Available online

JOURNAL OF CONTEMPORARY RESEARCH (JOCRES)

RESEARCH ARTICLE VOL. 1 (2) ISSN:2814-2241

www.unicrossjournals.com

Date Accepted: 30th Dec, 2022

Pages 208 - 216

VOLATILE AND MINERAL CONSTITUENTS OF LEAFEXTRACT OF *Andrographis* paniculata AND THEIR POTENTIAL HEALTH IMPLICATION

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Abstract

Andrographis paniculata is an annual herb belonging to the family Acanthaceae that grows abundantly throughout tropical and sub-tropical regions. *A. paniculata* is traditionally used for treating different aliments. This work seeks to determine the volatile compounds and mineral constituents of ethanol leaf extract of Cross River State grown *A. paniculata* using gas chromatography-mass spectrometry (GC-MS) and atomic absorption spectrophotometry (AAS). The results revealed the presence of twenty seven (27) volatile compounds, dominantly: 9,5-octadecadienoic acid methyl ester (11.26%), 14-octadecacenoic acid methyl ester (47.86%), Methyl stearate (16.91%), Methyl 18-methylnonadecanoate (1.36%), Hexadecanoic acid methyl ester (1.54%), 9,12-octadecadienoic acid (Z,Z) methyl ester (0.58%), 13-octadecenoic acid methyl ester (1.35%), 10-octadecenoic acid methyl ester (0.46%), Carbonic acid (IR)-(-)-methyl dodecyl ester (0.26%), Hexadecanoic acid 14-methyl methyl ester (0.12%), Oxiraneoctanoic acid,3-octyl methyl ester (0.15%), Di-n-octyl phthalate (0.14%), Oleic acid (0.17%) and the presence of minerals, including, magnesium which was observed to be the highest present in the dry sample and ethanol leaf extract (1266 and 2730 mg/kg, respectively), followed by Iron (461.1 and 1420 mg/kg), Zinc (62.0 and 51.7 mg/kg). Others include, Manganese (25.8 and 24.5 mg/kg), Copper (7.5 and 15.8 mg/kg) and Nickel (5.2 and 24.5 mg/kg). It was concluded that, leaves of *Andrographis paniculata* grown in Cross River State, Nigeria, have high electron/hydrogen donating organic compounds and minerals, which function as active antioxidants, anti-inflammation and could play essential role in ameliorating inflammatory and oxidative stress mediated metabolic diseases.

Key words: *Andrographis paniculata,* ethanol extract, volatile compounds, minerals, GC-MS, AAS

1. Introduction

Andrographis paniculata is an annual herb belonging to the family, Acanthaceae that grows abundantly throughout tropical and sub-tropical regions, and comprises of about 40 species. It is commonly known as Kalmegh, Green chireyta and “king of bitters”. It is native to peninsular India and Srilanka, and distributed in different regions of Southeastern Asia, China, America, West Indies and Christmas Island (Ukpanukpong *et al.,* 2018). *A. paniculata* is well-known and cultivated for its medicinal value; and grows well in most soil types thus, it is widely distributed (Latto *et al*., 2006). It is a widely used medicinal plant in various systems of medicine. The aerial parts and roots of the plant have been widely used as traditional medicine in China, India, Thailand and other Southeastern Asian regions to treat many maladies, and it is grown in Nigeria including Cross River State.

In Cross River State, *A. paniculata* is known as vinegar and "Kazu'kwel by the people of Obudu and Bekwarra. It is grown in homestead and used traditionally to treat fever, malaria, stomachaches, gastrointestinal tract upsets, menstrual pains, and sometimes used to drink palm wine with the intention of reducing the sugar content of the wine. Moreover, literature review shows that the presence of various chemical constituents in the aerial parts of *A. paniculata* grown in Asia and other part of the world confer medicinal potency on the plant and include, volatile compounds, glycosides, saponins, tannins, alkaloids, flavonoids, quinic acids xanthones and diterpenes (Hossain *et al.,* 2014).

These active chemicals of the plant are term secondary metabolites which are formed from diversions at allosteric point in the plant primary metabolic pathways. They are off-shoot metabolic products and could play role in health and disease conditions. The plant constituents are known to be influenced by certain factors, such that a plant of same species may still have some forms of variabilities from one another. These factors, include, soil type, temperature, rainfall and geographical location (Liu *et al.,* 2016).

Asian grown *A. paniculata* has been widely investigated and the active component, andrographolide isolated. However, *A. paniculata* grown in Nigeria and indeed Cross River State has little or no information on its constituents. Given that, the geographical area of Asia differs from that of Cross River or Nigeria, there is a possibility of the chemical constituents being influenced. Therefore, there is need to screen for volatile and mineral contents of *A. paniculata* grown in Cross River State, and their possible role in health and diseases.

2. Materials and Methods

Collection of Plant Material: Fresh leaves of *Andrographis paniculata* were harvested from local households across the three (3) senatorial zones (North, Central and South) in Cross River State, Nigeria. The plant was identified and authenticated in the department of Plants Science, University of Cross River State, Calabar, and the voucher number: CRUTECH/PSB/0031 deposited in the herbarium.

Preparation of Plant Extract: Fresh leaves of *A. paniculata* were rinsed to remove debris and air-dried at room temperature. The dried leaves were pulverized to powdered form using Loonier Grander Mill (Japan). Part of the pulverized plant material was used for dry leaf mineral analysis; other part was dissolved in absolute ethanol in a ratio of 1:4 and allowed for 24 hours. This was followed by filtration using cheese cloth and What Man No. 1 filter paper. The filtrate was oven-dried at 40°C to obtain a solvent free extract. 2 g of the ethanol leaf extract of *A. paniculata* was used for GC-MS and AAS analysis.

Determination of Volatile Organic Compounds

GC-MS Analysis of Ethanol Leave Extract: The ethanol leaves extract of *A. paniculata* was dissolved in a (solvent) in a ratio of 1:10 volume, then transferred to a sample vial; about 2µ of the sample was injected at 250°C in the injection port and then split at a ratio 5:1 before reaching the gas chromatography column which is conditioned at oven set initially at 110^0C. Held for 2 minutes at a rate of 10^0C/min raised to 200^0C held for 0 mins then at rate of 5^0C/mins to 280^0 and finally held for 9 minutes. The sample would be volatilized and separated into various components and then transferred to the mass selective detector. The resulting mass spectrum of the components were identified and qualified with a standard reference libraries in the data analysis software.

SHS-GC-MS Analysis Condition: The SHS-GC-MS analysis was performed in an Agilent 7890B-5977A gas chromatography mass spectrometer equipped with an Agilent 7697A Head space auto sampler (Agilent technologies Inc., Santa Clara, CA, USA). A J&W capillary column HP-5 MS UI of 30 m x0.250 mm with

0.25µm film (Aligent Technologies Inc.) was used for the separation. The ICS samples in a 20 mL headspace vial were heated at an equilibrium temperature of 130^{0}C for 40 mins, and the gas phase were injected into the GCMS for analysis. The injection time was 1.0 mins. A low shaker mode of the headspace vial was applied during sample heating.

Gas chromatography parameters were as follows: the carrier gas and make-up gas were high pure helium and nitrogen respectively. The career gas (Helium) was set at a flow rate of 1.0 mL mins$^-$1. The inlet temperature was 200^{o}C with a split ratio of 10:1 and the pressure was 11.6 psi. The column oven temperature was initially set at 60^{o}C for 5 mins, and then ramped to 200^{o}C at 5^{o}C mins$^-$1 for 5 mins and after that, it was warmed up to 300^{o}C at 10^{o}C mins$^-$1. MS parameters were as follows: Data were acquired in the electron impact (EI) mode, using the full scan mode from m/z 30-600 at 1562 amu/s sec. The iron source temperature and quadrupole temperature were 230 and 150^{o}C respectively. The identification of volatile compounds was based on the comparison of their GC retention time and mass spectra with retention index of n-alkane saturated alkanes and the reference spectra from the US national institute of standards and technology (NIST, 2014). The values were the mean of 3 replicates of each samples. Data were analyzed by using Agilent MassHunter analysis.

Determination of mineral contents

0.5g of the ethanol extract of *A. paniculata* was used for mineral composition, side by side with the pulverized sample.

Digestion and atomic absorption spectrophotometry: 0.5g of sample was weighed and transferred into a beaker; 2.5ml of hydrochloric acid (HCl) and 7.5ml of Nitric acid were added to the beaker. The beaker containing the mixture was put on a hot plate and set at a temperature range of 700^{o}C - 170^{o}C, observe when it was about to dry off or form precipitates. The beaker was removed and allowed to cool. Drops of distilled water were added and the solution was mixed and transferred to a funnel set with filter paper. The filtration was measured and made up to 50ml and finally transferred to the sample bottle for atomic absorption spectrophotometer analysis. Each elements had a standard solution. Each standard was prepared into three (3) serial concentrations which were used as working standards. The absorbance of each standard, blank and unknown sample was measured and the AAS software extrapolated and measured the unknown concentration. Actual concentration was calculated as shown below.

For each element;

$$\text{Actual Concentration} = \frac{\text{Instrument Reading x Final Volume x Dilution Factor}}{\text{Weight of Sample}}$$

Interpretation:

Instrument Reading = the mean concentration of the result (0.075) ppm
Final Volume of mixture used = 50mls
Dilution Factor = 1; 10 for magnesium
Weight of Sample used = 0.5g

For example, Copper (Cu) was calculated as follows;

$$\text{Actual Concentration} = \frac{0.075 \text{ x } 50 \text{ x } 1}{0.5} = 7.5 \text{ mg/Kg}$$

3. Results

Twenty seven (27) volatile organic compounds were identified in the ethanol leaf extract of *A. paniculata* and dominantly include: 14-octadecacenoic acid methyl ester (47.86%), Methyl stearate (16.91%), Methyl 18-methylnonadecanoate (1.36%), 9,5-octadecadienoic acid, methyl ester (11.26%), Hexadecanoic acid, methyl ester (1.54%), 9,12-octadecadienoic acid (Z,Z) methyl ester (0.58%), 13-octadecenoid acid, methyl ester (1.35%) 10-octadecenoid acid, methyl ester (0.46%) carbonic acid (IR)-(-)-methyl dodecyl ester (0.26%) Hexadecanoic acid, 14-methyl methyl ester (0.12%), Oxiraneoctanoic acid, 3-octyl methyl ester (0.15%), Di-n-octyl phthalate (0.14%) and others (**Table 1 and Figure 1).** Minerals profile revealed there was presence of Magnesium which was observed to be the highest present in the dry sample and ethanol leave extract (1266 and 2730 mg/kg, respectively), followed by Iron (461.1 and 1420 mg/kg), Zinc (62.0 and 51.7 mg/kg). Others include, Manganese (25.8 and 24.5 mg/kg), Copper (7.5 and 15.8 mg/kg) and Nickel (5.2 and 24.5 mg/kg) (**Table 2**).

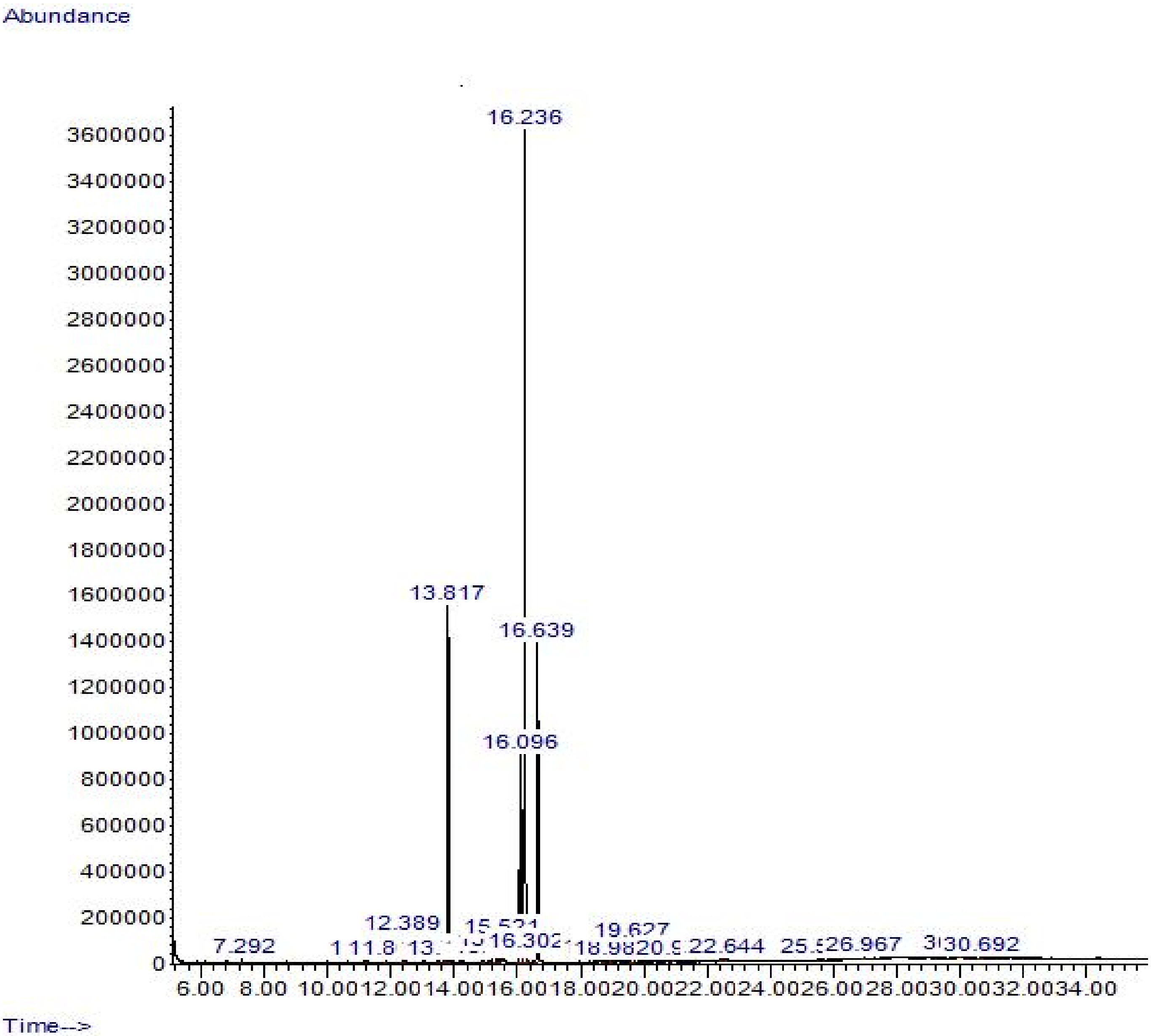

Figure 1: GC-MS TIC of ethanol leaf extract of *Andrographis paniculata*

Table 1: Volatile Constituents of ethanol leafextract of *Andrographis paniculata* using GC-MS

Serial No	Retention Time (Min)	Name of Compound	Molecular Formula	Molecular Weight	% Abundance
1	16.096	9,15-octadecadienoic acid, methyl ester, (Z,Z)	$C_{19}H_{34}O_2$	294.5	11.26
2	16.2362	14-octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	296.5	47.86
3	16.639	Methyl stearate	$C_{19}H_{38}O_2$	298.5	16.91
4	19.6267	Methyl 18-methylnonadecanoate	$C_{21}H_{42}O_2$	326.5	1.36
5	12.3886	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	1.54
6	15.3515	9,12-octadecadienoic acid (Z,Z)-methyl ester	$C_{19}H_{34}O_2$	294.5	0.58
7	15.5208	13-octadecenioc acid, methyl ester	$C_{19}H_{36}O_2$	296.5	1.35
8	16.3024	10-octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.5	0.46
9	14.9351	Carbonic acid (IR)-(-)-methyl dodecyl ester	$C_{23}H_{46}O_3$	370.6	0.26
10	15.1925	Hexadecanoic acid, 14-methyl, methyl ester	$C_{18}H_{36}O_2$	284.5	0.12
11	18.7025	Oxiraneoctanoic acid, 3-octyl-, methyl ester	$C_{19}H_{36}O_3$	312.5	0.15
12	22.5569	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	390.6	0.14
13	30.0285	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{48}O_{7}Si_{8}$	577.2	0.04
14	13.817	Hexadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	256.4	16.16
15	22.6438	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	354.6	0.15
16	30.0093	3,5-Dimethylbenzaldehyde thiocarbamoylhydrazone	$C_{10}H_{13}N_{3}S$	207.3	0.08
17	18.9817	8-Hexadecenal, 14-methyl-, (Z)-	$C_{17}H_{32}O$	252.4	0.17
18	30.6921	Pyridine, 1,2,3,6-tetrahydro-1-methyl-4-[4-chlorophenyl]-	$C_{12}H_{15}N$	173.3	0.20
19	25.5746	Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans-	$C_{20}H_{38}O_2$	310.5	0.12
20	20.9711	1-(Cyclopropyl-nitro-methyl)-cyclopentanol	$C_{10}H_{12}$	129.1	0.15

21	22.4557	Oleic acid	$C_{18}H_{34}O_2$	282.5	0.17
22	13.5007	2-Butenal, 2-ethenyl-10-Undecenal	$C_6H_{10}O$	98.1	0.16
23	11.8631	Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-, (1.alpha.,2.beta.,5.alpha.)-	$C_{10}H_{18}$	138.3	0.15
24	7.2923	Nonanoic acid, 9-oxo-, methyl ester	$C_{10}H_{18}O_3$	186.3	0.17
25	11.2639	Tetradecanoic acid, 12-methyl-, methyl ester, (S)-	$C_{16}H_{32}O_2$	256.4	0.07
26	26.9674	Cyclopentane, 1,1'-[3-(2-cyclopentylethyl)-1,5-pentanediyl]bis-	$C_{22}H_{40}$	304.6	0.11
27	13.703	Oxalic acid	$C_2H_2O_4$	90.0	0.12

Table 2: Mineral analysis of Dry Sample and Ethanol Leafextract of *Andrographis paniculata*

Mineral element	Dry sample (mg/kg)	Ethanol extract (mg/kg)
Copper (Cu)	7.5	15.8
Iron (Fe)	461.1	1420
Magnesium (Mg)	1266	2730
Manganese (Mn)	25.8	24.5
Nickel (Ni)	5.2	24.5
Zinc (Zn)	62.0	51.7

4. Discussion

This study evaluated the phytochemicals of ethanol leaf extract of *Andrographis paniculata* using gas chromatography mass spectrometer analysis. The mineral assessment of the plant leaves was part of this study. The results identified compounds of methyl esters in the medicinal plant, their names, percentage peak area, molecular weight, retention time and molecular formula as compared with the libraries. It was revealed that the leaves of *A. paniculata* contain of 27 volatile compounds. 14-octadecenoic acid (linoleic 47.86%) was observed to be the highest present, followed by methyl stearate (16.91%), methyl 18-methylnonadecanoate (16.16%) and 9, 15-octadecadienoic acid (11.25%).

Both 14-octadecenoic acid and 9, 15-octadecadienoic acid have been found to possess anti-arthritic, antihistaminic, anti-coronary and nematicide activities (Nishanthini *et al.,* 2014). 14-octadecenoic acid (linoleic acid) is a polysaturated omega-6 fatty acid. It is found commonly in diets such as sunflower oil, soyabeans, and corn. It is colorless or white oil that is virtually insoluble in water but in most organic solvents as reported by William (2016).

Its consumption is linked with reduced or decreased low-density lipoprotein cholesterol (bad cholesterol) and increased high-density lipoprotein cholesterol (good cholesterol) (Salisu *et al.,* 2019). It is typically occurring in nature as a triglyceride (ester of glycerin) rather than a free fatty acid (Mattes 2009).

The biological roles of the phytochemicals found in *A. paniculata,* especially, hexadecanoic acid known as Palmitic acid was found to have anti-inflammatory activity (Aparna *et al.,* 2012). It is an active substrate for phospholipase A (2) binding or interaction. It was concluded from the structural and kinetics studies that the fatty acid, hexadecanoic acid is an inhibitor of phospholipase A2 in a competitive manner which is a mechanism to control inflammation (Aparna *et al.,* 2012). Phospholipase A2 catalyzes phosphate A2 which is involved in the hydrolysis of ester Bonds at the Sn-2 position of membrane phospholipids and release fatty acids, such as arachidonic acids (AA) and phospholipids, which is the initiating step in the formation of potent inflammatory mediators (Aparna *et al.,* 2012). Moreover, Igile *et al.* (2018) have reported that moderate intake of palmitic acid display some levels of antioxidant and antiatherosclerotic properties.

The compound, 13- octadecenoic acid is responsible for cell signaling (Watson, 2006). Oleic acid is an omega-9- fatty acid, and it is also found in foods; highest levels found in olive oil and other edible oils. Oleic acid plays a role as an antioxidant, commonly acts in preventing type 2 diabetes and heart diseases, including hypertension and reducing cholesterol level (Lahey *et al*., 2014; Ochoa *et al.,* 2011). It has also been reported that Oleic acid increases fat burning, which is a good basis for weight loss (Ochoa *et al.,* 2011). Oleic acid performs role in myelination as well as preventing inflammation of the colon due to ulcer (Natali *et al.,* 2007). 1-(Cyclopropyl-nitro-methyl)-cyclopentanol, although the evidence that this compound helps alleviate nausea and vomiting resulting from chemotherapy or pregnancy is inconsistent Giacosa *et al*., (2015), there is evidence of having anti-inflammatory effect and improved digestive function (Anh *et al.,* 2020). According to Ostergaard and Larsen, (2007)), the stability of the carbonic ester derivatives of fatty acid-like structures expected to interact with the plasma protein, proved sufficient for possible combination with prodrug, an approach for optimization of drug pharmacokinetics.

Magnesium is an important mineral, playing a role in over 300 enzyme reactions in the human body. It also forms a component of chlorophyll in plant. Magnesium has been described to play a critical role in requiring metabolic processes, in protein synthe sis, membrane integrity, nervous tissue conducti on, neuromuscular excitability, muscle contracti on, hormone secretion, and in intermediary meta bolism (Laires *et al.,* 2014). Iron is one important element in the structural components of several compounds such as haemoglobin, cytochromes, ferridoxin, peroxidase, catalase and nitrite reductase. Iron is an essential element for almost all living organisms as it participates in a wide variety of metabolic processes, including oxygen transport, deoxyribonucleic acid (DNA) synthesis, and electron transport (Abbaspour *et al.,* 2014). Copper is an essential nutrient for the body. Together with iron, it enables the body to form red blood cells. It helps maintain healthy bones, blood vessels, nerves, and immune function, and it contributes to iron absorption. Sufficient copper in the diet may help prevent cardiovascular disease and osteoporosis (DiNicolantonio *et al.,* 2018).

Manganese which was also found in the plant, plays an important role in activating some energy components of chlorophyll molecule (Anibal *et al.,* 2017). Manganese functions as a cofactor in many enzymes and metalloproteins; of these the most widely known is manganese superoxide dismutase (MnSOD), whose primary function is detoxification of superoxide free radicals (Martinez-Finley *et al.,* 2013). Zinc is necessary to activate some of the enzymes in plant metabolism. Recently, Chinni *et al.* (2021) proposed that Zinc could serve as component of drug design for the treatment of COVID-19, and has impact on both male and female reproductive system (Sethuram (*et al.,* 2022).

5. Conclusion

Data from this study indicates that, leaves of *Andrographis paniculata* grown in Cross River State, Nigeria, have high electron/hydrogen donating organic compounds and minerals, which function as active antioxidants, anti-inflammation and could play essential role in ameliorating inflammatory and oxidative stress mediated metabolic diseases, including, type 2 diabetes, insulin resistance, obesity, high blood pressure, stroke, dyslipidaemia and other cardiovascular diseases.

Conflict of interest

The authors have not declared any conflict of interests.

Acknowledgements

Department of Chemistry, Ahmadu Bello University, Zaria is acknowledged for GC-MS and AAS facilities.

Funding

The research received no specific financial assistance or grant from any organization, institution or government.

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