International Journal of Herbal Medicine
Available online at www.florajournal.com

E-ISSN: 2321-2187
P-ISSN: 2394-0514
IJHM 2019; 7(4): 45-50
Received: 17-05-2019
Accepted: 20-06-2019

Frank NI Morah
Department of Chemistry, University of Calabar, Calabar Cross River, Nigeria

Amos F Ogar
Department of Chemistry, University of Calabar, Calabar Cross River, Nigeria

Eni I Eyong
Department of Chemistry, University of Calabar, Calabar Cross River, Nigeria

Helen A Nathaniel
Department of Chemistry, University of Calabar, Calabar Cross River, Nigeria

Marshal A Isong
Department of Chemistry, University of Calabar, Calabar Cross River, Nigeria

Correspondence
Frank NI Morah
Department of Chemistry, University of Calabar, Calabar Cross River, Nigeria

Chemical composition, anthelmintic, insecticidal and antimicrobial activities of *Vernonia colorata* leaf essential oil

Frank NI Morah, Amos F Ogar, Eni I Eyong, Helen A Nathaniel and Marshal A Isong

Abstract
Vernonia colorata is commonly employed in herbal medicine. The present study is therefore aimed at determination of the chemical composition, anthelmintic, insecticidal larvicidal and antimicrobial activities of its leaf essential oil. Fresh leaves of *Vernonia colorata* were ground and steam distilled to get the essential oil. The individual constituents of the essential oil were separated by gas chromatography and identified by mass spectrometry. Antimicrobial susceptibility test was carried out using agar disc diffusion method while the minimum inhibitory concentrations were determined through the dilution method. 100, 50 and 25µgcm^{-3} solutions of the essential oil were used for the determination of anthelmintic activity against *Lumbricus terrestris.* Ten adult *Anopheles* mosquitoes were kept in beakers containing different levels of the essential oil. The mosquitoes were observed for sixteen hours and the number of death recorded. From this the percentage mortality was calculated. In a similar way, the larvicidal activity of the essential oil was determined. A total of twenty four organic compounds were identified in the essential oil and these are being identified for the first time in *Vernonia colorata* leaf. The oil exhibited antimicrobial activity against *Candida albicans, Rhizopus nigricans, Streptococcus faecalis, Staphylococcus aureas, Pseudonomas aeruginosa* and *Salmonella typhi.* It exhibited anthelmintic activity against *Lumbricus terrestris.* The oil also exhibited strong insecticidal and larvicidal activity against *Anopheles* mosquito. The oil exhibited strong antimicrobial, insecticidal, anthelmintic activities. Twenty four compounds have been identified for the first time in *Vernonia colorata* leaf essential oil. With the observed antimicrobial and anthelmintic activity, the oil will serve as an alternative source of vermicide and new antimicrobial drugs. The oil will also assist in control of malaria through control of the mosquito vector.

Keywords: *Vernonia colorata,* antimicrobial activity, insecticidal and larvicidal activity, anthelmintic activity, essential oil, chemical composition

1. Introduction
Vernonia amygladina and *Vernonia colorata* are collectively called bitter leaf. They are edible leafy vegetables and African medicinal plants. The leaf of *Vernonia amygladina* ("bitter" bitter leaf) has much deeper green colour and more bitter taste than *Vernonia colorata* ("sweet" bitter leaf) [1, 2]. The leaves are used in traditional medicine as antipyretic, expectorant and laxative [3]. The leaf has been reported to have antiplasmodial [4, 5], anti-inflammatory [6] and antimicrobial activities [7, 8]. The root back is also reported to have strong antibacterial activity [2]. Although much work has been reported on *Vernonia amygladina,* there is scanty information on *Vernonia colorata* especially its essential oil. The present study is therefore focused at identification of the chemical constituents of *Vernonia colorata* leaf essential oil as well as its anthelmintic, antimicrobial, larvicidal and insecticidal activities.

2. Materials and Methods
Fresh *Vernonia colorata* leaves were plucked from a farm within the University of Calabar Senior Staff Quarters. It was authenticated by staff of the Herbarium unit, Botany Department, University of Calabar. The fresh leaves were rinsed with distilled water and subjected to steam distillation for $1\frac{1}{2}$h to obtain the essential oil as steam distillate.

2.1 Gc-Ms analysis: The chemical composition of the essential oil was determined using Agilent Hewett-packared 7980A gas chromatography-mass spectrometry with triple detector and auto injector (10µm syringe). Helium was used as the carrier gas at a constant rate of 1cm^3 min^{-1}. The column consists of a 30m length, 0.25µm diameter and thickness 250µm fused

silica capillary coated with polydimethylsiloxane. Ion source temperature is 25° C, pressure is 16.2 ps with 1µm injector in split mode with spin ratio of 1.50 with injection temperature of 300°C. The column temperature was raised at 35°C for 5min and raised to 150°C at the ratio of 40°C min^{-1}. The temperature was further raised to 250°C at a rate of 20°C min^{-1} and held for 5min before ionization. Microsoft solution provided by supplier of the instrument was used to control the system and to acquire the data. Identification of the constituent compounds was carried out by comparing the mass spectra obtained with those of standard mass spectra from National Institute of Standard and Technology (NIST) library [9].

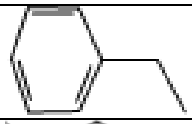
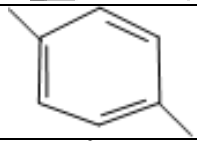
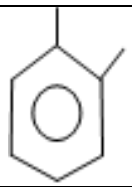
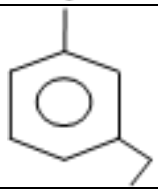
2.2 Anthelmintic test: Adult *Lumbricus terrestris* (common earthworm) was used for the anthelmintic test of the essential oil. They were collected few hours before test from decaying plantain stems. 100, 50, 25 and 0.0µgcm^3 solutions of the essential oil in distilled water were prepared. Four previously sterilized petri dishes for each of the three levels of the essential oil in water and the control were used. 25cm^{-3} of each of the solutions was placed in a petri dish. Also 25$cm^{-3}$of phosphate buffer saline in a petri dish served as the control. Five adult worms were placed in each of the petri dishes and observed at room temperature for 16h. The dead (ie non-motile) worms were counted and the percentage mortality as well as LC_{50} calculated.

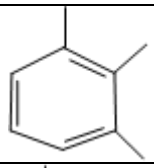
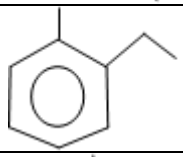
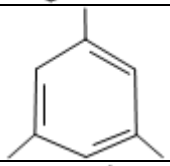
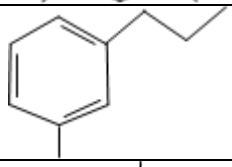
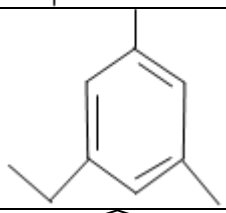
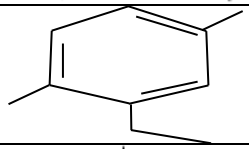
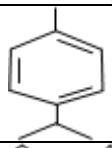
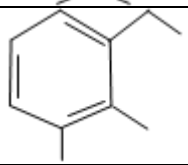
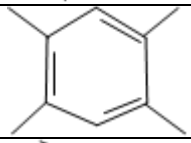
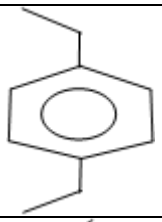
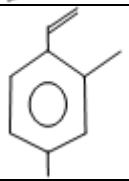
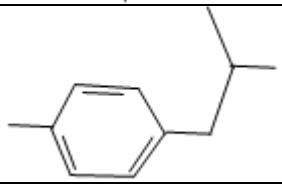
2.3 Insecticidal and larvicidal tests: Twenty milligram (20mg) of the essential oil was dissolved in 100$cm^3$of dimethylsulphoxide (DMSO) to obtain a solution of 200mg$dm^{-3}$of the stock solution. This was diluted with distilled water to get different concentrations of 50, 100, 150 and 200µgcm^{-3} solutions. They were separately placed in four different 500cm^3 beakers. A fifth beaker containing 0.00µgcm^{-3} of the oil was used as the control. Ten *Anopheles* mosquitoes were placed in each of the beakers. The number of dead mosquitoes after sixteen hours in each of the beakers was recorded. The entire process was repeated using *Anopheles* mosquito larvae in 100, 200 and 500 µgcm^{-3} solutions of the essential oil. The larval mortality was assessed and recorded after sixteen hours.

2.4 Antimicrobial susceptibility test: Agar disc diffusion method was used for the antimicrobial susceptibility test against *Staphylococcus aeures, Streptococcus faecalis, Pseudomonas aeruginosa, Salmonella typhi, Candida albicans* and *Rhizopus nigricans.* All these microbes are clinical isolates obtained from the Pathology Department, University of Calabar Teaching Hospital (UCTH). The microbes were maintained by the method from the National Committee for Clinical Laboratory Standards (NCCLS)[10]. The essential oil was diluted with distilled water to give solutions of 100, 50, 25 and 12.5 µgcm^{-3}. Mueller Hinton agar was used for both bacteria and fungi tests. Sterilized Whatmann filter paper discs were separately soaked in the solutions containing the different levels of the essential oil. These were placed in different agar plates which contained the different test organisms. They were incubated for 24h at 37°C. At the end of the incubation period, the zone of inhibition was measured for the different plates. 100mg of doxycycline was dissolved in 100cm^3 of water to give 100µgcm^{-3} solution which was used as the control [11]. For determination of minimum inhibitory concentration, (MIC), 25, 12.5, 6.25, 3.125, 0.781 and 0.39 µgcm^{-3} solutions of the essential oil were placed in different test tubes. 1$cm^3$of water was added the each of the test tubes. 4cm^3 of peptone water (Mueller Hinton broth) was added followed by addition of 24h broth culture of the microorganism. The test tubes were all sealed with sterile cork and incubated at 37°C for 24h and 48h for bacteria and fungi respectively. Thereafter the test tubes were observed for clearance and turbidity. The first test tube with high degree of clearance was taken as the minimum inhibitory concentration (MIC) while the one preceding MIC is regarded as the minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) for bacteria and fungi respectively [12, 13].

3. Results

Table 1: Gc-Ms analysis of essential oil from *Vernonia colorata* leaf

S/N	Compound Name	Retention Time (Minutes)	Molecular Formula	Relative Molecular Mass	Percentage composition	Chemical Structure
1	ethylbenzene	7.095	C_8H_{10}	106	1.785	
2	p-xylene	7.426	C_8H_{10}	106	8.163	
3	o-xylene	8.340	C_8H_{10}	106	3.823	
4	1-ethyl-3-methyl benzene	10.097	C_9H_{12}	120	105	

5	benzene,1,2,3-trimethyl-	11.536	C_9H_{12}	120	1.222	
6	1-ethyl-2-methyl benzene	12.549	C_9H_{12}	120	5.428	
7	mesitylene	13.700	C_9H_{12}	120	2.228	
8	benzene,1-methyl-3-propyl	14.945	$C_{10}H_{14}$	134	2.048	
9	benzene-1-ethyl-3,5-dimethyl	15.258	$C_{10}H_{14}$	134	1.248	
10	2-ethyl,1,4-dimethyl benzene	15.977	$C_{10}H_{14}$	134	1.228	
11	p-cymene	16.278	$C_{10}H_{14}$	134	3.951	
12	benzene,1-ethyl-2,3-dimethyl	13.632	$C_{17}H_{26}SO_2$	294	0.83	
13	benzene,1,2,4,5-tetramethyl	17.616	$C_{10}H_{14}$	134	2.762	
14	1,4-diethlybenzene	18.285	$C_{10}H_{12}$	132	3.024	
15	2,4-dimethylstyrene	18.680	$C_{10}H_{12}$	132	3.759	
16	benzene,1-methyl-4-(2-methylpropyl)	18.898	$C_{11}H_{16}$	148	1.143	

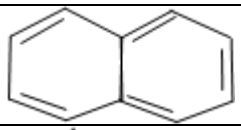
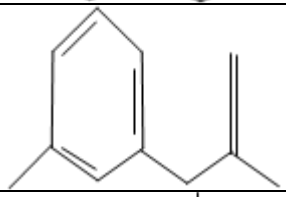
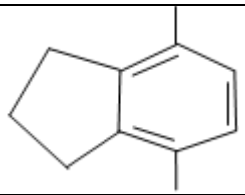
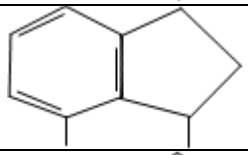
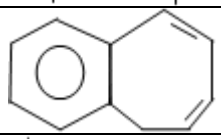
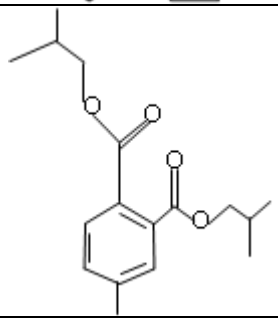
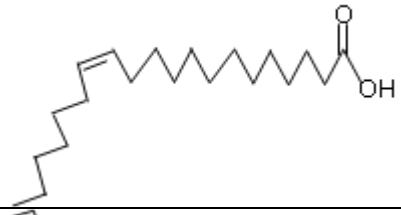
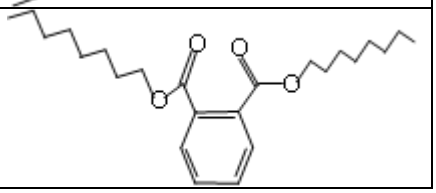
17	naphthalene	19.899	$C_{10}H_8$	128	2.984	
18	1-methyl-4-(1-methyl-2-propenylbenzene)	20.125	$C_{13}H_{18}$	146	3.313	
19	IH-indene,2,3-dihydro-4,7-dimethyl	20.494	$C_{11}H_{14}$	146	1.500	
20	1,6-dimethyl-2,3-dihydroindene	22.345	$C_{11}H_{14}$	146	1.758	
21	benzocycloheptatriene	23.834	$C_{11}H_{14}$	141	1.758	
22	1,2-benzene dicarboxylic acid, bis(2-methyl propyl) ester	36.163	$C_{16}H_{22}O_4$	149	2.336	
23	cis-13-eicosenoic acid	38.058	$C_{20}H_{38}O_2$	292	36.628	
24	Dioctylphthalate	43.150	$C_{24}H_{38}O_4$	299	2.062	

Table 2: Anthelmintic activity of *Vernonia colorata* leaf essential oil against *Lumbricus terrestris*

Concentration	No. of worms	No. of dead worms (16h)	% mortality
100ugcm^{-3}	5	5.00 ±6.00	100%
50ugcm^{-3}	5	3.66 ±1.11	73%
25ugcm^{-3}	5	1.33 ±0.73	26.70%
0. 00 ugcm^{-3}	5	0.00 ±0.00	0.00%

Values are means ±standard deviation of triplicate determination

Table 3: Insecticidal bioassay of *Vernonia colorata* leaf essential oil

Concentration	No. of mosquitoes	No. of dead ones	% mortality
50 ugcm^{-3}	10	4	40%
100 ugcm^{-3}	10	6	60%
150 ugcm^{-3}	10	9	90.00%
200 ugcm^{-3}	10	10	100.00%
0.00 ugcm^{-3}	10	0.0	0.0%

Table 4: Larvicidal bioassay of *Vernonia colorata* leaf essential oil

Concentration	No. of mosquitoes	No. of dead ones	% mortality
50ugcm^{-3}	10	4	40%
100ugcm^{-3}	10	6	60%
200ugcm^{-3}	10	9	90.00%
400ugcm^{-3}	10	10	100.00%
0.0ugcm^{-3}	10	0.0	0.0%

Table 5: Antimicrobial sensitivity test for *Vernonia colorata* leaf essential oil

Microbial isolates	**100ugcm^{-3}**	**50ugcm^{-3}**	**25ugcm^{-3}**	**12.5ugcm^{-3}**	Control
Candida albicans	20.0mm	18.0	15.5	10.0	21.0
Rhizopus nigricans	11.5mm	12.0	9.0	10.5	11.0
Staphylococcus aureus	9.5	10.0	9.0	9.0	10.0
Streptococcus faecalis	13	11.0	8.0	9.0	12.0
Pseudomonas aeruginosa	10	9.0	9.0	8.0	12.0
Salmonella typhi	11	10.0	11.0	9.0	15.0

Table 6: Minimum inhibitory, minimum bacterial/fungicidal concentrations of *Vernonia colorata* leaf essential oil

Microbial isolate	MIC	MBC	MFC
Candida albicans	0.791 ugcm^{-3}	-	
Rhizopus nigricans	0.781 ugcm^{-3}	-	
Staphylococcus aureus	0.891 ugcm^{-3}		-
Streptococcus faecalis	3.125 ugcm^{-3}		-
Pseudomonas aeruginosa	0.781ugcm^{-3}		-
Salmonella typhi	0.781ugcm^{-3}		-

The results are shown in tables 1 to 6. Table 1 shows that the leaf essential oil of *Vernonia colorata* contains twenty four organic compounds most of which are hydrocarbons while table 2 shows that the essential oil has pronounced anthelmintic property. Larvicidal and insecticidal activities of the essential oil are presented in Tables 3 and 4. The work shows that the oil is toxic to the *Anopheles* mosquito and its larvae. The results of antimicrobial susceptibility and minimum inhibitory concentrations of the essential oil against selected pathogens are presented in tables 5 and 6 respectively.

4. Discussion

4.1 Chemical composition: Gc-Ms analysis (Table 1) shows that *Vernonia colorata* leaf essential oil contains twenty four organic compounds. Twenty one of these are hydrocarbons, most of which are aromatic compounds. All these are being identified for the first time in *Vernonia colorata* leaf essential oil. These compounds include dioctypalmitate (2.06%), cis-13-eicosenic acid (36.23%), 1,2-benzenedicarboxylic acid bis(2-methylpropyl ester) (2.34%), 1-methyl-4-(1-methyl-2-proply)benzene (3.31%), naphthalene (2.98%), 2,4-dimethylstyrene (3.76%), 1,4-diethylbenzene (3.02%), 1,2,4,5-tetramethylbenzene (2.27%), o-cymene (3.95%), 1-methyl-3-proplybenzene (2.65%), mesitylene (2.23%), 1-ethyl-2-methylbenzene (5.92%), 1-ethyl-3-methylbenzene (3.19%), o-xylene (3.82%) and p-xylene (8.16%).

P-Xylene has antifungal, antibacterial and antioxidant properties [14]. 1-Ethyl-3-methylbenzene is also an antifungal agent. P-Xylene is a major raw material for the manufacture of terephthalic acid employed in the manufacture of Terylene fibres. Ethylbenzene is used in the manufacture of styrene which is polymerized to a common plastic known as polystyrene. 1, 2, 3-Trimethylbenzene is used in jet fuel to prevent formation of solid particles which might damage the engine. Propylbenzene is used as a non-polar solvent in the industry including printing and dying of textiles and manufacture of methylstyrene [15] while methyl-3-propylbenzene has antioxidant activity [9].

4.2 Anthelmintic activity: The work shows that *Vernonia colorata* leaf essential oil has anthelmintic activity against adult earthworm (*Lumbricus terrestris*). The earthworm was used because it shows anatomical and physiological resemblance with intestinal round worms and therefore serves as a suitable model for screening of anthelmintic drugs [14, 16]. Table 2 shows that none of the worms died in the control after sixteen hours while 26.7%, 73% and 100% died on exposure of the worms to the essential oil at the concentrations of 25, 50 and 100µgcm^{-3} respectively. This shows that the essential oil has some anthelmintic activity which is dose dependent. The observed LC_{50} after sixteen hours is 38µgcm^{-3}. The work therefore supports the use of fresh *Vernonia colorata* leaves as vermicide in traditional medicinal practice.

4.3 Insecticidal and larvicidal activity: Tables 3 and 4 show that *Vernonia colorata* leaf essential oil has both insecticidal and larvicidal activity against adult *Anopheles* mosquito and its larvae. Since the control showed no activity against the mosquito and its larvae, it is clear that the essential oil is responsible for the observed insecticidal and larvicidal activities. The LC_{50} after sixteen hours is 75 and 50µgcm^{-3} for the larvae and adult mosquitoes respectively. This shows that the essential oil is more toxic to the adult mosquito under the condition of the measurements. Dioctylphthalate identified in the essential oil is reported to be toxic to adult *Anopheles* mosquito and its larvae [17]. Naphthalene and cymene, also identified in the essential oil are known insecticides [18]. It is therefore the Dioctylphthalate, naphthalene, cymene and some other organic compounds identified in the oil that are responsible for the insecticidal and larvicidal properties of *Vernonia colorata* leaf essential oil against *Anopheles* mosquito.

4.4 Antimicrobial activity: Table 5 shows that *Vernonia colorata* leaf essential oil has both antibacterial and antifungal activities against *Staphylococcus aeures, Streptococcus faecalis, Pseudonomas aeruginosa, Salmonella typhi, Candida albicans* and *Rhizopus nigricans.* Table 6 further

shows that these microbes all have the minimum inhibitory concentration value, 0.781μgcm^{-3} except *Streptococcus faecalis* where it is 3.125 μgcm^{-3}. This shows that the essential oil has antibacterial activity against both gram negative and gram positive bacteria as well as fungicidal activity. The observed antimicrobial and anthelmintic activities are scientific evidence in support of the use of *Vernonia colorata* leaf in herbal medicine as antimicrobial agent and as vermicide. Some of the identified compounds are of known antimicrobial activity. For example p-xylene has antifungal and antibacterial activity. Also 1-ethyl-3-methylbenzene and 1, 2, 3-trimethylbenzene etc have antifungal and antibacterial activities [15].

5. Conclusion

Vernonia colorata leaf essential oil contains a number of organic compounds which are responsible for its biological activities and therapeutic applications. These compounds are being identified for the first time in *Vernonia colorata* leaf essential oil. A good number of these compounds will serve as useful drugs, insecticides and industrial raw materials. Although biological activity is commonly attributed to the major constituents of plants, such activities are commonly modulated by the minor constituents [18, 19].

6. Conflict of interest

The authors declare no conflict of interest

7. References

1. Etukudo I. Ethnobotany–Conventional and traditional use of plants, Verdit press Uyo, Nigeria, 2003.
2. Morah FNI, Ekpoko MM. Antimicrobial activity of *Vernonia colorata* and *Vernonia amygladina* stem bark. International Journal of Engineering and Science Research. 2015; 4(10):219-222.
3. Oliver-Bever P. Medicinal plants in tropical West Africa, Cambridge University Press, 1986.
4. Idris HM, Mann A, Kabiru AY, Busari MB. *In-vivo* antiplasmodial activity Gc-Ms analysis of *Vernonia colorata* (willd) Drake leaf. European Journal Medical Plants. 2016; 14(3):1-11.
5. Kraf C, Janeet-Siems K, Siems K, Jakupovic J, Mavis S, Bienzle U. Antiplasmodial evaluation of medicinal plants from Zimbawe, Phytotherapy Research. 2003; 17(2):123-128.
6. Cioffa G, Sanogo R, Diallo D, Romuisi J, Dethommas N. New compounds from an extract of *Vernonia colorata* leaves with anti-inflammatory activity. Journal of Natural Product. 2004; 67(3):389-394.
7. Rabe I, Stadem J. Isolation and identification of antibacterial compounds from *Vernonia colorata* Journal Ethnopharmacology. 2002; 80(1):91-94.
8. Reid KA, Jogar AK, Van Staden. Isolation of antibacterial vernodalin from traditionally used *Vernonia colorata*. South African Journal Botany. 2001; 67(1):71-73.
9. Morah FNI, Ashipu LB. Chemical composition and antimicrobial activity of essential oil from *Heinsia crinita*. American Journal of Essential Oil and Natural Product. 2017; 5(2):23-28.
10. NCCLC. National Committee for Clinical Standards. Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically, 4th ed. NCCLS approved standards, NCCLS documents M_7-A_4. 1997.
11. Morah FNI, Apebende GC. Chemical constituents, antimicrobial and anthelmintic activity of petroleum ether extract of aerial part of *Cleome rutidosperma*. International Journal of Herbal Medicine. 2018; 6(4):22-25.
12. Lauver H, Zerroug MM, Sahli F, Cker AN, Valentini G, Ferretti O. Composition and antimicrobial activity of *Ammoides pusilla* (Brot) Breister essential oil. Journal of Essential Research. 2003; 15:135-138.
13. Hammouche N, Leon-Golzates AJ, Navarro I, Bailila F, Benalkioua S, Martin-Cordero. Phytochemical profile and antibacterial activity of *Retama ratam* and *R. sphaerocarba* calodee from Algeria. Natural Product Com. 2017; 12:1857-1860.
14. Morah FNI, Apebende GC. Chemical composition, anthelmintic and antibacterial activity of essential oil of the aerial part of *Cleome rutidosperma*. International Journal of Advanced Science Research 2018; 3(4):48-54.
15. Morah FNI, Emehige ER, Mowang MM. Chemical composition and antimicrobial activity of *Nauclea latifolia* leaf essential oil. International Journal of Chemical and Biological Science. 2018; 11:44-50.
16. Morah FNI, Apebende GC. Chemical composition, anthelmintic and antimicrobial activity of the chloroform fraction of ethanol extract of aerial part of *Cleome rutidosperma*. Edorium Journal of Public Health. 2018; 5:1-6.
17. Morah FNI, Uduagwu DN. Chemical composition, antioxidant and larvicidal activity of *Alchornea laxiflora* (Benth) leaf extracts. Edorium Journal of Pharmacology. 2017; 1:1-8.
18. Begnault-Roger C. The potentials of botanical essential oils for insect pest control. Integrated Pest Management Revised. 1997; 2:25-34.
19. Morah FNI. Medicinal plants and health care delivery 45thInaugural lecture of the University of Calabar, Nigeria, 2009.