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Comparative *in vitro* dpph radical scavenging and growth inhibitory potentials of fractions of the methanol extract of the leaves of *Securinega Virosa*

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Key words: Antioxidant, *Securinega virosa, DPPH, growth inhibition, radicle length,* aqueous fraction, chloroform fraction.

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Abstract

Securinega virosa is a medicinal plant that has found numerous applications in the treatment of several diseases in parts of Nigeria. The preliminary phytochemical, *in vitro* antioxidant and growth inhibitory assays of fractions of the methanol extracts of leaves of S. virosa were carried out. The methanol extract obtained by maceration was fractionated into aqueous and chloroform phases and the active aqueous fraction were subsequently fractionated by vacuum liquid chromatography. All fractions were screened phytochemically and assayed for antioxidant (0.1-5 mg/mL) using 2, 2-diphenyl-1-picrylhydrazyl and growth inhibitory activity (1-30 mg/mL) using guinea corn seed radicle inhibition assay. Phytochemical screening of the fractions showed the abundance of alkaloids, phenolic compounds, flavonoids, saponins, terpenes, cardiac glycoside. The aqueous fraction was observed to exhibit a higher antioxidant activity over the chloroform faction as 78 and 33 % radical scavenging activity was recorded for the former and latter respectively at 5 mg/mL. Similar activities were also recorded for the aqueous faction in the growth inhibitory assay. The vacuum liquid chromatographic fractionations of the aqueous fraction further enhanced the antioxidant and growth inhibitory activities as the bulked sub-fraction AQF (4-7) which was the most active, recorded 88.86 % RSA and 100 % growth inhibition at 2 and 10 mg/mL respectively. The result has shown that the aqueous fraction and its vlc sub-fraction AQ (4-7) have high antioxidant and growth inhibitory properties .This could explain why the plant especially the leavesare used traditionally in treating cancer and other chronic diseases.

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Introduction

The human body consumes unestimated amounts of oxygen for the metabolism of biomolecules in order to produce energy. Although oxygen is essential for life, but its metabolites often referred to as reactive oxygen species (ROS) or free radicals which causes several ill health conditionssuch as AIDS and cancer (Kumpulainen and Salonen, 1999; Pourmorad *et al*., 2006). Antioxidants are radical scavengers which guard the human body against free radicals which may cause disorders in man.

The antioxidant potentials of medicinal plants is a reflection of certain phytochemicals it contains e.g phenolic, flavonoids. Research has shown that synthetic antioxidants have several negative health effects on man (Abramovic and Abram, 2006; Kowalski, 2007; Azizkhani and Zandi, 2009), hence the need for natural antioxidants with little or no side effects has become necessary.In the search for novel antioxidant for the formulation of drugs, several traditionally known antioxidant plants such as vegetables, spices and fruits have been screened (Koleva *et al*., 2002; Oke and Hamburger, 2002).

However, there is need for more of this screening exercise to be carried to ascertain the antioxidant potentials of more useful plants.

The plant S*ecurinega virosa* which is nicknamed "cure all" in Nigeria because of the medicinal value of all its parts, belongs to the Euphorbiaceae family . It is a shrub widely distributed in tropical Africa. In the three major languages of Nigeria(Hausas, Yorubas and Ibos), the plant is referred to as "tsuwaawun karee", "iranje" and "njisinta" respectively (Neuwinger, 1996).

Some ethno medicinal uses of this plant especially the leaves, includes the treatment of abdominal pain, dysmenorrhea, diarrhoea , stomach ache, rheumatism, pneumonia, epilepsy (Neuwinger, 1996), veneral disease (Dalziel, 1936) and cancer (Soladoye *et al*., 2010). Although, the antioxidant activities of the leaf extract of *S. virosa* has been reported (Uzama *et al*, 2013; Dickson *et al*, 2006), there is however no reports on the antioxidant and growth inhibitory activities of fractions of *S. virosa*.

This study is therefore aimed atreporting for the first time, the radical scavenging and growth inhibitory effects of the partitioned and vaccum liquid chromatographic fractions of the methanol extract of the leaves of *S. virosa*.

Materials and methods

Collection, processing and extraction of plant material

The leaves of *S.virosa* collected in November, 2017 were processed, air dried for 3 days and later dried in the oven maintained at 40°C after which it was grounded to powder form using a laboratory electric milling machine (Chris Norris, England).

About 1.0kg of the powdered sample were extracted by cold maceration using 90 % methanol The total methanol extract was concentrated to dryness under vacuum at 40°C using rotary evaporator (Eyela, Tokyo Rikikai Co. Ltd, Japan).

Partitioning of the methanol extract of S.virosa

About 50g of the crude methanol residue were re-dissolved in methanol-water (1:1) and fractionated exhaustively with chloroform (200 mL ×4) volumes in a separating funnel. The chloroform layer (lower) was collected at intervals, followed by the aqueous fraction. This was repeated until a clear lower layer was obtained. The aqueous and the chloroform fractions were concentrated to dryness on a rotary evaporator and their respective yields noted.

Determination of radical scavenging activity of the aqueous and chloroform fractions of the methanol extract of leaves of S.virosausing DPPH radical scavenging assay

This was carried out using methods previously described by Liyana-Pathiranan and Shahidi, (2005) with little modification in concentrations (0.1-5.0 mg/mL). This procedure was carried out in triplicate and was repeated for the vlc factions (0.1-1 mg/mL).

Determination of antiproliferative effects of the aqueous and chloroform fractions of S.virosa using seeds of Sorghum bicolor

These were carried out using standard methods earlier described by Ayinde *et al*, 2010 and Ikpefan *et al*, 2013. The antiproliferative test were carried out at concentrations between 1-30mg/mL for the aqueous and chloroform fractions.

The experiment was repeated for the subsequent vacuum liquid chromatographic fractions all in triplicate (1-10 mg/mL).

Vacuum liquid chromatography (VLC) of the chloroform fraction

About 20.5g of the aqueous fraction was subjected to vacuum liquid chromatography with eluting 300 mL eluting solvents of $CHCl_3$ (100 %), $CHCl_3$-$CH_3COOC_2H_5$ (1:1, 1:3), $CH_3COOC_2H_5$ (100 %), and $CH_3COOC_2H_5$-CH_3OH (3:1, 1:1, 1:3), CH_3OH (100 %) and CH_3OH (3:1) Analytical thin layer chromatographic analyses of the nine (9) vacuum Liquid Chromatographic fractions of the aqueous fraction was carried out on a pre-coated aluminum plate of Silica gel GF_{254} using $CHCl_3$-CH_3OH (4:2). After development, the plates were sprayed with concentrated H_2SO4 and subsequently heated for 5min at 110°C. The colour spots were noted and their R_f values were recorded. Fractions were bulked into three AQ(1-3), AQ(4-7), and AQ(8-9) based on similar tlc profile. The bulked fractions were further subjected to antioxidant radical scavenging and growth inhibitory test.

Preliminary phytochemical screening

Phytochemical screening of the aqueous and chloroform fractions were carried out using standard methods previously described by Khandelwal (2006). The bulked vlc fractions from the aqueous fraction were also screened.

Determination of radical scavenging and growth inhibitory activitiesof the bulked vacuum liquid chromatographic sub-fractions obtained from the aqueous fraction

These were carried out using methods earlier described above using concentrations of 0.1-2.0 mg/mL and 1-10 mg/mL respectively.

Statistical analysis

The data's obtained were expressed as Mean ± SEM and one-way Analysis of Variance ANOVA statistical test using Graph Pad Instat R version 2.17 (UK) was used to test for significance. $P< 0.05$ was considered Significant.

Results

Results of the radical scavenging activity of the aqueous and chloroform fractions of the methanol extract of the leaves of *securinega virosa* on guinea corn radicle.

Table 1. Results of the phytochemical screening of the partitioned and bulked vacuum liquid chromatographic fractions of the aqueous fraction.

	Partitioned fractions		Bulked vacuum liquid chromatographic sub-fractions of the aqueous fraction		
Phytochemical groups	Aqueous fraction	Chloroform fraction	AQ (1-3)	AQ (4-7)	AQ (8-9)
Alkaloids	++	-	-	+	+
Anthraquinones	-	-	-	-	-
Tannins/Phenolic compounds	+++	-	-	++	+
Flavonoids	+	-	-	+	-
Saponins	+	-		+	+
Steroids	-	++	+	-	-
Terpenes	+	+++	+	+	-

Key: +++: appreciable amount; ++: moderate amount; + : minute amounts; - : not detected.

The results of the DPPH radical scavenging ability showed that the aqueous fraction had a higher DPPH scavenging ability over the chloroform fraction in a concentration dependent manner. At the 2 and 5 mg/mL, the chloroform fraction exhibited 18 and 33 % scavenging ability while the aqueous fraction gave 53 and 78 % at the same concentrations (Figure 1).

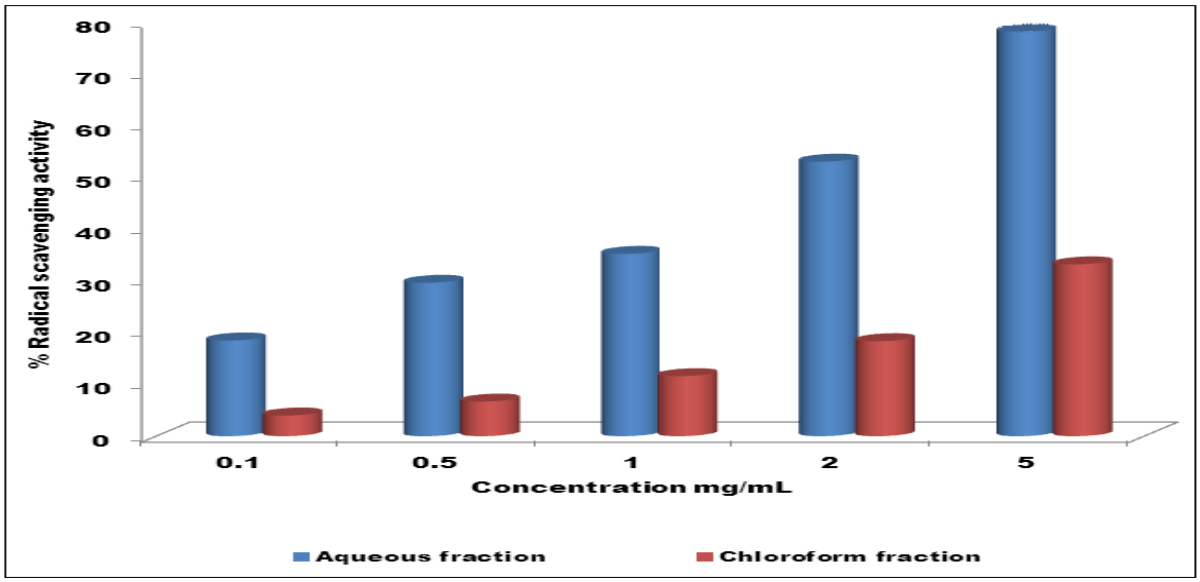

Fig. 1.DPPH-radical scavenging potential of the aqueous and chloroform fractions of the methanol extract of leaves of *S. virosa*. Values are Mean ± S.E.M, n = 3.

Results of the effects of the aqueous and chloroform fractions on germinating seed radicle length

Both fractions where observed to produce a concentration dependent effects on radicle length of seeds of *Sorghum bicolor*. At 24 h, the chloroform fraction at 1 mg/mL recorded a radicle length of 7.90 mm compared to 9.08 mm produced by the control seeds which implies about 12 % reduction in radicle length. This reduction in radicle length continues up to 96 h were radicle lengths of 9.53 and 5.47 mm were recorded at 20 and 30 mg/mL against 54.90 mm produced by the control seeds which implies 83 and 90 % radicle length reduction respectively (Figure 2).

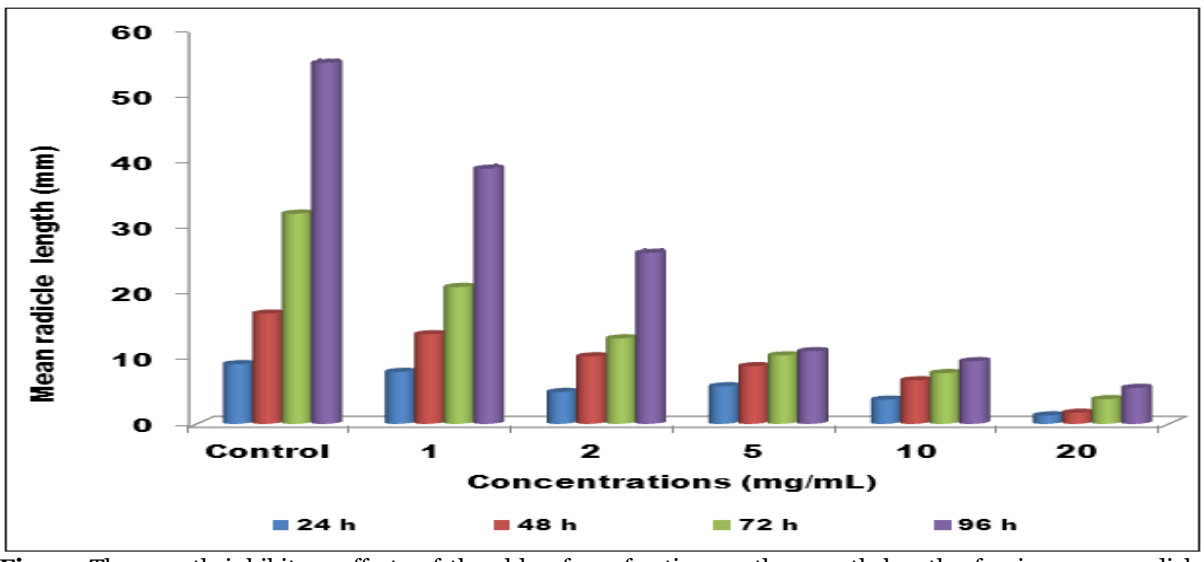

Fig. 2. The growth inhibitory effects of the chloroform fraction on the growth length of guinea corn radicle length. Values are Mean ± S.E.M, (n = 20).

Similar trend of results were recorded for the aqueous fraction but a higher radicle length reduction were produced when compared to the chloroform fraction. At the end of 96 h ,87 and 95 % radicle reduction were recorded at 20 and 30 mg/mL which is about 4 and 5 % higher than the chloroform fraction (Figure 3).

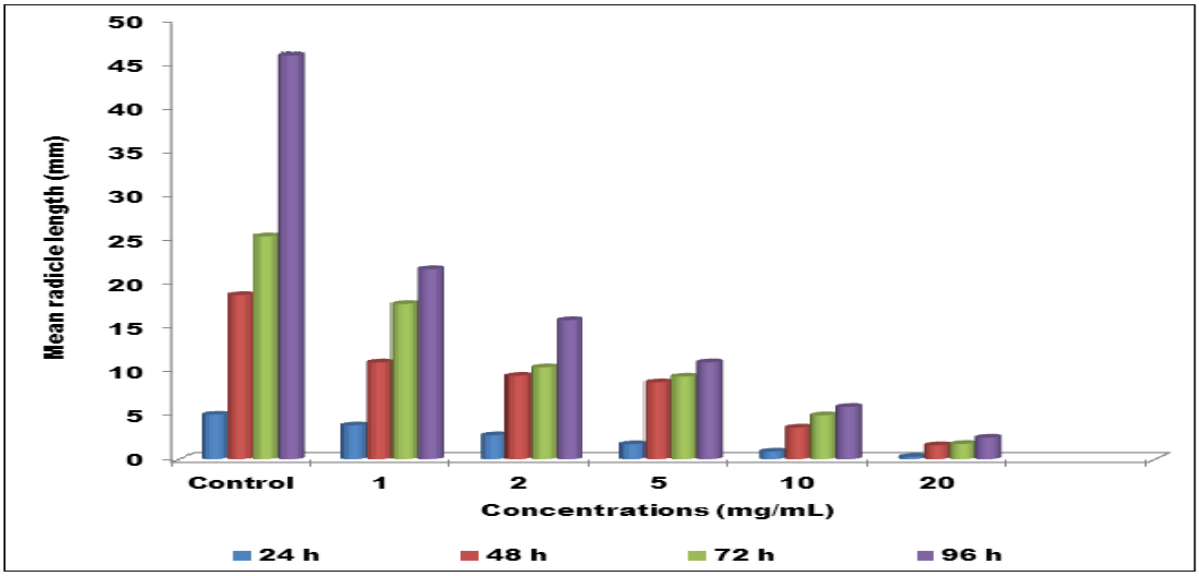

Fig. 3. The growth inhibitory effects of the aqueous fraction on the growth length of guinea corn radicle length. Values are Mean ± S.E.M, n = 20.

Results of the DPPH radical scavenging activities of the bulked VLC factions obtained from the aqueous

TheDPPHradical scavenging effects of the bulked vacuum liquid chromatographic fractions were also observed toincreased withincrease in the concentrations in all the fractions.The vlc sub-fraction AQ (4-7) gave the highest activity as it recorded 88.86 % radical scavenging activity at 2 mg/mL which is about 5 % than that of the control (garlic acid) which gave 93.13 %. However, bulked fraction AQ (1-3) and AQ (8-9) gave 25.40 and 43.05 % respectively at the same concentration. (Figure 4).

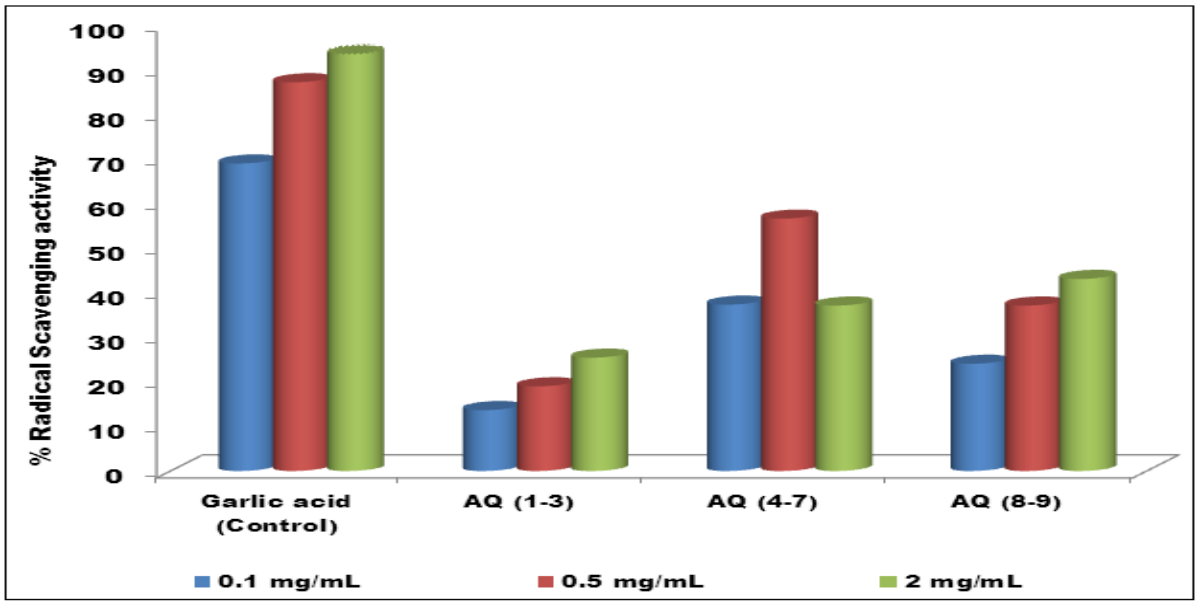

Fig. 4.*In vitro*DPPH radical scavenging activities of the bulked vacuum liquid chromatographic fractions obtained from the aqueous fraction *S.virosa*.

Result of the growth inhibitory effects of the vlc sub-fractions on radicle length of S.bicolor

There was also a marked in the growth inhibitory effects with increased in concentrations as earlier observed in the results of the aqueous and chloroform fractions. The control seeds at 24 and 96 h recorded 12.33 and 48.96 mm while seeds treated with 10 mg/mL of the bulked fractions AQ (1-3) and AQ (8-9) gave 4.80 and 18.72 mm, 3.10 and 10.53 mm which imply 61, 62, 74 and 78 % radicle length reduction respectively. However, at similar concentration, bulked fraction AQ (8-9) inhibited seed germination (100 %) throughout the entire period of the experiment (Figure 5).

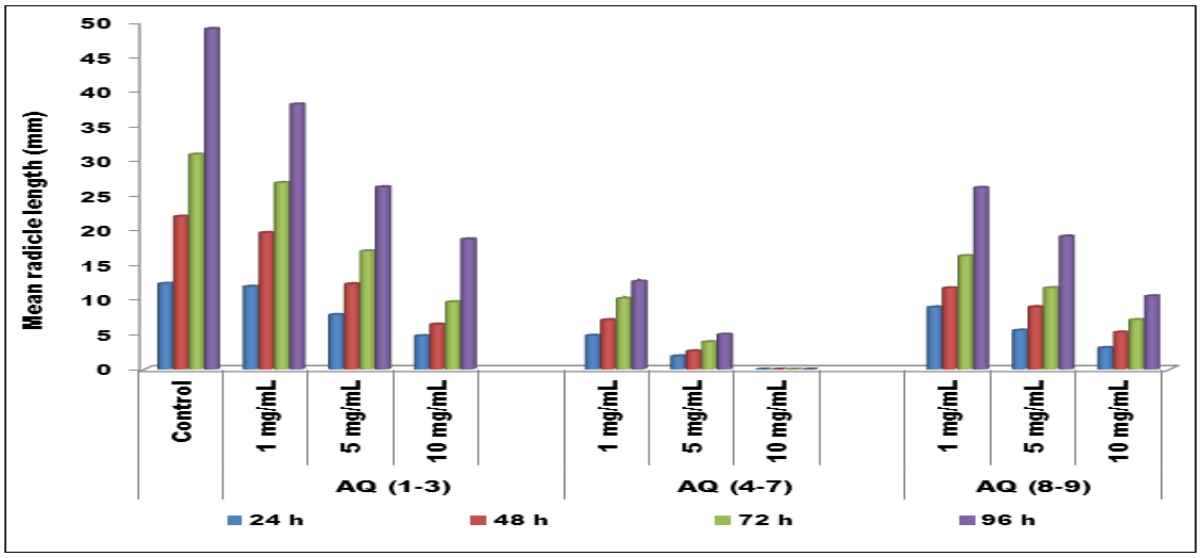

Fig. 5. The growth inhibitory effects of the vlc sub-fraction of the aqueous fractionon the growth length of guinea corn radicle length. Values are Mean ± S.E.M, n = 20.

Discussion

For many centuries now, medicinal plants still maintain their medicinal value which could be as a result of the secondary metabolites they contain. Majority of medicinal plant serves as reservoir of natural antioxidant which helps to prevent the occurrence of many diseases including cancer.

Although there are many antioxidant assays, DPPH assay which was developed by Blois and modified by Brand-Williams *et al*, 1995, is the most popular and frequently used because of its simplicity, efficiency, rapidness and relatively cheap. The growth inhibitory assay like other bench-top assay methods is reproducible, simple and inexpensive. Also, it can be used to monitor extracts, fractions, and compounds with probable herbicidal, alleopathic or antitumor properties (McLaughlin, 1991). The choice of *Sorghum bicolor* seeds for this experiment is due to their rapid growth (24 h), unlike other seeds. Their rapid growth is likened to those of cancerous cell. The inhibition of seed growth by extracts, fractions or pure compounds could be as a result of the interference of some cell division process, hence resulting in a reduction in mitotic index (Celik and Aslanturk, 2010).

The methanol extract of the leaf of *S.virosa* was selected for partitioning because of the earlier result recorded in a previous work where it exhibited a higher cytotoxic and growth inhibitory effect over the root bark (Ikpefan *et al*, 2013).

The higher DPPH-radical scavenging and growth inhibitory activity exhibited by the aqueous fraction over chloroform fraction was further enhanced by the vacuum liquid chromatographic purification. For example, the aqueous fraction at 2 mg/mL gave 53.01 % radical scavenging activity which was increased to 88.86 % recorded by sub-fraction AQ (4-7) at the same concentration. Similarly, while the aqueous fraction recorded an average radicle length of 5.97mm

at the end of 96 h, its bulked vlc sub-fraction totally inhibited the germination of the seed by 100 % at the same concentrations.

The higher radical scavenging and growth inhibitory activity recorded by the aqueous fraction and its sub-fraction of *S. virosa* could be due to the presence of phenolic compounds which are known for their ability to attack diseases causing free radicals and inhibition of carcinogenesis (Zheng and Wang, 2001; Miliauskas *et al.*, 2004). Phytochemical screening of the fractions revealed the presence of various phytochemicals including tannins and flavonoids which are known to possess free radicals scavenging activity, hence prevent the development diseases. The aqueous fraction and its vlc sub-fraction AQ (4-7) were observed to possess more of these phytochemicals.
The results obtained from this work have further validated the ethnomedicinal use of this plant in treating various forms of diseases. The next phase of this work will focus more on the isolation and characterization of the active constituents from the active vlc bulked fraction.

References

Abramovic H, Abram V. 2006. Effect of added rosemary extract on oxidative stability of Camelina sativa oil. Acta Agriculturae Slovenica **87,** 255-261.

Ayinde BA, Omogbai EKI, Ikpefan EO. 2011.Comparative cytotoxic and antiproliferative effects of persea americana mill (lauraceae) leaf, stem and root barks. Nigerian Journal of Pharmaceutical Sciences**10,** 16 –26.

Azizkhani M, Zandi P. 2009. Effects of some natural antioxidants mixtures on margarine stability. World Academy of Science, Engineering and Technology**49,**93-96.

Blois MS. 1958.Antioxidant determinations by the use of a stable free radical. Nature**26,**1199–1200.

Brand-Williams W, Cuvelier ME, Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. Lebenson Wiss Technol. **28,** 25–30.

Celik TA, Aslantürk OS. 2010. Evaluation of cytotoxicity and genotoxicity of Inula viscosa leaf extracts with Allium test. Journal of Biomedical Biotechnology. 10, 189-252.

Uzama D, Bwai MD, Orijajogun OY, Olajide O, Sunday AT. 2013. The antioxidant potentials and phytochemical properties of the Hexane, Ethyl acetate and Ethanolic extracts of Securinega virosa (Euphorbiaceae) leaves. Journal of AppliedPharmaceuticalScience. **3(05),** 131–135.

Dalziel JM. 1936. The useful plants of West Tropical Africa. Watmonghs, Idle, London, 354-355 p.

Dickson RA, Houghton PJ, Hylands PJ, Gibbons S. 2006. Antimicrobial, resistance-modifying effects, antioxidant and free radical scavenging activities of Mezoneuron benthamianum Baill., Securinega virosa Roxb. & Willd. and Microglossa pyrifolia Lam. Phytotherapy Research **20(1),** 41–45.

Ikpefan EO, Ayinde BA, Gita TD. 2013. In vitro comparative cytotoxic and growth inhibitory effects of the methanol extracts of the leaf, stem and root barks of Cnidoscolus acontifolius(Mill.) Johnst (Euphorbiaceae). International Journal Bioassays **2(2),** 445-449.

Liyana-Pathiranan CM, Shahidi F. 2005. Antioxidant activity of commercial soft and hard wheat (Triticum aestivum L) as affected by gastric pH conditions. Journal of Agriculturaland Food Chemistry**53,** 2433–2440.

Khandelwal KR. 2006. Preliminary Phytochemical screening.Practical Pharmacognosy, 6th ed. Pune, India: Nirali Prakashan, 149-539.

Koleva II, Van Beek TA, Linssen JPH, de Groot A, Evstatieva LN. 2002. Screening of plant

extracts for antioxidant activity: a comparative study on three testing methods. Phytochemical Analysis, **13,** 8–17.

Kowalski R. 2007. GC analysis of changes in the fatty acid composition of sunflower and olive oils heated with quercetin, caffeic acid, protocatechuic acid and butylated hydroxyl-anisole. Acta Chromatographica**18,** 15-23.

Kumpulainen JT, Salonen JT. 1999. Natural Antioxidants and Ant carcinogens in Nutrition, Health and Disease. UK: The Royal Society of Chemistry; 178–187 p.

McLaughlin JL. 1991. Crown gall tumors on potato discs and brine shrimp lethality: Two single bioassays for plant screening and fractionation. In: Hostettmann K, editor. Methods in Plant Biochemistry.**6,** London: Academic Press. 1–31 p.

Miliauskas G, Yenkutonis PR, Van Beek TA. 2004. Screening of radical scavenging activity of some medicinal and aromatic plants extracts. Food Chemistry**85,** 231–237.

Neuwinger JD. 1996. Translated by Porter A. African ethno botany poison and drugs. Chapman and Hall, Weinhelm, 495-499 p.

Oke JM, Hamburger MO. 2002. Screening of some Nigerian medicinal plants for antioxidant activity using 2, 2-diphenyl- picryl- hydrazylradical. African Journal of Biomedical Research**5,** 77–79.

Pourmorad F, Hosseinimehr SJ, Shahabimajd N. 2006. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. African Journal Biotechnology **11,**1142–1145.

Soladoye MO, Amusa NA, Raji-Esan SO, Chukwuma EC, Taiwo AA. 2010. Ethnobotanical survey of anti-cancer plants in Ogun State, Nigeria. Annals of Biological Research1**(4),** 261-273.

Zheng W, Wang SY. 2001. Antioxidant activity and phenolic compounds in selected herbs. Journal of Agricultural and Food Chemistry**49(11),**5165-5170.