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Chemical composition and antimicrobial activity of *Nauclea latifolia* leaf essential oil

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Abstract

The work is aimed at the determination of the chemical composition and antimicrobial activity of *Nauclea latifolia* leaf essential oil. The fresh leaves were ground and steam distilled to get the essential oil. Part of the essential oil was used for gas-chromatographic separation. The individual constituents were identified by mass spectrometry using GC-MS instrument. The antimicrobial susceptibility test of the essential oil was carried out using agar disc diffusion method while broth method was employed for determination of minimal inhibitory, minimal bactericidal and minimal fungicidal concentrations of the essential oil against the test pathogenic microbes. The present work shows that *Nauclea latifolia* leaf essential oil contains forty seven compounds which are being identified for the first time in *Nauclea latifolia*. The present work also shows that the essential oil has greater activity against gram negative bacteria than gram positive bacteria. It also has antifungal activity.

Key words: *Nauclea latifolia*, leaf essential oil, chemical composition, antimicrobial activity

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1. Introduction

Nauclea latifolia (Rubiaceae) is a versatile African medicinal plant. It is a savanna shrub or tree which grows abundantly in Nigeria. It also strives in rain forest region. The leaves and stem are used in herbal medicine for control of malaria [1-4], oral sepsis and dental caries [5-8]. It also has anti-viral activity [9], anthelmintic and diuretic activities [10]. Children eat the ripe fruits. These fruits have been shown to be good livestock feed [11] and they contain a lot of nutrients. A lot of alkaloids have been isolated from this plant. These include naucleofoline [12], naucleofine [13], naucleatine and naucleafine [14]. It also contains several monoterpene indole alkaloids naucleamide A to E [15].

Seed contain 12.4% of yellow oil with refractive index (1.44), viscosity (38cst), melting point (10-16°C), specific gravity (O.82), moisture content (5.71%), iodine value (140), enthalpy of combustion (41kJg^{-1}), acid value (4.5), ester value (189) and free fatty acid as oleic (1.37). The high iodine value of 140 indicates that it is a drying oil and will be suitable for the manufacture of paints, varnishes, linoleum and water proofing of fabrics etc. [16]. A lot of microbial pathogens have developed resistance to the conventional antibiotics. The use of medicinal plants for the control of human and livestock infections is increasing in popularity. This is because these herbal drugs are cheap, readily available and environmental friendly [17].

Nauclea latifolia has been employed in herbal medicine for the control of different microbial infections. The present work is therefore aimed at extraction and determination of chemical constituents of its leaf essential oil. It is also aimed at evaluation of antifungal and antibacterial activity of essential oil from *Nauclea latifolia* leaf.

2. Material and Methods

Nauclea latifolia leaves were harvested from behind college of medicine block, University of Calabar. It was authenticated by staff of the Herbarium unit, Botany Department, University of Calabar, Calabar, Nigeria. They were rinsed with distilled water, ground and subjected to steam distillation to obtain the essential oil as steam distillate. The distillation lasted for 1 ½h.

The chemical composition of the essential oil was determined using Agilent Hewlett-packared 7980A gas chromatography- mass spectrometer with triple detector and auto injector (10 µm syringe). Helium was used as carrier gas at a constant rate of 1cm^3 min^{-1}. The column consists of a 30 m length, 0.25 µm diameter and thickness of 250 µm fused silica capillary coated with poly-dimethyl-siloxane. Ion source temperature is 25°C, pressure is 16.2ps with 1µm injector in split mode with split ratio of 1:50 with injection temperature of 300°C. The column temperature was raised at 35°C for 5 min and raised to 150°C at the rate of 40°C min^{-1}. The temperature was further raised to 250°C at a rate $20°Cmin^{-1}$. 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 the supplier of instrument was used to control the system and to acquire the data. Identification of compounds was carried out by comparing the mass spectra obtained with those of the standard mass from National Institute of Standard and Technology (NIST) library.

Antimicrobial susceptibility test was done using agar disc diffusion method. The following microorganisms were used: the gram negative *Escherichia coli* and *Pseudomonas aeruginosa*, gram positive *Staphylococcus aureus* and *Streptococcus feacalis* and the fungi, *Candida albicans* and *Aspergillus niger*. All these microbes are clinical isolates. These microbes were cultured and maintained using methods of Cruicksharak [19]. Essential oil was diluted with hexane to give solutions of 6.25, 12.5, 25, 50 and 100 μgcm^{-3}. Nutrient agar and Saboarand's agar were used for bacteria and fungi respectively. Sterilized filter paper discs were separately soaked in the solutions containing different levels of the essential oil. They were placed on different plates constituting the different test organisms. They were incubated at 37°C for 24h for bacteria and 48h for fungi. After incubation, the zone of inhibition was observed for the different plates.

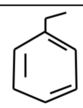
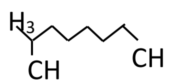
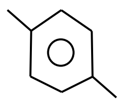
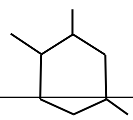
For the determination of minimal inhibitory concentration (MIC), 50, 25, 12.5, 6.25 and 3.13 μgcm^{-3} of the essential oil was placed in the different test tubes and $1cm^3$ of hexane was added to each of them. Peptone water (Muccller Hinton broth) $4cm^3$ was added followed by addition of $4cm^3$ of 24h–broth culture of the microorganism. The test tubes were all sealed with sterile corks and incubated at 37°C for 24h. Thereafter the test tubes were observed for clearance or turbidity. The first test tube with high degree of clearance is taken as the minimum inhibitory concentration, MIC, while the one preceding the MIC is regarded as minimal bactericidal concentration, MBC, or minimal fungicidal concentration (MFB) for bacteria and fungi respectively. The procedure was separately carried out for *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus feacalis*, *Candida albicans* and *Aspergillus niger*.

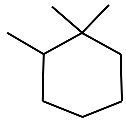
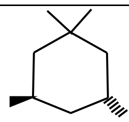
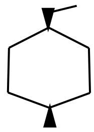
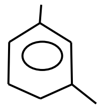
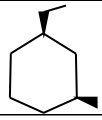
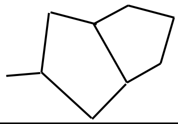
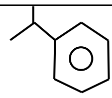
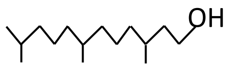
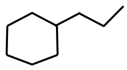
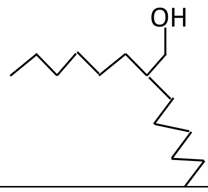
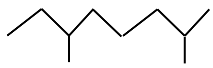
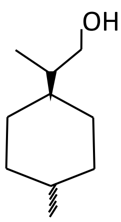
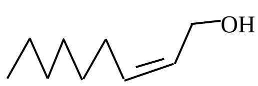
3. Results and Discussions

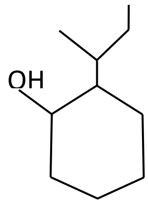
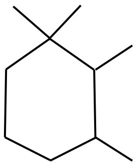
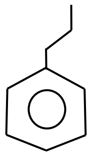
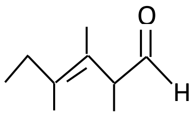
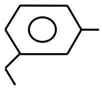
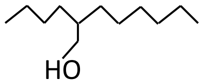
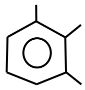
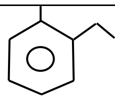
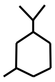
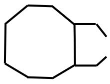
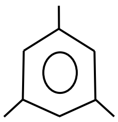
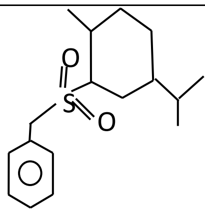
Table 1 shows GC-MS analysis of *Nauclea latifolia* leaf essential oil. The antimicrobial sensitivity of essential oil on selected microbial pathogens is shown in the Table 2 while minimal inhibitory, minimal bactericidal and minimal fungicidal concentrations are shown in Table 3.

Forty seven compounds were identified in *Nauclea latifolia* leaf essential oil. Thirty five of these compounds are hydrocarbons. All these compounds are being reported for the first time in *Nauclea latifolia*. These include the ethylbenzene (3.48%), 2-methyloctane (3.57%), p-xylene (16.24%), cis-1-ethyl-3-methylcylohexane (1.89%), nonane (6.79%), 2-methyloctahydropenetalene (2.39%), propyl-cyclohexane (1.92%), 2,6-dimethylloctane (2.72%), 1-ethyl-3-methylbenzene (3.98%), 1,2,3-trimethylbenzene (4.21%), mesitylene (7.29%), 1-ethyl-3-methylbenzene (3.98%), decane (4.10%), 1,2,5-trimethylbenzene (2.14%) and undecane (6.50%).

Table 1: Gas Chromatography-Mass Spectroscopy Analysis of Essential Oil from *Nauclea latifolia*

S/N	Compound Name	Retention Time (Minutes)	Molecular Formula	Relative Molecular Mass	Percentage composition	Chemical Structure
1	Ethylbenzene	5.137	C_8H_{10}	106	3.01	
2	p-methyl octane	5.268	C_9H_{20}	128	3.92	
3	p-xylene	5.425	C_8H_{10}	106	16.238	
4	1,2,4-trimethylcyclohexane	5.600	C_9H_{18}	126	0.525	

5	1,1,2-trimethyl Cyclohexane	5.769	C_9H_{18}	126	0.767	
6	1,1,3,5-tetramethyl cyclohexane trans-	5.931	$C_{10}H_{20}$	140	0.548	
7	Cis-1-ethyl-4-methyl-cyclohexane	5.963	C_9H_{18}	126	1.888	
8	m-xylene	6.307	C_8H_{10}	160	5.095	
9	Nonane	6.676	C_9H_{20}	128	6.796	
10	Cis-1-ethyl-3-methyl-cyclohexane	6.832	C_9H_{18}	126	1.379	
11	Pentalene, octahydro-2-methyl	7.383	C_9H_{16}	124	2.398	
12	1-octadecyne	7.589	$C_{18}H_{34}$	250	0.588	
13	(1methylethy1)-Benzene	7.820	C_9H_{12}	120	0.749	
14	3,7,11-trimethyl-1-Decanol	7.920	$C_{15}H_{12}O$	228	1.358	
15	Propyl cyclohexane	8.008	C_9H_{18}	126	1.923	
16	2-Hexyl-1-octanol	8.178	$C_{14}H_{30}O$	214	0.615	
17	2,6-dimethyloctane	8.415	$C_{10}H_{22}$	142	2.721	
18	β,4-dimethyl-trans-cyclohexane ethanol	8.627	$C_{10}H_{20}O$	156	0.838	
19	(z)- 2-Nonen-1-ol	8.802	$C_9H_{18}O$	142	1.876	

20	2-(1-methylpropyl)-cyclohexanol	9.128	$C_{10}H_{20}$	156	0.942	
21	1,1,2,3-tetramethyl cyclohexane	9.409	$C_{10}H_{20}$	140	1.806	
22	Propylbenzene	9.516	C_9H_{12}	120	0.539	
23	2,3,4-Trimethylhex-3-enal	9.115	$C_9H_{16}O$	140	0.857	
24	1-ethyl-3-methyl benzene	9.409	C_9H_{12}	120	3.980	
25	2-butyl-1-octanol	9.522	$C_{12}H_{26}O$	186	1.190	
26	1,2,3-trimethyl benzene	9.766	C_9H_{12}	120	4.206	
27	6,10,13-trimeyhltetradecanol	10.097	$C_{17}H_{16}O$	256	1.236	
28	1-ethyl-2-methyl benzene	10.460	C_9H_{12}	120	0.933	
29	m-Menthane,(1S,3R)-(+)	10.598	$C_{10}H_{20}$	140	0.874	
30	1,2-diethylcyclooctane	10.904	$C_{12}H_{24}$	168	0.742	
31	Mesitylene	11.536	C_9H_{12}	120	7.279	
32	Decane	11.911	$C_{10}H_{22}$	142	4.602	
33	(5-Isopropyl-2-methylcyclohexyl) Sulfonylmethyl)benezene	13.406	$C_{17}H_{26}O_2S$	294	0.912	
34	1-Hexadecyne	13.632	$C_{16}H_{30}$	222	0.990	

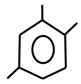
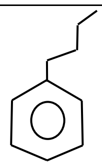
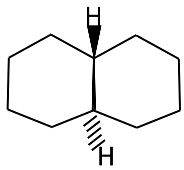
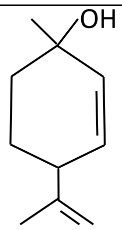
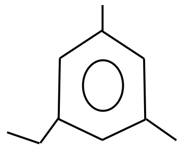
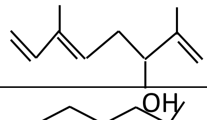
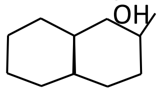
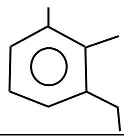
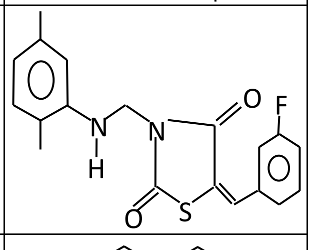
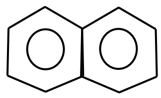
35	1,2,4-trimethyl benzene	14.801	C_9H_{12}	120	0.990	
36	4-methyldecane	14.945	$C_{11}H_{24}$	156	16.183	
37	Butyl-cyclohexane	15.583	$C_{10}H_{20}$	1.40	1.070	
38	Trans-decahydro Naphthalene	15.884	$C_{10}H_{18}$	138	1.253	
39	Cis-p-mentha-2,8-dien-1-ol	17.704	$C_{10}H_{16}O$	152	0.807	
40	1-ethyl-3,5-dimethyl benzene	18.079	$C_{10}H_{14}$	134	0.69	
41	1,5,7-octatrien-3-ol, 2,6-dimethyl	19.218	$C_{10}H_{16}O$	152	0.683	
42	Naphthalene, decahydro-2-methyl	19.993	$C_{11}H_{20}$	152	1.026	
43	Undecane	24.741	$C_{11}H_{24}$	156	0.683	
44	1-ethyl-2,3-dimethylbenzene	25.485	$C_{10}H_{14}$	134	2.497	
45	3-(2,5-dimethylanilinomethyl)-5-(3-fluorobenzylidene)-2,4-thiazolidinedione	26.568	$C_{18}H_{17}O_2N_2SF$	-	0.559	
46	Naphthalene	35.131	$C_{10}H_8$	128	0.735	
47	Dodecane	39.428	$C_{12}H_{26}$	170	0.746	

Table 2: Antimicrobial sensitivity for *Nauclea latifolia* leaf essential oil

Clinical isolate	100 μgcm^{-3}	50 μgcm^{-3}	25 μgcm^{-3}	12.5 μgcm^{-3}	6.25 μgcm^{-3}	Control
Escherichia coli	10mm	9 mm	8 mm	8 mm	7 mm	10 mm
Staphylococcus aureus	8 mm	7 mm	6 mm	6 mm	7 mm	6 mm
Pseudomonas aeruginosa	12 mm	10 mm	10 mm	9 mm	7 mm	11 mm
Streptococcus feacalis	13 mm	12 mm	11 mm	11 mm	8 mm	11 mm
Candida albicans	7 mm	7 mm	6 mm	6 mm	6 mm	6 mm
Aspergillus niger	8 mm	6 mm	6 mm	6 mm	6 mm	6 mm

Table 3: MIC and MBC/MFC of *Nauclea latifolia* leaf essential oil

Isolate	MIC	MBC/MFC
Escherichia coli	1.57μgcm^{-3}	3.13μgcm^{-3}
Staphylococcus aureus	12.5μgcm^{-3}	25.0μgcm^{-3}
Pseudomonas aeruginosa	1.57μgcm^{-3}	3.13μgcm^{-3}
Streptococcus feacalis	6.25$\mu g\ cm^{-3}$	12.5$\mu g\ cm^{-3}$
Candida albicans	1.57$\mu g\ cm^{-3}$	3.13$\mu g\ cm^{-3}$
Aspergillus niger	3.13$\mu g\ cm^{-3}$	6.25$\mu g\ cm^{-3}$

The principal constituents of the essential oil are of known industrial applications. P-xylene with the highest percentage is a major raw material for the manufacture of tetraphthalic acid, employed for the manufacturing of the terelyne fibres. Ethyl benzoate is used in the industry for production of styrene which [15] polymerized to a common plastic known as polystyrene [20]. Nonane is a component of kerosene, fuel additive and a component of biodegradable detergents. 1,2,3-trimethylebenzene is used in jet fuel to prevent formation of solid particles which might damage the engine while undecane is a sex attractant for a number of insects and an alert signal for variety of ants. Propylbenzene is used as non-polar solvent in industries including printing and dying of textiles and manufacture of methyl styrene.

Table 2 shows that the essential oil has some inhibitory effect on the test organisms. It is shown in table 3 that minimal inhibitory concentration (MIC) of 1.57μgcm^{-3} is observed for the *Escherichia coli* and *Pseudomonas aeruginosa* both of which are gram negative bacteria. The MIC of 6.52 and 12.8μgcm^{-3} are recorded for the gram positive *Streptococcus feacalis* and *Staphylococcus aureus* respectively. This shows that the gram negative bacteria are more sensitive to the *Nauclea latifolia* essential oil than the gram positive ones. The *Nauclea latifolia* leaf essential oil can therefore not serve as a broad spectrum antibacterial agent. The essential oil is also very effective against test fungi. *Candida albicans* has M/C of 1.57μgcm^{-3} while the *Aspergillus niger* has MIC of 3.13μgcm^{-3}. These chemical constituents of the essential oil are responsible for its highly efficient biological activities. Although biological activity of an essential oil is often attributed to its major constituents, such biological activities are known to be modulated by the minor constituents through several antagonistic, synergistic and additive effects [22].

4. Conclusions

Nauclea latifolia leaf essential oil contains forty seven constituents which are being identified for the first five in *Nauclea latifolia*. Essential oil has both antifungal and antibacterial activities. It is more active against the gram negative bacteria than the gram positive bacteria. A good number of these identified compounds will serve as an important industrial raw materials and useful drugs.

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