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Phytochemistry and antioxidant activity of *Amaranthus viridis* L (Green leaf)

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Abstract

Phytochemistry and antioxidant activity of *Amaranthus viridis* L (Green leaf) were undertaken with standard methods. The leaf sample after being screening for phytochemicals, contained tannins, saponins, flavonoids, alkaloids, steroids, phenolic compounds, proteins and anthraquinones at different concentrations. A further study using Gas Chromatography-Mass Spectrophotometry (GC-MS) analytic method on the leaf sample revealed a total of twenty-two detailed compounds among which are 3-Hydroxy-N-methylphenethylamine, Erucic acid, n-Hexadecanoic acid, 1,2-Propanediol,3-chloro-, and Cystamine. 3-Hydroxy-N-methylphenethy lamine had the highest retention time; Erucic acid had the highest molecular weight while n-Hexadecanoic acid had the highest peal area. Most of the compounds have important applications in body care products and cosmetics, pharmaceutical or food industries. *A. viridis* leaf extract exhibited a better antioxidant activity against the ascorbic acid used as control in the present study. The phytochemicals present in the leaf sample could be behind its antioxidant activity. The study has shown the *phytochemistry and antioxidant activity of A. viridis* leaf sample.

Keywords: Antioxidant activity; *Amaranthus viridis;* Phytochemistry; GC-MS analysis

1. Introduction

Leafy vegetables have played crucial roles in complementing diets for humans and animals [1-7]. Edible vegetables are embodiment of nutritional materials for the body. Vegetables also contribute significant fiber to human diet. In recent years, studies have shown that fiber has the capacity to lower cholesterol and also strengthen the passage of bowels through the body. Apart from the contributions of vegetables in the nutrition of humans and animals, they are also employed in traditional medicine [8-14]. They are among the major raw materials of traditional medicine practice [15-33]. Different authors have defined traditional medicine in different perspectives. However, each perspective inculcated the use of medicinal plants as core raw materials [15, 17, 20, 25, 29-30, 34-37]. Studies have revealed the ameliorative potential of some vegetables against some disease conditions [20, 25, 38-39]. It has been reported that these vegetables contain bioactive compounds which are physiologically active against disease causing pathogens [35-41]. These compounds have been collectively known as phytochemicals [15, 28, 34-37]. Further details on phytochemicals have revealed molecules that possess the potential to ameliorate, cure or prevent some disease conditions [35-43]. It has also been reported that some of the phytochemical molecules form the basis for modern pharmacopeia as synthesized model of compounds [44].

Amaranthus viridis L. popularly called green in Nigeria, which belongs to Amaranthaceae family is among the vegetables employed in traditional medicine. It is distributed in the warmer climates of the world [8, 10, 32]. The plant is believed to have originated from Asia but it is sometimes addressed as a cosmopolitan weed found in temperate as well as

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tropical and subtropical part of the world [8, 32-33]. In African, *A. viridis* is cultivated deliberately for use [8, 10-11, 33] and can also be found as weed [33]. The plant is described as ascending annual or short-lived perennial herb that grows up to 1 m tall. It has alternate leaves which possess petiole and can grow up to 10 cm [8, 32]. The leaves of *A. viridis* are glabrous and have light green lower surface and dark green upper surface. Lessen of *A. viridis is* used against labour pain and as antipyretic [15, 34]. The leaves of the plant are used against rashes, psoriasis, and eczema. The fruits, leaves, and flowers of *A. viridis* serve as vermifuge in veneral diseases, antiulcer, analgestic, laxative, as an antileprotic, as anti-inflammatory agents of urinary tract, against high blood sugar as well as against high cholesterol [35-37].

Studies have revealed that some leafy vegetables are also effective in facilitating the molecules that act as body defense mechanisms [14, 45]. These molecules are called antioxidants and they counter the activities of reactive oxygen species [45-46]. Excessive production of reactive oxygen species against antioxidants results in a condition known as oxidative stress. Oxidative stress has been recognized as the root of most generative diseases of the body [45-48]. Antioxidants that act as defense mechanisms are major cellular redox buffers ascorbate and glutathione (γ-glutamyl-cysteinylglycine, GSH) as well as tocopherol, carotenoids, and phenolic compounds; and enzymatic components such as superoxide dismutase, catalase, guaiacol peroxidase, enzymes of ascorbate-glutathione cycle ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase [47-49].

This study investigated the detailed phytochemistry and antioxidant activity of *A. viridis* L using its leaf sample.

2. Material and methods

2.1. Sample Collection and Identification

Leaves of *A. viridis* used for the present study were harvested from the plant in Imo State University School Farm and identified by a Botanist in the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. They were washed, dried in the sun for few weeks before they were milled and stored for further studies.

2.2. Preparation of Extract

The method as described by Ezekwe *et al*. [50] was used in the preparation of aqueous extract. Ten grams of the milled sample was extracted by maceration in 50 mL of water for three days with frequent agitation at a speed of 280 rpm at 28°C in the dark. The sample was then centrifuged for 10 minutes at 2000 rpm. The combined supernatants were collected, filtered through Whatman No 1 filter paper and was then concentrated. The concentrate was placed in a vacuum desiccator for complete removal of solvent. The yield extract was thus used for some of phytochemical screening, gas chromatography-mass spectrophotometry (GC-MS) analysis and assessment of antioxidant activity.

2.3. Qualitative phytochemical Screening

Tannins, phlobatannins, alkaloids, saponins, flavonoids, steroids, terpernoids, cardiac glycosides, phenolic componunds, proteins, redcing sugars, and anthraquinnones were screened on the leaf extract.

2.4. Test for tannin

The method as described by Amadi *et al*. [51] was used for the screening tannins. Two millilitres (2 mL) of extract was mixed with an equal volume of bromine water. The formation of greenish to red precipitates was taken as positive result for the presence of tannins.

2.5. Test for phlobatannins

The method as described by Amadi *et al*. [51] was used for the phytochemical screening of phlobatannins. Five millilitres (5 mL) of the extract was added to 2.0 mL of 1% Hydrochloric acid (HCl), and red precipitate was then as evidence for the presence of phlobotannins.

2.6. Test for saponins

The method as described by Amadi *et al*. [51] was used for the screening of saponins. Two drops of olive oil was added to 1 mL of the extract and shaken vigorously. The formation of emulsion was taken as positive result for saponins.

2.7. Test for flavonoids

The method as described by Amadi *et al*. [51] was used for the screening of flavonoids. One millilitre (1 mL) of the extract was mixed with an equal volume of sodium hydroxide (NaOH) and shaken properly. The formation of precipitate was taken as positive result for flavonoids.

2.8. Test for alkaloids

The method as described by Amadi *et al*. [51] was used for the screening of alkaloids. One millilitre of extract was shaken with 5 mL of 2% hydrochloric acid on a steam bath and was the filtered. The filtrate was then treated with Meyer`s reagent. A cream coloured precipitate was taken as positive result for alkaloids.

2.9. Test for steroid

Salkowski test was used for the screening of steriods. 1 mL of the extract was dissolved in 2.0 mL of chloroform in a test tube. Through one side of the test tube, 1 mL of concentrated H_2SO_4 was carefully added. A reddish brown colour at the interphase was taken as positive test for steroid nucleus.

2.10. Test for terpenoids

One gram of seed sample was shaken in a test tube with 10 mL of methanol, and then filtered. 5 mL extract was then mixed with 2 mL of chloroform and 3 mL of sulphuric acid was added. Formation of reddish brown color indicates the presence of terpenoids in the selected plants.

2.11. Cardiac glycosides

Half gram of sample was dissolved in 1.0 mL pyridine. 5 drops of 2% sodium nitroprusside and 3 drops of 20% NaOH were added. The presence of deep red colour which fades to brown yellow was taken as positive result for cardiac glycosides.

2.12. Test for phenolic compounds

Phloroglucinol test was used.One percent of $FeCl_3$ was added to 2 mL of the extract and the presence of blue, violet, purple, green or red-brown colour was taken as positive result for phenolic compounds.

2.13. Test for proteins

Four millilitres of the extract was mixed with 5.0 mL of distilled water and was allowed to stay for 3 hours, and was then boiled. 2 mL of the boiled extract was then added to 01 mL of million`s reagent and shaken vigorously. A pinkish precipitate was taken as positive test for protein.

2.14. Test for anthroquinones

One gram of the extract was placed in a dry test tube and 20 mL of chloroform was added. This was heated in steam bath for 5 min. The extract was filtered while hot and allowed to cool. 2 mL of the filtrate was nixed with equal volume of 10% ammonia solution and shaken vigorously. The presence of brick pink colouration was taken as positive result for anthraquinones.

2.15. Gas Chromatography-Mass Spectrophotometry (GC-MS) Analysis of the Extracts

The method as described by Ezekwe *et al*. [50] was for GC-MS. GC-MS analysis of the aqueous extracts was carried out using AOC-20i auto sampler and gas chromatograph interface to a mass spectrometer (GC-MS) instrument. Employing the following conditions; column Elite-1 fused silica capillary column (30 mm×0.25 mm ID×1µM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml/ min, and an injection volume of 0.5µl, Split ratio of 10:1), with injector temperature 250°C; and ion-source temperature 280°C. The oven temperature was programmed from 110°C (Isothermal for 2 min), with an increase of 10°C/min to 200°C, then 5°C/min to 280°C, ending with a 9 mins isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450Da. Total GC running time was 36 mins. The plant extract was dissolved in aqueous and filtered with polymeric solid phase extraction (SPE) column and analyzed in GC-MS for different components.Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62 000 patterns. The spectrum of the unknowncomponents was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test were ascertained.

2.16. Determination of Antioxidant Activity

DPPH (1, 1-Dipheny l-2-picrylhydrazyl) radical scavenging assay: This was carried out with the method of Brand-Williams *et al.* [52], following the procedure as described by Ezekwe *et al.* [50]. The free radical scavenging activity was measured by DPPH assay method. Four mg of DPPH (0.1 mM) was dissolved in 100 mL of distill water to obtain working solution. One mL of each extract was mixed separately with 2.0 mL of 0.1 mM DPPH followed by 30 min incubation in dark. The reduction of the DPPH free radical was measured by taking the absorbance at 517 nm. Colour of DPPH was reduced from purple to yellow. The antioxidant activity of each extracts was evaluated by calculating the inhibition % of free radical formation using the formula:

% inhibition = $[(A-A_1)/A] \times 100$; A= absorbance of the blank (DPPH); A1= absorbance of the extract (DPPH+ extract).

3. Results and discussion

Table 1 Phytochemical screening of *A. viridis* leaf extract

Phytochemicals	Status
Tannins	++
Phlobatannins	-
Saponins	++
Flavonoids	+
Alkaloids	+
Steroids	+
Terpenoids	-
Cardiac glycosides	-
Phenolic compounds	+
Proteins	+
Reducing sugars	--
Anthraquinones	+

+=Present in low concentration;++= present in high concentration; and - = absent.

Phytochemicals screened in the presents study include tannins, phlobatannins, saponins, flavonoids, alkaloids, steroids, terpenoids, cardiac glycosides, phenolic compounds, proteins, reducing sugars and anthraquinones. From Table 1, the concentrations of tannins and saponins were high, while the concentrations of flavonoids, alkaloids, steroids, phenolic compounds, proteins and anthraquinones were low. Phlobatannins, terpenoids, cardiac glycosides, and reducing sugars were not found in *A. viridis* leaf extract. The phytoactivity and physiological effects of some of these phytochemicals have been reported by different authors. Tannins have been reported to possess anti-inflammatory, antioxidant, analgesic as well as wound healing properties [53-54]. Saponins are known to possess bitter taste, and are foamy in nature [55]. Saponins have reported to possess antibacterial properties [55-56]. Dietary flavonoids have been reported to oppose coronary heart disease [57]. Alkaloids possess reductive effect towards fever and headache [55]. They also possess analgesic and antibacterial properties. Steroids form the nucleus of all the steroid hormones and are involved as precursors to those hormones. The insecticidal property of most phenolic compounds has been reported by different authors.The role of proteins in body building and energy generation has been reported [12]. Proteins also serve precursor for growth of microorganisms [58]. Anthraquinones are known to repel birds. They are also useful in pulp, textile and dye industries [54, 58].

A more detailed study of the compounds found in *A.viridis* leaf extract using GC-MS analysis (Table 2), revealed a total of twenty-two compounds with their retention time, molecular formula, molecular weight, and peak areas (as shown in the Chromatograph of Figure 1). 3-Hydroxy-N-methylphenethylamine had the highest retention time; Erucic acid had the highest molecular weight while n-Hexadecanoic acid had the highest peal area. Some of these compounds are particularly important when their uses and effects in the biological system are considered. 1,2-Propanediol, 3-chloro- is an industrial important compound used in chemical productions. It could also irritate the mucous membrane, skin and eye. The compound is suspected to be a human carcinogenic compound. Cystamine is a tranglutaminases inhibitor and a symmetric organodisulphide [59]. It has a radioprotective effect and displays synergistic effects in combination with cysteine [60-61]. Paul and Snyder [62] reported the therapeutic application of Cysteamine and Cystamine in

neurodegenerative and neuropsychiatric diseases. 8-Nonen-2-one is a class of flavoring agent. Hexadecanoic acid methyl ester has applications in food, syrups and perfume industries. n-Hexadecanoic acid is among the fatty acids that can be found naturally in both plants and animals. It can also be created artificially in the laboratory. It has wide application in personal care products and cosmetics. Phytol is a diterpenoid natural compound with pharmacological applications [63]. It is a chlorophyll component with anti-inflammatory and metabolic properties. Phytol also has a remedy effect on the central nervous system [63]. Tetradecanoic acid has surfactant properties and is applied the beauty and cosmetic industries. cis-11-Eicosenoic acid has engine, pharmaceutical and cosmetic applications. Erucic acid is a monounsaturated omega-9 fatty acid, which could result in a heart problem called myocardial lipidosis, over on long exposure.

Antioxidant studies as presented in Figure 2, showed percentage inhibitions of the *A. viridis* leaf extract taken at 10, 20, 30 and 40 mg/L concentrations as 4.98%, 18.44%, 25.01%, and 444.26%. These inhibitions were against 1.12%, 15.73%, 23.60% and 40.45% observed for the ascorbic acid used as control. In generality, *A. viridis* leaf exhibited better antioxidant activity than the control in the present study and could possess antioxidant potential. Plants with antioxidant activity have been reported by different authors [14, 64].

Table 2 GC-MS of *A.viridis* leaf extract

S/N	RT	Name of Compound	Molecular Formula	MW	Peak Area %
1	2.435	1,2-Propanediol, 3-chloro-	$C_3H_7ClO_2$	110	4.84
2	2.611	N-Dimethylaminomethyl-N-methylformamide	$C_5H_{12}N_2O$	116	0.85
3	3.820	Cystamine	$C_4H_{12}N_2S_2$	152	2.80
4	4.510	Benzeneethanamine	$C_8H_{11}N$	121	2.02
5	5.339	Cyclobutane, methylene-	C_5H_8	68	1.21
6	8.019	Spirio-10-(2,11-dioxabicyclo [4.4.1]undeca-3,5-diene)-2'-(oxirane), 1,3,7,7-tetramethyl-	$C_{14}H_{20}O_3$	236	1.18
7	8.548	2,2'-Thiodisuccinic acid	$C_8H_{10}O_8S$	266	1.49
8	9.013	Bicyclo [3.1.1]heptane, 2,6,6-trimethyl-, (1α,2β,5α)-	$C_{10}H_{18}$	138	5.03
9	9.062	8-Nonen-2-one	$C_9H_{16}O$	140	1.54
10	9.308	Oxirane, hexadecyl-	$C_{18}H_{36}O$	268	2.04
11	9.629	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	2.00
12	9.805	2,2'-Thiodisuccinic acid	$C_8H_{10}O_8S$	266	1.81
13	9.982	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	26.95
14	10.752	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	1.60
15	10.827	Phytol	$C_{20}H_{40}O$	296	11.80
16	11.078	9,12,15-Octadecatrienal	$C_{18}H_{30}O$	262	20.04
17	11.196	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	4.68
18	12.217	cis-11-Eicosenoic acid	$C_{20}H_{38}O_2$	310	2.64
19	12.351	Adipamide	$C_6H_{12}N_2O_2$	144	0.92
20	13.030	cis-10-Nonadecenoic acid	$C_{19}H_{36}O_2$	296	0.87
21	13.282	Erucic acid	$C_{22}H_{42}O_2$	338	2.82
22	19.080	3-Hydroxy-N-methylphenethylamine	$C_9H_{13}NO$	151	0.82

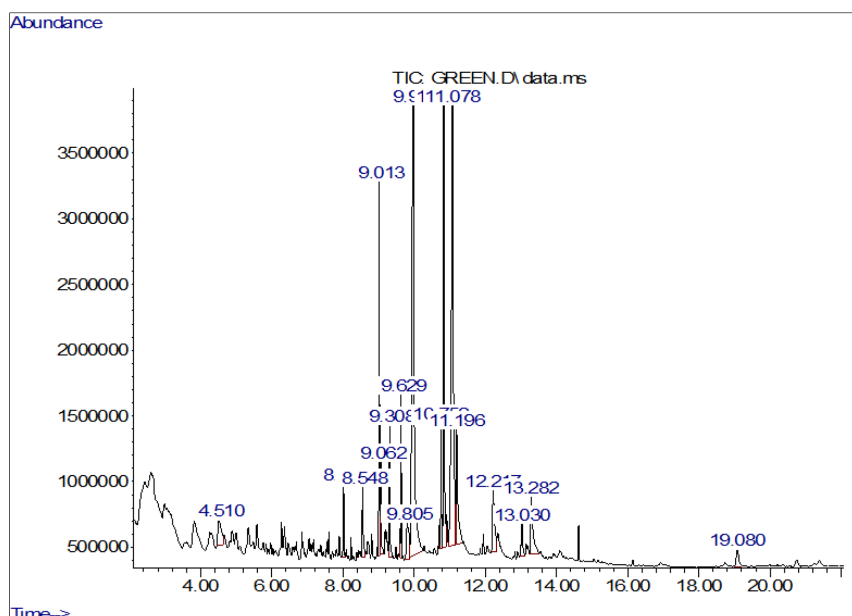

Figure 1 Chromatograph for *A. viridis*leaf extract

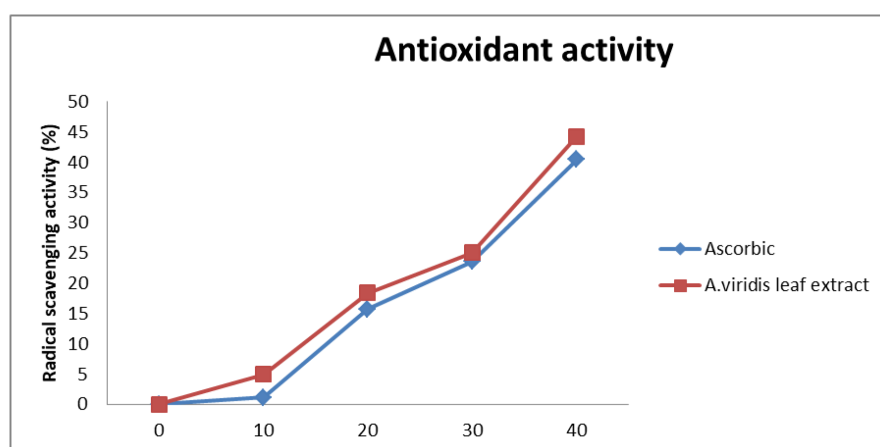

Figure 2 Antioxidant activity of *A.viridis* leaf extract

4. Conclusion

This study has shown that *A. viridis* leaf contain tannins, saponins, flavonoids, alkaloids, steroids, phenolic compounds, proteins and anthraquinones at different concentrations with about twenty-two detailed compounds revealed by further studies through GC-MS. The leaf sample also exhibited better antioxidant potential against the ascorbic acid used as the control in this present study. This study has revealed the phytochemistry and antioxidant activity of *A. viridis* L.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no such as conflict of interest with regards to this work. All the authors are aware that the work has been sent to World Journal of Advanced Research and Reviews, and they all gave their consent to it.

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