African Journal of Basic & Applied Sciences 8 (1): 34-40, 2016
ISSN 2079-2034

DOI: 10.5829/idosi.ajbas.2016.8.1.1160

Phytochemical Composition, Gas Chromatography-Mass Spectrometric (GC-MS) Analysis and Anti-Bacterial Activity of Ethanol Leaf-Extract of *Ageratum conyzoides*

[1]P.M. Aja, [2]O.C. Enechi, [2]V.E.O. Ozougwu, [1]E.A. Onya-Mmaghiri, [1]K.A. Agu, [1]A. Ali Ikechukwu and [3]O.L. Nweke

[1]Department of Biochemistry, Faculty of Sciences, Ebonyi State University Abakaliki, Nigeria
[2]Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria.
[3]Department of Medical Biochemistry, Faculty of Basic Medicine, Ebonyi State University Abakaliki, Nigeria

Abstract: The study was designed to evaluate the phytochemical composition, GC-MS analysis and anti-bacterial activity of ethanol leaf-extract of *Ageratum conyzoides*. The phytochemical and antibacterial activity of *Ageratum conyzoides* leaf extract were carried out using standard methods while the GC-MS analysis was done using gas chromatography-mass spectrometric method. The result of phytochemical analysis revealed the presence of alkaloids, tannins, saponins, glycoside, flavonoids, resins, terpenoids and phenol. The result of GC-MS analysis showed the presence of 23 chemical constituents which include: 5-(1-methylidene)-1,3-methylidenecyclopentane (14.6%), nonane (18.2%), propan-2-ylcyclohexane(8.9%), (1-methylethyl) benzene (9.1%) and hexanoic acid (4.3%) as the major chemical constituents. The susceptibility test of the ethanol leaf-extract against septic wounds organism, showed higher value of 41.00 mm zone of inhibition on *Staphylococcus*, 26.00mm on *Escherichia coli*, 25.00mm on *Klebsiella*, 23.00mm on *Streptococcus* and low value of 20. 00mm on *Pseudomonas* after the antimicrobial analysis test on the organisms. This indicates that *A. conyzoides* is rich in bioactive compounds and sensitive to organisms of septic wounds and could be used for treatment/cure of diseases.

Key words: GC-MS analysis · Chemical constituents · Phytochemicals · *Ageratum conyzoides* · Ethanol leaf-extract and anti-bacterial

INTRODUCTION

The use of plants and the products for different purposes has been with man from the beginning. Apart from food, plants are often being used as medicine [1]. Plants used as medicine are known as medicinal plants [2]. Medicinal plants often exhibit a wide range of biological and pharmacological activities such as anti-inflammatory, anti-bacterial and anti-fungal properties [3]. Extracts, syrups, infusions and concoctions prepared from different parts of these plants are used to remedy different ailments. Such ailments include typhoid, anemia, malaria, headache, burns and wound [4]. The efficacy of medicinal plants against ill health is possible due to certain numerous biologically active compounds found in it such as nutrients and phytochemicals, which have physiological actions in the body of living organisms [5-7].

Ageratum conyzoides is an annual herbaceous plant with a long history of traditional medicinal uses in many countries in the world, especially in the tropical and subtropical regions. It belongs to the family of *Asteraceae*. It is an erect, branched, slender, hairy and aromatic plant, which grows to approximately 1m in height. It is native to Central America, Southeast Asia, South China, India and West Africa. *Ageratum conyzoides* has been known since ancient times for its curative properties and has been utilized for the treatment of various ailments, such as typhoid, anemia, malaria, headache, burns and wounds, analgesic, inflammation,

Corresponding Author: P.M. Aja, Department of Biochemistry, Faculty of Sciences, Ebonyi State University Abakaliki, Nigeria.

asthma, spasmodic arthosis, dysnea, pneumonia and haemostatic effects, Stomach ailments, gynecological diseases, leprosy and other skin diseases [8]. In Nigeria, different tribes have different names for it, Igedes of the middle belt, Yorubas of the southwest and Igbos of the southeast of the country calls it "Ufuopioko", "Imiesu" and "Nriewu" respectively [9]. While in Onicha Igbeze in Ebonyi State of Nigeria, it is called "Nsiigube". The plant is widely employed in traditional medicine within the above mentioned geopolitical zones in Nigeria. It is the only plant used in the treatment of HIV/AIDS by Igede people in Nigeria [10].

The use of herbs for the treatment of disease is almost universal among industrialized and non-industrialized societies and often more affordable than purchasing synthetic drugs. Despite the use of *Ageratum conyzoides* leaf for the treatment of various diseases, there is still paucity of documented data / information available regarding Gas chromatography–Mass spectrometric (GC/MS) analysis of the chemical constituents. This study therefore evaluates the phytochemical composition, Gas chromatography–mass spectrometric (GC/MS) analysis of the chemical constituents and anti-microbial activity of ethanol leaf-extract of *Ageratum conyzoides*.

MATERIALS AND METHODS

Materials: The materials used in this research work were *Ageratum conyzoides* leaf, microorganisms (*Escherichia coli*, *Staphylococcus*, *Streptococcus*, *Salmonella, Klebsiella* and *Pseudomonas*) and Chlorophenical.

Plant Collection: The fresh leaves of *Ageratum conyzoides* which was collected by hand picking in the month of November, 2014 from Ishiagu in Ivo L. G. A. Ebonyi State, Nigeria. The plant was identified by a taxonomist in the Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria. Some parts of the plant were also deposited in the herbarium for reference purpose.

Preparation of Plant Sample: The leaves were destalked, washed and shade dried at ambient temperature with constant turning to averts fungal growth. The dried leaves were later milled to obtained the vegetable leaf meals (VLMs) using an electric blender and was stored in 4°C temperature in refrigerator in well labeled air-tight containers for analysis.

Preparation of *Ageratum conyzides* Ethanol Leaf-Extract: Exactly 40grams of dried powdered leaves of *Ageratum conyzoides* were extracted successively with 300ml of ethanol in an orbital shaker for 24hours at room temperature. The extract was filtered using what-man N0.1 filter paper to remove extractable substances at every 3hrs interval. The combined extracts were then evaporated with rotary evaporator and the dried extracts were stored at 4°C in air-tight sterile container in refrigerator.

Methods

Preliminary Phytochemical Analysis: The preliminary phytochemical screening for the presence of tannins, sapanins, alkaloids, cardiac glycosides, flavonoids and others were carried out on the ethanol leaf-extract of *Ageratum conyzoides*.

Test for the Presence of Tannins: This was carried out by the method of Harborne [11].

Principle: Tannins are secondary metabolites of plant species and consist of sugar and non-sugar parts. They are capable of undergoing hydrolysis when inserted into dilute acids or boiling water to give rise to products such as polyhydroxyl phenolic compounds. They are reactive following the possession of functional groups called hydroxyl group (OH). They participate in redox reaction to give characteristics colour change on the reagent applied.

Procedure: One milliliter (1ml) of crude extract of the sample was collected using syringe and dispensed into test tube. Then, one milliliter (1ml) of ferric chloride ($FeCl_3$) was added to the test tube. A dirty green precipitate was observed which showed the presence of tannins.

Test for the Presence of Saponins: This was carried out by the method of Harborne [11].

Principle: Saponins are glycosides with distinctive foaming characteristics. They consist of a polycyclic aglycone that is either a choline steroid or triterpeniod attached through C_3 and an ether bond to a sugar side chain. The aglycone is referred to as the sapogenin and steroid saponins are called saraponins. The ability of saponins to foam is caused by the combination of the non-polar sapogenin and the water soluble side chain (hydrophilic part), which have hydroxyl groups (^{-}OH) as functional group.

Procedures:

Frothing Test: Two milliliters (2mls) of the extract were diluted with 5ml of distilled water in a test tube. The mixture was stirred vigorously for about 5mins and was allowed to stand for 30minutes. Frothing which persisted for this duration indicated the presence of saponins.

Emulsion Test: An emulsion is any thick liquid in which tiny drops of oil or fat are evenly distributed. Two to Five (2-5) drops of olive oil were added to 3mls of the sample in a test tube, stirred vigorously and allowed to stand for 30mins. Emulsification that was observed for this duration indicated the presence of saponins.

Test for Presence of Alkaloids

Principle: Alkaloid can be detected as loose complexes following their ability to react with some reagents by producing characteristics colour changes depending on the type of reagent used. Alkaloids have an amino group (NH_2) as their functional group as in nicotine.

Procedure: Two milliliters (2mls) of the extract was collected using syringe and was dispensed into a test tube, the test tube was heated for 2mins and 5mls of hydrogen (HCl) was added and heated again and allowed to cool. The mixture was divided into A and B. To A, 2 drops of Meyer's reagent was added and white precipitate was observed which showed the presence of Alkaloids. To B, 2 drops of Dragendroff's reagent was added and the formation of red precipitate was observed which confirmed the presence of alkaloids.

Test for the Presence of Flavonoids: This was carried out by the method of Harborne [11].

Principle: Flavonoids are colourless or pale yellow glycosides that are not soluble in non- polar solvents. They are compound that are oxidize by ethyl-acetate. They react with polar solvent to produce colour changes in accordance with the level of redox reactions that are likely to take place. Flavonoids also reacts with sodium hydroxyl group (NaOH) to form a yellow colour following the reaction of the hydroxyl group (ˉOH) with the ketone functional group.

Procedure: Five milliliters (5ml) of the extract was collected using syringe and was dispensed into a test tube. Exactly 10mls of distilled water, 5mls of dilute ammonium hydroxide (NH_4OH) and few drops of tetraoxosulphate (VI) acid (H_2SO_4) were added in the test tube. A yellow colouration was observed which showed the presence of flavonoids.

Test for the Presence of Cardiac Glycoside: This was carried out by the method of Harborne [11].

Principles: Cardiac glycosides are organic compounds that are capable of undergoing hydrolysis in the presence of dilute acids, alkali or enzymes.

Procedure: Two milliliters (2mls) of the extract was collected into a test tube and 5ml of glacial acetic acid was added and then 2mls of $FeCl_3$ and 2mls of concentrated ferric acid were added too. A brown ring formation at inter phase of the mixture indicated the presence of deoxy sugar characteristics of cardiac glycosides.

Test for the Presence of Steroids and Triterpenoids: This was carried out by the method of Harborne [11].

Principle: Steroids are class of organic compounds with a chemical structure that contains the core of gonane or a skeleton derived from them. Usually, methyl groups are present at carbon one (C-1) and carbon three (C-3), an alkyl side chain at carbon seventeen (C-17) may also be present. Formation of red colouration if steroids is present or yellow colouration if triterpenoids is present upon addition of concentrated tetraoxosulphate (VI) acid to unknown sample in Salkwoki reagent test as a result of reaction with the functional group.

Procedure: Five milliliters (5mls) of ethanol leaf extract of *Ageratum conyzides* was collected and dispensed into test tube, then two milliliters (2mls) of chloroform was added to the tube and then concentrated tetraoxosulphate (VI) acid (H_2SO_4) was also added. The mixture was stirred thoroughly and allowed to stand for some minutes. A red colour appeared at the lower layer of the mixture, which indicates the presence of steroids while a yellow colour was observed at the upper layer indicates the presence of triterpenoids.

Test for the Presence of Glycosides. This was done according to the method of Van-Burden and Robinson (1981).

Principle: Glycosides are compounds (organic compounds) that are capable of undergoing hydrolysis in the presence of dilute acids, alkali or enzymes. They are composed of sugar and non-sugar molecules.

Procedure: Five milliliters (5mls) of ethanol leaf extract of *Ageratum conyzides* was collected using syringe and was dispensed into test tube and then 2mls of H_2SO_4 was added to the tube and was heated in boiling water for 15minutes. The content of the tube was divided into two test tubes A and B and 2drops of Fehling solution were added to A and B and then heated to boiling. A brick red precipitate indicates the presence of glycosides.

Test for the Presence of Phenols: This was carried out by the method of Harborne [11].

Principle: Aqueous solution of phenol is weakly acidic and turns blue litmus paper slightly red. Phenol is easily neutralized by sodium hydroxide to form sodium phenate or phenolate.

Procedure: One milliliter (1ml) of the extract was collected using syringe and dispensed into test tube. One milliliter (1ml) of $FeCl_3$ was added to ethanol and water sample in a test tube, a dirty green precipitate showed the presence of phenols.

GC-MS Analysis of Ethanol Extract of *Ageratum conyzoides* Leaf

Procedures: GC-Ms analysis of the ethanol extract of *Ageratum conyzoides* leaf was performed using Shimadzu Japan gas chromatography QP2010 plus with a fused gas chromatography (GC) column (2010) coated with poly-methyl silicon (0.25nm x 50m) and the conditions were as follows: Temperature programming from 80-200°C held at 80°C for 1minute, rate 5°C/min and at 200°C for 20min. Field ionization detector (FID) Temperature of 300°C, injection temperature of 220°C, carrier gas nitrogen at a flow rate of 1ml/min, split ratio of 1:75. Gas chromatography mass spectrum was conducted using GCMS-QP 2010 plus Shimadzu Japan with injector temperature of 220°C and carrier gas presence of 116.9kpa. The column length is 30m with a diameter of 0.25mm and flow rate of 50ml/min. Elutes were automatically passed into a mass spectrometer with a dictator voltage set at 1.5 Kv and sampling rate of 0.2 sec. The mass spectrum was also equipped with a computer fed mass spectra bank. German Hermlez 233M-Z centrifuge was used.

Component Identification: Chemical constituent of the extract was identified by matching the peak with computer Wiley Ms libraries and confirmed by those comparing mass spectra of the peaks and those from literature.

Antimicrobial Analysis: The Bioassay for extracts was carried out as described by Prescott *et al.* [12]. Seven grams (7g) of Nutrient Agar (NA) were weighed into 250ml conical flask electrical weighing balance and 250ml of distilled water was added to it to dissolve it. It was shaken and heated slightly to make a complete solution using an oven. The solution were sterilized in an autoclave for 15 minute at 121°C and 5mls of the Agar was poured into sterilized petri-dish 25mls each under the laminar flow hood and allow to gel.

Agar Plates Inoculation: Serial dilution method were adopted, the organisms to be used were removed from the fridge and sub-cultured in a prepared nutrient broth for 24 hours to have a day old organism. To the solidified nutrient Agar, 5 walls were made in each plates and 0.5 ml of the diluted *A. conyzoides* ethanol leaf-extract was collected with pipette into 4 walls in each of the plates, why the control (Chlorophenical) was also collected with pipette into one wall in each of the plates. The various zones of inhibition in cm were observed, measured and recorded after 24hours.

RESULTS

Result of Phytochemical Screening of *A. conyzoides* Leaf: The result revealed the presence of tannins, saponins, alkaloids, phenols, glycosides, steroids, terpenoids and cardiac glycosides in the sample as shown in Table 1.

Result of GC-MS Analysis of *A. conyzoides* Ethanol leaf-Extract: The ethanol extract of the leaf of *Ageratum conyzoides* showed 23 peaks (Figure 1) from the GC-MS analysis. These peaks indicate the presence of 23 compounds (1-23) in the extract (Table 2). The composition of the extract comprises of 5-(1-methylidene)-1,3-methylidenecyclopentane (14.6%), nonane (18.2%), propan-2-ylcyclohexane(8.9%), decane (3.2.%), (1-methylethyl) benzene (9.1%), nonasne (2.8%), 1-methyl-4-(prop-1-en2-yl) cyclohexa-1,3-diene (2.8%), 1-methyl-3-(propan-2-yl) benzene (4.2%), (1-ethenyl-2,4-dimethylbenzen 4.3%), 1-methyl-3-(propan-2-yl) benzene (4.3%), oct-2-ene (2.7%), octane (3.1%), heptane (4.3%),

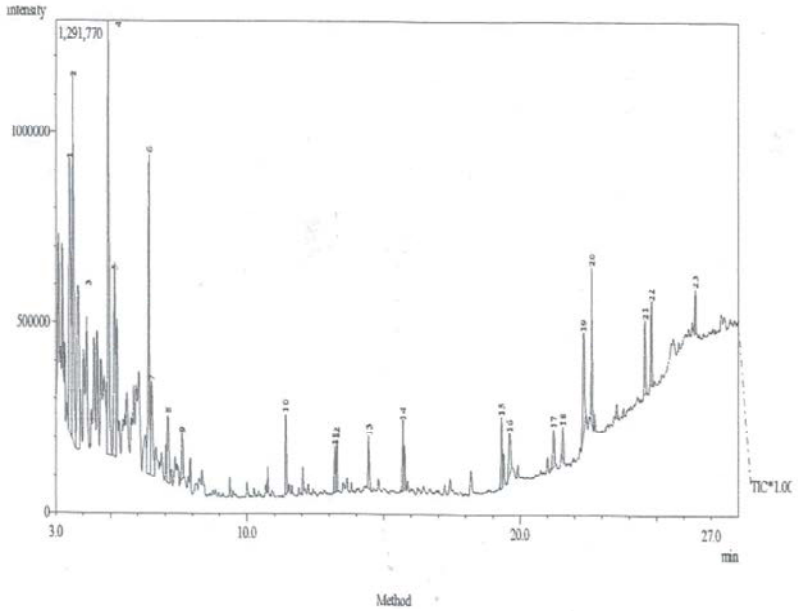

Fig. 1: GC-MS Chromatogram of *Ageratum conyzoides* Ethanol leaf-Extract

Table 1: Phytochemical Screening of *A. conyzoides* Ethanol leaf-Extract.

Phytochemicals	Remarks
Tannins	Positive
Alkaloids	Positive
Steroids	Positive
Saponnins	Positive
Cardiac glycosides	Positive
Glycosides	Positive
Terpenoids	Positive
Phenols	Positive

Table 2: GC-MS Analysis of *A. conyzoides* and Mass Spectral Data of Ethanol Fraction from *A. conyzoides* Showing Molecular Formula, Molecular Weight, Percentage Content, Retention Time and Base Peak

Peak	Compounds	Molecular Formula	Molecular Weight	Retention Time	Percentage Content (%)	Base Peak
1	5-(1-methylidene)-1,3-methylidenecyclopentene	C_6H_8	106	3.508	14.6	91
2	Nonane	C9H20	128	3.625	18.2	43
3	Propan-2-ylcyclohexane	$C_{10}H_{17}$	106	3.817	14.6	91
4	Decane	$C_{10}H_{22}$	152	4.917	8.9	43
5	(1-methylethyl) benzene	C_9H_{12}	143	5.150	3.2	105
6	Nonasne	C_9H_{19}	142	6.408	9.1	43
7	1-methyl-4-(prop-1-en-2yl)cyclohexa-1,3-diene	$C_{10}H_{16}$	152	6.508	9.1	119
8	1-methyl-3(propan-2yl)benzene	$C_{10}H_{14}$	134	7.108	1.4	119
9	1-ethyl-2,4-dimethylbenzene	$C_{10}H_{11}$	132	7.625	4.8	117
10	1-methyl-3-(propan-2-yl)benzene	$C_{10}H_{13}$	204	11.4	4.2	41
11	Oct-2-ene	C_8H_{15}	168	13.2	4.3	41
12	Octane	C_8H_{17}	142	13.3	2.7	43
13	Heptanes	C_7H_{15}	155	14.5	3.1	43
14	Oct-2-ene	C_8H_{15}	168	15.7	4.3	43
15	Non-2-ene	C_9H_{17}	155	19.3	4.3	41
16	Heptanoic acid	$C_7H_{13}O_2$	298	19.6	2.7	41
17	Penta-2,4-dienoic acid	$C_5H_6O_2$	264	21.2	4.3	55
18	Penta-2,4-dienoic acid	$C_5H_{16}O_2$	228	21.6	4.3	74
19	Non-2-ene	C_9H_{17}	196	22.17	4.3	55
20	Non-2-ene	C_9H_{17}	125	22.6	3.7	43
21	Heptanoic acid	$C_7H_{13}O_2$	129	24.6	5.5	43
22	Hexanoic acid	$C_6H_{12}O_2$	116	24.8	4.3	43
23	Hex-3-enoic acid	$C_6H_{10}O_2$	133	26.4	4.3	43

Table 3: Antimicrobial Effect Ethanol- Extract of *Ageratum conyzoides*

Ethanol Crude Extract and Sensitivity of Extracts after 24 hrs (mm) On	
Escherichia coli 26mm	
Streptococcus	23mm
Staphylococcus	41mm
Salmonella	20mm
Klebsiella	25mm
Pseudomonas	20mm

oct-2-ene (2.7%), non-2-ene (2.7%), heptanoic acid (3.5%), penta-2,4-dienoic acid (4.7%), penta-2,4-diene acid (4.7%), non-2-ene (3.7%), heptanoic acid (5.5%), hexanoic acid (4.3%) and hex-3-enoic acid (4.3%) as the chemical constituents. The result also showed that the ethanol leaf extract of *A. conyzoides* has nonane (18.2%) as the highest chemical constituent and oct-2-ene (2.7%) as the lowest chemical constituent.

Result of Antimicrobial Screening of *Ageratum conyzoides*: Table 3 showed that the ethanol leaf-extract of *Ageratum conyzoides* was sensitive to the following organisms after 24 hours. *Escherichia coli (26mm), Streptococcus (23mm), Staphylococcus (41mm), Salmonella (21mm), Klebsiella (25mm) and Pseudomonas (20mm)*.

DISCUSSION

The results revealed that *Ageratum conyzoides* is rich in tannins, steroids, glycosides, saponins, phenols, alkaloids, terpenoids, cardiac glycosides and glycoside. Aja *et al*. [13] revealed that *Talinum triangulare* leaf and *Moringa oleifera* leaf are good source of phytochemicals. Nwali *et al*. [6] showed that *Bryophylum pinnatum* leaf contained low levels of phytochemicals. Aja *et al*. [14] had reported that *Cajanus cajan* leaf and seed are very rich in phytochemicals. Aja *et al.* [15] also had reported the rich phytochemical contents of *Dissotis rotundifolia* leaf and root. Phytochemicals are known to have antifungal, anticancer, antibacterial, anti-inflammatory and well as pronounced effect on nerve system respectively [5].

The result from GC-MS analysis revealed mainly 23 chemical compounds (Table 2). The result also showed that the ethanol leaf extract of *A. conyzoides* has nonane (18.2%) as the highest chemical constituent and oct-2-ene (2.7%) as the lowest chemical constituent (Table 2). Hex-3-enoic acid (4.3%) and 5-(1-methylidene)-1, 3-methylidenecyclopentane (14.6%) possess antibacterial activities against *Escherichia coli* and *Staphylococcus*. Aja *et al.* [13] and Nweke *et al*. [16] had reported sixteen and ten chemical constituents of GC-MS of *Moringa oleifera* and *Vitex doniana* leaves. The constituent compounds in ethanol extract are long chain aliphatic carboxylic acids, (saturated and unsaturated) and their derivatives including alcohols, aldehydes as well as benzene carboxylic acid esters and a steroidal compounds. It is pertinent to identify the possible roles of these constituent compounds in the curative properties attributed to the plant by herbal medical practitioners.

Raheela *et al.* [8] evaluated antimicrobial activity from the seeds of *Moringa oleifera*. The seed extract of *Moringa oleifera* was assayed for the evaluation of antimicrobial activity against bacterial (*Pasturella multocida, Escherichia coli, Bacillus subtilis* and *Staphlocuccus aureus*) and fungal (*Fusarium solani* and *Rhizopus solani*) strains. The zones of growth inhibition showed greater sensitivity against the bacterial strains as compared to the fungal strains. Minimum inhibitory concentrations (MIC) extracts revealed that *Pasturella multocida* and *Bacillus subtilis* were most sensitive strains [8]. Rhaman *et al.* [2] investigated antibacterial activity of leaf juice and extracts of *Moringa oleifera* using disc diffusion and minimum inhibitory concentration (MIC) determination method against human pathogenic bacteria. The fresh leaf juice (10 µl), powder from fresh leaf juice, cold water extract of fresh leaf, 1175 µg disc-1, displayed a potential antibacterial activity against all the tested four Gram-negative bacteria (*Shigella shinga, Pseudomonas aeruginosa, Shigella sonnei and Pseudomonas spp.*) and six Gram-positive bacteria (*Staphylococcus aureus, Bacillus cereus, Streptococcus-B-haemolytica, Bacillus subtilis, Sarcina lutea and Bacillus megaterium*). However, ethanol extract (1175 µg) of fresh leaves exhibited inhibitory effect against all the tested Gram-negative bacteria and Gram-positive bacteria except in *S. aureus* and *Streptococcus-B. haemolytica* [2].

Conclusion: The result of GC-MS analysis showed that ethanol leaf-extract of *Ageratum conyzoides* has twenty three chemical constituents and has showed anti-bacterial properties.

REFERENCES

1. Edeoga, H.O., D.E. Okwu and B.O. Mbaebie, 2005. Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol., 4: 685-688.
2. Rahman, M.M., M.M. Islam Sheikh, S.A. Sharmin, M.S. Islam, A. Rahman, M.R. Mizanur and M.F. Alam. 2009. CMU Journal, 8(2): 219-228.

3. Irvine, F.R., 1961. Woody plants of Ghana. Oxford University Press, New York. London, pp: 61.
4. Kobayashi, N., J. Tsuda, M.K. Agemi and J. Vacelet, 1991. Antidepressant, Inflammatory and Treatment of Cell, New York, Academic Press, pp: 673.
5. Menut, C.S., J.I. Sharma and C. Luthra, 1993. Aromatic Plants of Tropical Central Africa, Chemical Composition of Essential oils of *Ageratum houstonianum* Mill. And *Ageratum conyzoides* from Cameroon. Flavour Fragrance Journal, 8(1): 1-4.
6. Nwali, B.U., A.N.C. Okaka, U.A. Ibiam and P.M. Aja, 2012. Phytochemical Composition of *Bryophyllum pinnatum* Leaves, International Journal of Advanced Biological Research (IJABR), 2(3): 1-3.
7. Ugwu Okechukwu, P.C., Nwodo, Okwesili F.C. Joshua, Parker E. Bawa, aburbakar, C. Ossai Emmanuel and E. Odo Christian, 2013. Phytochemical and Acute Toxicity Study of *Moringa oleifera* Ethanol Leaf Extract. International Journal of Life Sciences Biotechnology and Pharma Research, 2(2): 66-7.
8. Raheela, J., S. Muhammad, J. Amer and A. Muhammad, 2008. Pakistian Journal of Botany, 40(4): 1349-1358.
9. Van-Burden, L. and J.H. Robinsin, 1981. Phytochemical Techniques of plant analysis 2nd edition, New York, pp: 100-150.
10. Burkill, H.M., 1985. The useful plants of west Tropical Africa, vol.1. New Royal Botanic Gardens.
11. Harborne, J.B., 1973. Text book of Phytochemical Method, 1st Edition, Champmaan and Hall Ltd. London, pp: 110-113.
12. Prescott, L.M., J.P. Harley and D.A. Klein, 2003. Microbiology 6a edition, McGraw-Hill Education. United States Prince, L and Prabakaran, P. (2011) Asian J. Plant Sci. Res., 1(1): 8.
13. Aja, P.M., N. Nwachukwu, U.A. Ibiam, I.O. Igwenyi, C.E. Offor and U.O. Orji, 2014. Chemical Constituents of *Moringa oleifera* Leaves and Seeds from Abakaliki, Nigeria, American Journal of Phytomedicine and Clinical Therapeutics, 2(3): 310-321.
14. Aja, P.M., E.U. Alum, N.N. Ezeani, U.A. Ibiam and C. Egwu, 2015. Comparative Phytochemical Evaluation of *Dissotis rotundifolia* Root and Leaf, Global Veterinaria, 14(3): 418-424.
15. Aja, P.M., E.U. Alum, N.N. Ezeani, B.U. Nwali and N. Edwin, 2015. Comparative Phytochemical Composition of *Cajanus cajan* Leaf and Seed, International Journal of Microbiological Research, 6(1): 42-46.
16. Nweke, O.L., N. Nwachukwu, P.M. Aja, K.N. Agbafor, A.C. Nwaka and R. Uchenna Ezeilo, 2015. Phytochemical and Gas Chromatograpy-Mass Spectrophotometeric (GC-MS) Analyses of *Vitex doniana* Leaf from Abakaliki, Ebonyi State, IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS), 10(5): 33-38.