

Original Research Paper

Phytochemical Analysis and Antioxidant Activity of Partition Fractions of *Hibiscus sabdariffa* and *Vernonia amygdalina*

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Abstract: Abstract: The calyx of *Hibiscus sabdariffa* and the leaf of *Vernonia amygdalina* are plant parts which are widely consumed in Nigeria. These plant parts are known for their numerous medicinal uses which is a function of the phytochemicals present in them. This study was undertaken to investigate and compare the phytochemical constituents and antioxidant activities of the petroleum ether, chloroform, ethyl acetate, acetone and methanol fractions of the calyx of *H. sabdariffa* and the leaf of *V. amygdalina* to ascertain their medicinal values. Phytochemical studies were done using standard methods such as Fehling's test, alkaline reagent test, Keller-Kiliani test and spectrophotometric techniques. The antioxidant activities were carried out through DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging effects. The preliminary phytochemical analysis of the extracts of *Hibiscus sabdariffa* and *Vernonia amygdalina* revealed the presence of varying amounts of alkaloids, saponins, tannins, terpenoids, cardiac glycosides, phenols and flavonoids. The petroleum ether, chloroform, ethyl acetate and methanol fractions of *Vernonia amygdalina* contained alkaloids while only the chloroform fraction of *Hibiscus sabdariffa* contained alkaloids. The difference in alkaloidal content of the chloroform fractions of both plant extracts was not statistically significant at $P < 0.05$. The methanol fraction of both plants contained saponins with *Vernonia amygdalina* showing a higher percent of saponin compared to that of *Hibiscus sabdariffa* although the difference was not statistically significant at $P < 0.05$. *Vernonia amygdalina* contained more flavonoids in all fractions than *Hibiscus sabdariffa*. The difference in concentration of flavonoids between the fractions of both plant was statically significant at $P < 0.05$. The phenolic content of the petroleum ether, chloroform and methanol fractions of *H. sabdariffa* were higher than that of *V. amygdalina* but the difference was not statistically significant at $P < 0.005$ while the phenolic content of the ethyl acetate and acetone fractions of *Vernonia amygdalina* were statistically higher than that of *Hibiscus sabdariffa*. Amongst all the fractions, the methanolic fraction of *Hibiscus sabdariffa* showed the highest antioxidant activity of 90.57 % at a concentration of 0.25 mg/ml. The activity decreased with an increase in the concentration. The result of the phytochemistry and antioxidant activity of the calyx of *Hibiscus sabdariffa* and the leaf of *Vernonia amygdalina* ascertained; the medicinal values of these plants, the folkloric claims and their wide consumption.

Keywords: *Hibiscus sabdariffa*, *Vernonia amygdalina*, Phytochemical, Roselle, Rosella

INTRODUCTION

Phytochemistry is the study of phytochemicals which are chemicals derived from plants. The term is often used to describe the large number of secondary metabolic compounds found in plants, many of which are known to provide protection against insect attacks and plant disease

Physical evidence of the use of herbal remedies some sixty thousand years ago has been found in a burial site of a Neanderthal man uncovered in 1960 in a cave in northern Iraq (Solecki, 1975).

The knowledge of plant-based drugs developed gradually and was passed on, thus, laying the foundation for many systems of traditional medicine all over the world. In some communities, herbal medicine is still a central part of their medical system (Desmet, 1995). Medicinal plants are widely distributed throughout the world but most abundantly in tropical countries. It is estimated that about 25% of all modern medicines are directly or indirectly derived from higher plants (WHO, 2005).

Over the years, many orthodox medicines have been obtained from plants and they have been found to be advantageous as they are relatively available, cheap and safe. Examples of drugs derived from plants are; penicillin from *Penicillium notatum*, quinine from *Cinchona succirubra*, morphine from *Papava somniferum*, nicotine from *Nicotiana tabacum*, vincristine from *Catharanthus roseus*, theophylline from *Theobroma cacao*, physostigmine from *Physostigma venenosum*, reserpine from *Rauwolfia serpentina*, artemisinin from *Artemisia annua* and digoxin from *Digitalis*

purpurea

Although it is generally believed that most herbal preparations are safe for consumption, some herbs like most biologically active substances could be toxic and present with undesirable side effects (Bisset, 1994).

To determine the phytochemical constituents and antioxidant activity of the calyx of *Hibiscus sabdariffa* and leaves of *Vernonia amygdalina*

Hibiscus sabdariffa is known as Zobo in Western Nigeria while the Yorubas in Nigeria call the white variety “Isapa” and the Hausas call it “Zoborodo”. The *Roselle* is known as the *Rosella* fruit in Australia. It’s close relative, *Hibiscus Cannabinus* is also known as *meshta* on the Indian subcontinent, *Tengamora* in Assam, *Gongura* in Telugu, *lalchatni* or *Kutrum* in Mithula. *Mathipuli* in Kerala, *Chin baung* in Burma, *krajeab* in Thailand, *Bissapin* in Senegal, Guinea Bissau, Mali, Burkina Faso, Ghana, Benin, Niger, Congo and France, *Wonjo* in Gambia, *Chaye-Torosh* in Iran, *Karkade* in Egypt, Saudi Arabia and Sudan, *Omutete* in Namibia, *Sorrel* in the Caribbean and in Latin America, *Flor de Jamaica* in Mexico, *Saril* in Panama, *rosella* in Indonesia, *asam paya* in Malaysia and *Luoshen Hua* in Chinese. In Zambia the plant is called *Lumanda* in Gbemba, *Katolo* in Kikaonde and, *wusi* in Chilunda (Chau et al., 2000).

Vernonia amygdalina Del is commonly called “bitter leaf” because of its bitter taste. The bitterness can, however, be abated by boiling or by soaking the leaves in several changes of water. In Nigeria, it is known as “Ewuro” in Yoruba language, “Onugbu” in Igbo language, “Oriwo” in

Bini language, “Ityuna” in Tiv, “Chusar doki or fatefate” in Hausa and “Etidot” in Cross River State of Nigeria. The bitter taste has been ascribed to anti-nutritional factors such as alkaloids, saponins, tannins, and glycosides (Osuji *et al.*, 1995).

The economic uses of the plant are as garden ornamentals, potherbs, as source of dye, medicine and drink. In Africa, the water extract of *Hibiscus sabdariffa* is taken as a hot or cold drink which is commonly known as ‘Zobo’ in Nigeria, Wanjo in the Gambia, Karkadayin in Egypt and Sudan (Chau *et al.*, 2000).

The leaves are used as green leafy vegetable and may be consumed either as a vegetable (leaves are macerated in soups) or aqueous extracts used as tonics for the treatment of various illnesses. In the wild, chimpanzees have been observed to ingest the leaves when suffering from parasitic infections (Huffman, 2003). Many herbalists and native doctors in Africa recommend its aqueous extracts for their patients as treatment for varieties of ailments ranging from emesis, nausea, diabetes, loss of appetite, dysentery and other gastrointestinal tract problems to sexually transmitted diseases and diabetes mellitus among others (Argheore *et al.*, 1998)

The leaves can be taken as an appetizer and the water extract as a digestive tonic (Singha, 1995). These are largely consumed by the female Hausas in their belief that it makes them more sexually attractive (Owoeye *et al.*, 2011). In Northern Nigeria, it has been added to horse feed to provide a strengthening or fattening tonic called ‘Chusar Doki’ in Hausa (Dalziel, 1937) while the leaves have also been used in Ethiopia as hops in preparing

tela beer (Getahun, 1976). Also, the leaves are widely used for fevers and are often known as a quinine– substitute in Nigeria and some other African countries (Oliver, 1960) while the young leaves are used in folk medicine as antihelmintic, antimalarial, laxative/ purgative, enema, expectorant, worm expeller and fertility inducer in subfertile women.

MATERIALS AND METHOD

PLANT COLLECTION

Dried parts of *Hibiscus sabdariffa* (calyx) and *Vernonia amygdalina* (leaves) were collected from Tanke, Ilorin, Kwara State, Nigeria. The plant materials were taxonomically identified and authenticated by Mr. Femi at The Herbarium, Department of Plant Biology, University of Ilorin, Ilorin, Kwara State, Nigeria. They were dried under the shade until all the water molecules had evaporated and plants became well dried for grinding. After drying, the plant materials were ground into fine powder using mechanical blender and transferred into airtight containers which were properly labelled.

EXTRACTION

Solvent Extraction

280g of powdered leaves of *Vernonia amygdalina* and 450g of powdered calyx of *Hibiscus sabdariffa* plant materials were weighed and uniformly packed into an extraction bottle each and extracted with 600 ml each of petroleum ether, ethyl acetate, acetone, chloroform and methanol successively. Extraction was done exhaustively and the solvent was separated from the extract on a rotary evaporator followed by drying in a dessicator.

Dried extracts were kept in a refrigerator at 4°C for their future use. The percentage yield of the different fractions were calculated using the equation;

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powered drug}} \times 100 \text{----(1)}$$

Solubility profile

The solubility profile of all the fractions of the extracts were carried out by dissolving each extract in petroleum ether, chloroform, ethyl acetate, acetone, methanol and distilled water to determine the solvent(s) in which they are soluble.

Qualitative phytochemical analysis

The extracts were tested for the presence of bioactive compounds by using following standard methods.

Test for carbohydrates

Fehling's test: 1 ml each of Fehling A and Fehling B reagents were mixed together and added to 2ml of the extract and gently boiled. A brick-red precipitate appearing at the bottom of the test tube indicated the presence of reducing sugars (Sofowora, 1993).

Molisch's test: The extract was mixed with 2ml of Molisch's reagent and the mixture was shaken properly. After that, 2ml of concentrated H₂SO₄ was poured carefully along the side of the test tube. Appearance of a violet ring at the interphase indicated the presence of carbohydrate (Sofowora, 1993).

Test for phenols and tannins

The extract was mixed with 2ml of a 2% solution of FeCl₃. A blue-green or black color indicated the presence of phenols and tannins (Sofowora, 1993).

Test for flavonoids

Alkaline reagent test: The extract was mixed with 2ml of a 2% solution of NaOH. An intense yellow color formed which disappeared on addition of few drops of diluted acid indicated the presence of flavonoids (Harbone, 1973).

Test for saponins

The extract was mixed with 5ml of distilled water in a test tube and shaken vigorously. The formation of a stable foam for at least 30 minutes was taken as an indication of the presence of saponins (Harbone, 1973).

Test for glycosides

Keller-kilani test: The extract was mixed with 2 ml of glacial acetic acid containing 1-2 drops of 2% solution of FeCl₃. The mixture was then poured into another test tube containing 2ml of concentrated H₂SO₄. A brown ring at the interphase indicated the presence of cardiac glycosides (Trease and Evans, 1989).

Test for steroid

The extract was mixed with 2ml of chloroform and concentrated H₂SO₄ was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing the crude extract with 2 ml of chloroform. Then 2 ml of each of concentrated H₂SO₄ and acetic acid were poured into the mixture. The development of a greenish color indicated the presence of steroids (Trease and Evans, 1989).

Test for terpenoids

The extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated

H₂SO₄ was added and heated for about 2 minutes. A grayish colour indicated the presence of terpenoids (Trease and Evans, 1989).

Test for alkaloids

The extract was mixed with 2 ml of 1% HCl and heated gently. Wagner's reagent was then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids (Harbone, 1973).

QUANTITATIVE PHYTOCHEMICAL ANALYSIS

Total phenolic content

The amount of phenol in the aqueous extract was determined by the Folin-Ciocalteu reagent method (Aiyegroro *et al.*, 2010). 3.6 ml of 10 % Folin-Ciocalteu reagent and 2.9 ml of 2% solution of sodium bicarbonate were added to 3.6ml of plant extract. Different concentrations (10 µg to 1000 µg) of gallic acid (standard) were prepared the same way as the extracts. The resulting mixture was incubated for 30 minutes at room temperature and the absorbance of the standard and samples were measured at 765 nm. A graph of the absorbance of standard versus concentration was plotted as the standard curve. All the tests were performed in triplicates. The results were determined from the standard curve and were expressed as gallic acid equivalent (mg/g of extracted compound).

Total flavonoid content

A colorimetric method (Aiyegroro *et al.*, 2010) using aluminium chloride was used to determine the flavonoid content. 0.5 ml of the extract was mixed with 2 ml of methanol and 2.5 ml of 10 % aluminium chloride and allowed to stand for 30

minutes at room temperature. Different concentrations (10 µg to 1000 µg) of quercetin (standard) were prepared the same way as the extracts. The absorbance was measured at 420 nm. A graph of the absorbance of standard versus concentration was plotted as the standard curve. All the tests were performed in triplicates. The flavonoid contents were determined from the standard curve and were expressed as quercetin equivalent (mg/g of extracted compound)

Total alkaloid content

0.1 g of the extract was weighed into 4 ml of 20 % acetic acid in ethanol and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath at 55 °C to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise into the extract until precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide solution and then filtered (Harbone, 1973).. The residue which is the crude alkaloid was weighed and calculated according to the equation:

$$\text{Amount of alkaloid} = \frac{\text{Weight of precipitate}}{\text{Weight of sample}} \quad \text{---(2)}$$

Total saponin content

0.1 g of the extract was dissolved in 2 ml of 20% ethanol and transferred into a separator funnel. 2 ml of diethyl ether was added and shaken vigorously. The ether layer was discarded, while the purification process was repeated. 6 ml of n-butanol was added to the extract in ethanol and the extracts were washed twice with 1 ml of 5% aqueous sodium chloride. The remaining solution was

evaporated on a water bath. After evaporation, the sample was dried in the oven to a constant weight (Kim *et al.*, 2003). The saponin content was calculated according to the equation:

$$\text{Amount of saponin (mg/g)} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \text{ ---(3)}$$

Antioxidant activity

Different concentrations (0.0078125 - 0.5 µg/ml) of each extract were added, at an equal volume, to methanolic solution of DPPH (100 µM). The mixture was allowed to react at room temperature and in the dark for 30 minutes. Different concentrations (10 µg to 1000 µg) of vitamin C (standard) were prepared the same way as the extracts. Three replicates were made for each test sample. After 30 minutes, the absorbance (A) was measured at 518 nm and converted into the percentage antioxidant activity using the following equation:

$$\% \text{ scavenged} = \frac{\text{Absorbance of standard} - \text{Absorbance of extract}}{\text{Absorbance of standard}} \times 100 \text{ --- (4)}$$

The IC₅₀ values which denote the concentration of sample which is required to scavenge 50 % of DPPH free radicals were calculated by linear regression of plots, where the abscissa represented the concentration of the tested plant extracts and the ordinate the average percent of scavenging capacity from three replicates (Nabari *et al.*, 2001)

THIN LAYER CHROMATOGRAPHY

Spotting

A small portion of the different fractions of extracts was dissolved in appropriate solvents in different sample bottles. TLC plates were spotted with the

dissolved extracts using capillary tubes at the baseline of 1cm from the lower end of the plate. The plate was then allowed to dry before putting it into the chromatographic tank.

Development of Chromatogram

Different solvent systems were prepared (table 1) and poured into the chromatographic tank to a depth of about 0.5 cm. The tank was covered with a lid to allow saturation of the tank with the solvent. The spotted plate was carefully inserted into the tank using a pair of forceps and covered to allow proper development of the plate until the solvent was about ¾ above the baseline. The plate was removed from the tank and the solvent marked. The developed chromatogram was dried and sprayed with dilute H₂SO₄. After spraying, it was dried in an electric oven to visualize the spots produced (Harry and Christopher, 1989).

Table 1. **Solvent systems of the fractions and their ratio**

	Mobile phase (solvent system)	Ratio
V.amygdalina Pet.Ether	CHCl ₃ :EtOAc:CH ₃ OH	1:0.3:0.3
V.amygdalina CHCl ₃	CHCl ₃ :EtOAc	1.5:1.5
V.amygdalina EtOAc	CHCl ₃ :EtOAc:CH ₃ OH	1:0.3:0.3
V.amygdalina Acetone	CHCl ₃ :EtOAc:CH ₃ OH	1:0.3:0.3
V.amygdalina CH ₃ OH	CHCl ₃ :EtOAc:CH ₃ OH	1:0.3:0.3
H.sabdariffa CHCl ₃	Pet.Ether:CHCl ₃ : EtOAc	4:1:1
H.sabdariffa EtOAc	Pet.Ether:CHCl ₃ :EtOAc:CH ₃ OH	0.5:2:0.3:0.1
H.sabdariffa Acetone	Pet.Ether:CHCl ₃ :EtOAc:CH ₃ OH	0.5:2:0.3:0.1
H.sabdariffa CH ₃ OH	Pet.Ether:CHCl ₃ :EtOAc:CH ₃ OH	0.5:2:0.3:0.1

Statistical analysis

Data obtained were represented as Mean ± Standard Error of Mean (SEM). Analysis of Variance (ANOVA) was used to statistically analyze the data obtained after the experiment by comparing the data

obtained for each extract of *H. sabdariffa* with its *V. amygdalina* counterpart. $P < 0.05$ was regarded as significant.

RESULTS AND DISCUSSION

Percentage extractive value

Table 2. Percentage yield of *Hibiscus sabdariffa* and *Vernonia amygdalina*

Extract	<i>Hibiscus sabdariffa</i>			<i>Vernonia amygdalina</i>		
	Plant weight (g)	Extract weight (g)	yield (%)	Plant weight (g)	Extract weight (g)	yield (%)
Petroleum ether	450	1.556	0.346	280	2.366	0.845
CHCl ₃	450	3.999	0.889	280	7.642	2.729
EtOAc	450	9.388	2.086	280	2.881	1.029
Acetone	450	17.655	3.923	280	2.414	0.862
CH ₃ OH	450	23.566	5.237	280	23.284	8.316

Solubility Profile

For *Hibiscus sabdariffa*, the petroleum ether extract was soluble in all solvents except water. The chloroform extract was insoluble in petroleum ether and water but was soluble in the other solvents while the ethyl acetate extract was insoluble in petroleum ether and chloroform but soluble in other solvents. The acetone extract was insoluble in ethyl acetate, chloroform and petroleum ether but the methanol extract was only soluble in chloroform,

The methanol fractions had the highest extractive value in both *H. sabdariffa* (5.24%) and *V. amygdalina* (8.32%) respectively while the petroleum ether fraction of both plant extracts had the least percentage yield (table 2).

methanol and water (table 3).

For *V. amygdalina*, the petroleum ether extract was soluble in all solvents except for methanol and water. The chloroform and ethyl acetate extracts were soluble in all solvents except for petroleum ether and water. The acetone extract was insoluble in petroleum ether, chloroform and water but was soluble in the other solvents. Methanol extract was soluble in all except petroleum ether as shown in table 3.

Table 3. Solubility profile of *H. sabdariffa* and *V. amygdalina*

Extract	Solvents											
	Petroleum ether		CHCl ₃		EtOAc		Acetone		CH ₃ OH		H ₂ O	
	HS	VA	HS	VA	HS	VA	HS	VA	HS	VA	HS	VA
PET.ET	+	+	+	+	+	+	+	+	+	-	-	-
CHCl ₃	-	-	+	+	+	+	+	+	+	+	-	-
EtOAc	-	-	-	+	+	+	+	+	+	+	+	-
Acetone	-	-	-	-	-	+	+	+	+	+	+	-
CH ₃ OH	-	-	+	+	-	+	-	+	+	+	+	+

KEYS: EtOAc = Ethyl acetate, + = Soluble, - = Insoluble, HS = *Hibiscus sabdariffa*, VA = *Vernonia amygdalina*

Phytochemical analysis

The preliminary phytochemical analysis of the petroleum ether, chloroform, ethyl acetate, acetone

and methanol extracts of *Hibiscus sabdariffa* and *Vernonia amygdalina* revealed the presence of varying amount of alkaloids, saponins, tannins, cardiac glycosides, flavonoids and shown in the

tables 4.

For *V.amygdalina*, the petroleum ether extract contained steroids, alkaloids and flavonoids. The chloroform extract contained steroids, alkaloids, tannins and flavonoids. The ethyl acetate extract contained steroids, alkaloids, flavonoids and cardiac glycosides.

The acetone extract contained steroid, flavonoids and cardiac glycosides. The methanol extract contained steroid, saponins, alkaloids, flavonoids

and cardiac glycosides as shown in Table 4.

For *H.sabdariffa*, the petroleum ether extract contained steroids and terpenoids. The chloroform extract contained steroids,terpenoids, alkaloids, cardiac glycosides and flavonoids. The ethyl acetate and acetone extracts contained terpenoids, saponins, flavonoids and cardiac glycosides. The methanol extract contained terpenoids and saponins as shown in Table 4.

Table 4. Phytochemical screening of fractions of *Vernonia amygdalina* and *Hibiscus sabdariffa*

Phytochemicals	Petroleum ether		CHCl ₃		EtOAc		Acetone		CH ₃ OH	
	VA	HS	VA	HS	VA	HS	VA	HS	VA	HS
Steroids	+	+	+	+	+	-	+	-	+	-
Terpenoids	-	+	-	+	-	+	-	+	-	+
Saponins	-	-	-	-	-	+	-	+	+	+
Alkaloids	+	-	+	+	+	-	-	-	+	-
Tannins	-	-	+	-	-	-	-	-	-	-
Flavonoids	+	-	+	+	+	+	+	+	+	-
Cardiac glycosides	-	-	-	+	+	+	+	+	+	-
Carbohydrates	+	-	+	+	+	-	-	-	+	+

KEY: + = Present, - = Absent, HS = *Hibiscus sabdariffa*, VA = *V. amygdalina*,

Quantitative determination of phytochemicals

Alkaloids

For *V.amygdalina*, the petroleum ether extract contained 61.20 % of alkaloid. The chloroform extract contained 68.30 % of alkaloid. The ethyl acetate extract contained 58.60 % of alkaloid while the acetone extract contained no alkaloid. The methanol extract contained 85.40 % of alkaloid as shown in Figure 1. For *H.sabdariffa*, only the chloroform extract contained 64.10 % of alkaloid while the petroleum ether, ethyl acetate, acetone and methanol extract contained no alkaloid as shown in Figure 1.

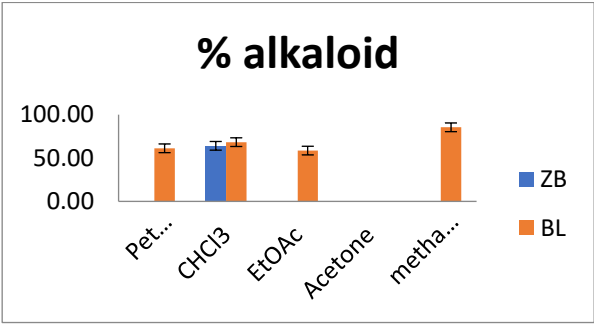


Figure 1. % alkaloid content of fractions of *V. amygdalina* (BL) and *H. sabdariffa* (ZB)

Saponin

The petroleum ether and chloroform extract of both plants contained no saponin. For *V. amygdalina*, only the methanol extract contained 27.43 % of saponin as shown in Figure 2. The ethyl acetate and acetone extracts contained no saponin. For *H. sabdariffa*, the ethyl acetate and acetone extracts contained 47.73 % and 53.90 % of saponins respectively while the methanol extract contained 22.3 % of saponin as shown in Figure 2.

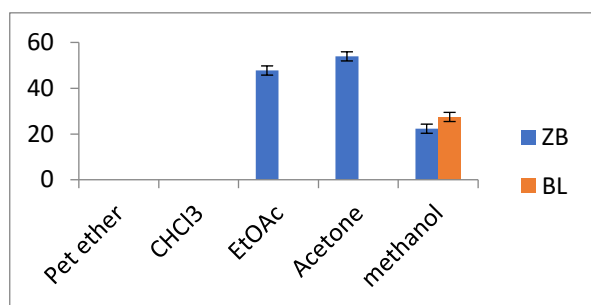


Figure 2. % Saponin content of *V. amygdalina* (BL) and *H. sabdariffa* (ZB)

Flavonoids

All extracts of both plants contained flavonoids in varying quantities. For *Vernonia amygdalina*, the petroleum ether extract contained 21.57mg/g of flavonoids while the chloroform extract contained 27.69mg/g. The ethyl acetate extract contained 8.16mg/g while the acetone and methanol extracts contained 16.35 and 10.51 mg/g respectively (Figure 3). For *Hibiscus sabdariffa*, its petroleum ether extract contained 12.34 mg/g while its chloroform extract contained 18.00mg/g. The ethyl acetate extract contained 0.55 mg/g, and the acetone and methanol extracts contained 2.51 and 2.59mg/g of flavonoids respectively (Figure 3).

Phenols

All extracts of both plants contained varying amounts of phenols. For *Hibiscus sabdariffa*, the petroleum ether and chloroform extracts contained 3.38 mg/g and 6.72 mg/g of phenol respectively. The methanol extract contained 7.91 mg/g while the ethyl acetate and acetone extracts contained 6.00 mg/g and 7.27 mg/g respectively (Figure 4). For *Vernonia amygdalina*, the amount of phenol in the petroleum ether and chloroform extracts were 3.34 mg/g and 4.97 mg/g respectively. The methanol extract contained 7.35 mg/g of phenol while the ethyl acetate and acetone extracts contained 12.99 mg/g and 13.01 mg/g respectively (Figure 4).

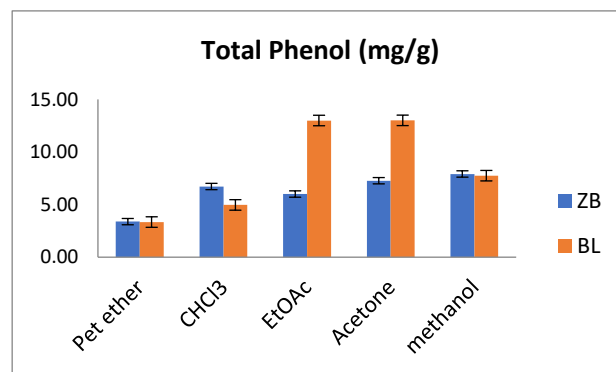


Figure 4. Phenol content of *V. amygdalina* (BL) and *H. sabdariffa* (ZB)

Antioxidant activity

All extracts of both plants showed antioxidant activity at varying degrees and concentrations (figures 4.5 – 4.13). For *Hibiscus sabdariffa*, the petroleum ether extract showed its highest antioxidant activity (59.35 %) at a concentration of 2 mg/ml while the chloroform extract showed its highest antioxidant activity (80.08 %) at a

concentration of 8 mg/ml. The ethyl acetate extract showed its highest antioxidant activity (88.13 %) at a concentration of 1 mg/ml while the acetone extract showed its highest activity (87.97 %) at a concentration of 0.125 mg/ml and the methanol extract showed its highest activity (90.57 %) at a concentration of 0.25 mg/ml.

For *Vernonia amygdalina*, the petroleum ether extract showed its highest antioxidant activity

(77.24 %) at a concentration of 0.5 mg/ml while the chloroform extract showed its highest activity (27.97 %) at a concentration of 0.5 mg/ml and the ethyl acetate extract showed its highest activity (86.18 %) at a concentration of 0.25 mg/ml. For the acetone extract, its highest antioxidant activity (89.11 %) was at a concentration of 0.125 mg/ml while the highest antioxidant activity of the methanol extract (89.43 %) was at a concentration of 0.5 mg/ml as shown in Figures 4.5 – 4.13.

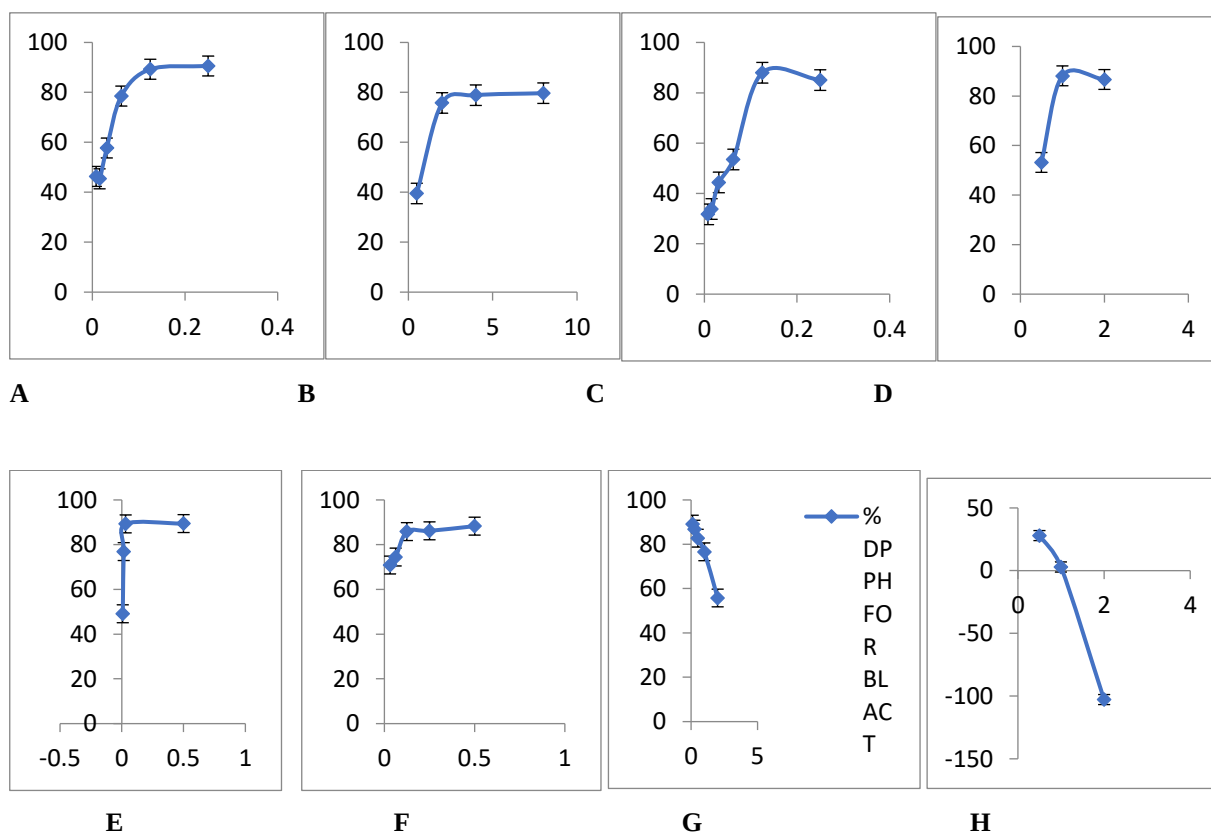


Figure 5. % DPPH scavenging activity of methanolic (A), chloroform (B), acetone (C), and ethyl acetate fractions (D) of *Hibiscus sabdariffa* and methanolic (E), ethyl acetate (F), acetone (G), chloroform (H) fractions of *Vernonia amygdalina*.

Thin layer chromatography

The number of spots and Rf values for the fractions are shown in table 4.7 below;

Plant	Extract	Number of spots	Rf values of spots
V.amygdalina	PET.Ether	2	0.22, 0.24
	CHCl ₃	3	0.30, 0.51, 0.68
	EtOAc	4	0.37, 0.45, 0.52, 0.59
	Acetone	5	0.20, 0.48, 0.52, 0.59, 0.65
	CH ₃ OH	2	0.47, 0.63
H.sabdariffa	PET.Ether		
	CHCl ₃	2	0.14, 0.56
	EtOAc	2	0.48, 0.56
	ACETONE	2	0.52, 0.64
	CH ₃ OH	2	0.56, 0.68

DISCUSSION

The methanolic fraction of both plants had the highest percentage yield, which implies that there contain more polar constituents as also observed in the results of the phytochemistry in which that of *V. amygdalina* contain saponins, steroids, alkaloids, cardiac glycosides and flavonoids while *H. sabdariffa* contains terpenoids, saponins and cardiac glycosides. Flavonoids and saponins are polyphenolic compounds which are very polar thus justifying the solubility and polarity of the fractions. This result is in line with that reported by Burda *et al.* (1994) who reported that *V. amygdalina* contains more polar constituents while Hong *et al.* (1990) also reported the presence of more polar constituents in for *H. sabdariffa*.

The result of preliminary phytochemical analysis of these plant extracts is in agreement with earlier literatures (Wong *et al.*, 2002) for *H. sabdariffa*, and for *V. amygdalina* (Burda *et al.*, 1994; Tona *et*

al., 2004) that these plants contain alkaloids, tannins, flavonoids, steroids, terpenoids, saponins and cardiac glycosides.

The presence of flavonoids found in both methanol fractions is likely responsible for the high free radical scavenging effects observed in both methanol fractions. Flavonoids are a major group of compounds that act as primary antioxidants or free radical scavengers (Polterait, 1997). This confirms the antioxidant activity of the plant extracts as reported by Winslow and Krol (1998).

The solubility of the petroleum ether fraction of *H. sabdariffa* in acetone and methanol shows that it contains the terpenoids and steroids with medium molecular weight while the solubility of the petroleum ether in acetone confirms the presence of flavonoids in the fraction. This result is in line with the report of Burda *et al* (1994). The chloroform fraction of *H. sabdariffa* was equally insoluble in water and petroleum ether but was soluble in other

solvents since it contains mostly alkaloids while that of *V. amygdalina* was soluble in all solvents except for petroleum ether and water. The ethyl acetate extract of *H. sabdariffa* was soluble in more polar solvents indicating presence of polar phytochemicals as reported by Wong *et al.* (2002) as it was insoluble in petroleum ether and chloroform.

Vernonia amygdalina contains the highest percentage of alkaloids compared to *Hibiscus sabdariffa* since most of the fractions contained alkaloids. This secondary metabolite has been associated with numerous physiological activities in mammalian cells in various studies (Sofowora, 1993). Many plants containing alkaloids and flavonoids have diuretic, analgesic, antispasmodic and anti-inflammatory effect (Owoeye *et al.*, 2002).

Saponins are polar compounds and thus cannot be found in non-polar solvents, thus explaining the absence of it in the petroleum ether and chloroform fractions of both plants. The ethyl acetate, acetone and methanol fractions of *H. sabdariffa* contains saponin. Only the methanol fraction of *Vernonia amygdalina* contains saponin. *Vernonia amygdalina* methanol fraction contains higher percent of saponin compared to that of *Hibiscus sabdariffa* but the difference is not statistically significant at $P < 0.05$. Saponin reduces the uptake of glucose and cholesterol at the gut through intra-luminal physicochemical interaction thus could confer a chemoprotection against heart diseases (Lewis *et al.*, 1995). Saponin also exhibit anti-inflammatory properties (Lewis *et al.*, 1995). Therefore, the methanol fraction of *Vernonia amygdalina* and the ethyl acetate, acetone and methanol fractions of *Hibiscus sabdariffa* could be

useful in protection of the heart against diseases and inflammation.

Vernonia amygdalina contains more flavonoids in all fractions than *Hibiscus sabdariffa*. The difference in concentration between the chloroform, ethyl acetate and acetone fractions of both plants is statistically significant at $P < 0.05$. The highest concentration was found in the chloroform fraction of both extracts. The ethyl acetate fractions in both extracts contains the least amount of flavonoid. Since the *Vernonia amygdalina* extracts contained larger amount of flavonoid compared to *Hibiscus sabdariffa*, it is a better source of flavonoid and mostly from the chloroform and petroleum ether fractions. Flavonoids in human diet may reduce the risk of various cancers as well as prevent menopausal symptoms (Ross and Kasum, 2002; Padayatty *et al.*, 2003). According to epidemiological studies, the consumption of flavonoid is effective in lowering the risk of coronary heart diseases (Rice Evans *et al.*, 1997), thus the chloroform and petroleum ether fractions of both plants could be useful in treating coronary heart disease and prevention of various forms of cancer.

The phenolic content of the ethyl acetate and acetone fractions of *Vernonia amygdalina* are higher than that of *Hibiscus sabdariffa* with the difference statistically significant at $P < 0.05$. This is justified by the presence of flavonoids in both fractions. Phenols are one of the major groups of non-nutritive dietary components that have been associated with the inhibition of cancer, atherosclerosis, as well as for age-related degenerative brain disorder (Chang *et al.*, 2006; Wang *et al.*, 2009). The high polyphenol content in

both plant fractions especially the ethyl acetate and acetone fractions of *Vernonia amygdalina* is beneficial to consumers' health as a potential source of natural antioxidant.

Amongst all the fractions, the methanolic fraction of *Hibiscus sabdariffa* showed the highest antioxidant activity which decreases with increase in concentration. The chloroform fractions of *Vernonia amygdalina* showed the minimum antioxidant activity which also decreases with increase in the concentration of the fractions. It was also observed that for majority of the fractions, the antioxidant activity decreased with a decrease in the concentration of the fractions.

The DPPH test provides information on the reactivity of test compounds with stable free radical. DPPH gives a strong absorption band at 517nm in the visible region (Ross and Kasum, 2002). When the odd electron becomes paired off in the presence of a free radical scavenger, the absorption reduces and the DPPH solution is decolourised as the colour changes from deep violet to light yellow. The degree of reduction in absorbance measurement is indicative of the radical scavenging power of the extract which is its antioxidant activity as observed in both plants (Ross and Kasum, 2002). Both plants exhibit potent antioxidant activity as they both possess high DPPH radical scavenging activity. This therefore implies that *Hibiscus sabdariffa* and *Vernonia amygdalina* may be useful in reducing the risk of various cancers

CONCLUSION

The result of the analysis of the calyx of *Hibiscus sabdariffa* and the leaf of *Vernonia amygdalina*

indicated that *Vernonia amygdalina* contained larger amounts of alkaloids, flavonoids and saponins in comparison with *Hibiscus sabdariffa* which on the other hand had higher saponin content and antioxidant activity.

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