calcium for normal growth and development of the skeleton and regulates muscle contraction such as the cardiac muscles and also aid normal blood clotting as supported by [42]. According to [43], calcium can be used for treating osteoporosis and osteopenia. Copper composition in *G. suaveolens, V. amygdalina, E. heterophylla and X. aethiopica* were 0.20, 0.24, 0.21 and 0.28. The composition of copper was highest in *X. aethiopica* with a concentration of 0.28 ug/ml. Copper, though present in all four plants is shown in little quantities. It is reliable in the treatment of copper deficiency as it is required in trace quantities. According to [44], copper is very important as a trace element for all living organisms because it is a key component of the respiratory enzyme and cytochrome C oxidase required for metabolism.

Iron composition in *G. suaveolens, V. amygdalina, E. heterophylla and X. aethiopica* were 4.80, 3.40, 2.90 and 10.50 Mg/Kg. The composition of iron was highest in *X. aethiopica* with a concentration of 10.50 Mg/Kg. According to [45], iron is useful in the synthesis of haemoglobin and myoglobin and also in the treatment of iron deficiencies such as iron deficient anaemia, hence, these plants may be used in both nutritive and treatment regimens. The medicinal importance of magnesium in accordance with [46] is used medically as ordinary laxatives, antacids and to stabilize abnormal nerve excitation or vascular spasms in diseases such as eclampsia.

Manganese composition in *G. suaveolens, V. amygdalina, E. heterophylla and X. aethiopica* were 0.73, 0.66, 1.19 and 0.86 Mg/Kg. The composition of Manganese was highest in *X. aethiopica* with a concentration of 1.19 Mg/Kg. According to [47], manganese which is present as a coenzyme in several biological processes is an important human dietary element. This biological processes include free radical defense systems, bone formation, and macronutrient metabolism. Chromium composition in *G. suaveolens, V. amygdalina, E. heterophylla and X. aethiopica* were 0.05, 0.09, 1.01 and 0.03 Mg/Kg. Though present in little quantities, it was highest in *V. amygdalina* with a concentration of 0.09 Mg/Kg. The U.S. National Institutes of Health chromium accepts as a trace element it plays very important roles in the action of insulin, a hormone essential for the metabolism and storage of carbohydrate, fat and protein. Its actual mechanisms of its actions in the human system, however, have not been defined fully, leaving the question whether chromium is of essence to healthy people [48]. Potassium composition in *G. suaveolens, V. amygdalina, E. heterophylla and X. aethiopica* were 136.20, 177.10, 80.20 and 74.10 Mg/Kg. It was highest in *V. amygdalina* with a concentration of 177.10 Mg/Kg. In medicine, the benefits of potassium are resting cellular-membrane potential and the propagation of action potentials in neuronal, muscular and cardiac tissue. Potassium ion, K^+, larger than Na^+ ions due to electrostatic and chemical properties, and as a result, the ion channels and pumps in membranes of the cells can differentiate between the two ions by actively pumping or passively passing one of the two ions while blocking the other as supported by [49, 50]. Only *X. aethiopica* is known to contain nickel with a composition of 0.10 Mg/Kg, though, the United States Institute of Medicine (1997) did not established that nickel is

essential for the human body, so neither an adequate intake nor a recommended dietary allowance (RDA) have been established.

Nitrogen composition in *G. suaveolens, V. amygdalina, E. heterophylla and X. aethiopica* were 0.5690, 0.3456, 0.2950 and 0.2571 % and it was highest in *G. suaveolens* with a percentage of 75.00 %. According to [51], when ammonia is extracted from plants, it is used for protein synthesis. Phosphorus composition in *G. suaveolens, V. amygdalina, E. heterophylla and X. aethiopica* were 54.404, 56.411, 18.135 and 54.404 Mg/Kg. It was less in *E. heterophylla* with a concentration of 18.135 Mg/Kg. Phosphorus plays a very vital role in the structure of DNA and RNA. Also, cellular energy with adenosine triphosphate (ATP), necessary for every cellular process that uses energy are transported by phosphate using living cells. Calcium phosphate is also used for the reinforcement of bones.

From the results, *G. suaveolens, V. amygdalina and E. heterophylla* has DPPH scavenging activities (figure 1), ABTS scavenging activities (figure 2), OH^- scavenging activities (figure 3) and FRAP (figure 4). DPPH is a radical widely used in food industry to evaluate scavenging activities of food product [51]. ABTS method is accurate, easy and rapid. It has several advantages as it does not require high temperatures to generate ABTS radicals, avoids unwanted side-reactions and antioxidant activity can be studied over a wide range of pH values. This method is able to determine both lipophilic (inorganic media) and hydrophilic (in buffered media) antioxidant properties [52]. The ability of the plants to scavenge DPPH radical and ABTS radical indicates that the plants have antioxidant properties. Hydroxyl radicals functions as highly reactive species as primary poisons and as sources of secondary toxic substances. Free radicals have shown to be causative agent of ailments such as inflammation, diabetes, cardio vascular disease, liver cirrhosis, aging, cancer, Alzheimer and acquired immunodeficiency syndrome. It has been reported that OH- free radical is the chief cause of inflammation [53]. Substances with natural antioxidant are alleged to play a vital role in interfering with the oxidation process by reacting with free radicals, chelating catalytic metals and scavenging oxygen in biological systems [54]. Hydroxyl radicals produced in or near DNA have contributed to significant biological effects such as mutagenesis and cytotoxicity. The scavenging activity of OH^- by *G. suaveolens, V. amygdalina and E. heterophylla*, finds application in prevention of cancer from DNA mutation. According to [55] Ferrozine, a compound closely related to 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), a complexing substance used in spectrophotometric analysis, has been widely employed, with surplus ascorbic acid, to measure iron limiting factor of Fe (II)-TPTZ, and hence, colour formation is the reducing ability of the sample. This assay is commonly used to measure the antioxidant content in industries and solvent systems including ethanol, aqueous acetone, methanol, aqueous alcohol, and benzene [55]. The quantitative phytochemical constituents, non- phytochemical constituents and scavenging activity of the plants gave evidence that these plants are used in the treatment of gastric and duodenal tumours, inflammation, lung cancer, stomach cancer, ovarian cancer and estrogen-dependent breast cancer in humans. They are also known to reduce blood cholesterol levels by inhibiting intestinal cholesterol absorption [55, 56].

4. Conclusion

The *invitro* research work established that the plants can be used to treat diseases especially those due to oxidative stress. It is therefore recommended that more research need to be carried out *Invivo* to further prove their anti-oxidative potentials.

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