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Chemical composition and antimicrobial activity of essential oil from *Heinsia crinita* leaf

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Abstract
The aim of this work is to determine the chemical constituents and antimicrobial activity of essential oil from *Heinsia crinite* leaf. The fresh leaves of *Heinsia crinita* were ground and steam-distilled to get the essential oil. Constituents of the essential oil were separated by gas chromatography while its individual constituents were identified by mass spectrometric analysis using a GC-MS instrument. The antimicrobial susceptibility test of the essential oil was carried out through agar disc diffusion method while the broth method was employed for determination of MIC, MBC and MFC of the essential oil against the test pathogenic microorganisms. The isolated essential oil of *Heinsia crinita* leaf contains forty organic compounds which are being identified for the first time in this plant species. Some of these compounds like phytol and oleic acid are known to have biological activities. The oil inhibited the growth of *Escherichia coli, Salmonella typhi, Staphylococccus aureus, Bacillus subtilis, Aspergillusnigar* and *Saccharomyces cereviseae.* The oil has antimicrobial activity against gram positive and gram negative bacteria as well as fungi.

Keywords: *Heinsia crinita*, essential oil, chemical composition, antimicrobial activity

1. Introduction
Heinsia crinita (Rubiaceae) is an edible vegetable which is used in making soup in parts of Southern Nigeria. The fruits are edible with reddish or yellow sweet pulp when ripe [1]. It is used in treatment of craw-craw and other skin diseases [2] as well as other ailments like infertility, hypertension, acute bacterial infections, sore throat and catarrh [3, 4, 5], The presence of saponins, flavonoids, tannins, glyocosides and proteins in the aqueous methanol and hexane extracts of *Heinsia crinita* leaves had been reported [4]. The aqueous extract of *Heinsia crinita* has been reported to have higher total phenol and flavonoids than the methanol extract [6]. The identified phenolics includegallic acid, catechin, chlorogenic acid, caffeic acids, quelitin, quercetinankaempterol [6]. Also identified from the leaf hexane extract are 11-tetradece-1-o1 acetate heptacobanoic acid, heptadecanoic acid, phytol, oleic acid and nonadecanoicacid [7]. To the best of our knowledge, there is no report on the essential oil of *Heinsia crinita* leaf. The present work is thereforefocused on the chemical composition and antimicrobial activity of essential oil from *Heinsia crinita* leaf.

2. AIM
Heinsia crinita is used as a remedy for different fungal and bacterial infections. The present work is therefore aimed at isolation and determination of the chemical constituents of its essential oil. It is also aimed at evaluation of its antifungal and antibacterial activity against selected pathogenic microorganisms.

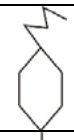
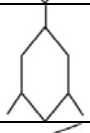
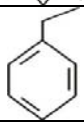
3. Materials and methods
Heinsia crinita leaves were harvested from Uwanse, Calabar South local Government Area of Cross River State, Nigeria. The plant was authenticated by the Herbarium Unit of the Botany Department of the University of Calabar. The fresh leaves were rinsed with deionized water, pulverized and immediately steam distilled for two hours. The essential oil was separated from the distillate with a separatoryfunnel. The volatile oil was analyzed with Gas Chromatography-Mass Spectrometry using an Agilent, Hewlett-Packared (7890A) with triple detector with an injector (10µm syringe). Helium gas was used as the carrier gas. The operating conditions include length, 30cm^3, internal diameter, 0.25µm; thickness, 250µm treated with phenylmethylsilox.

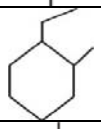
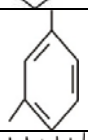
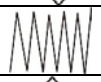
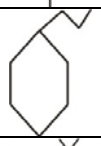
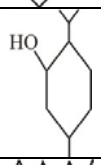
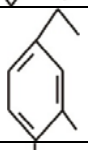
Ion source temperature, 250^{0}Cpressure, 16.2ps; 1µm injector in split mode with split ratio of 1:50 with injection temperature of 300^{0}C.The column temperature was raised at 35^{0}C for 5min and changed to 150^{0}C at rate of 40^{0}C/min, the temperature was raised to 250^{0}C at a rate of 20^{0}C/min and held for 5min before ionization. Microsoft solution software provided by the supplier was used to control the system and to acquire the data. Identification of the compounds was carried out by comparing the mass spectra with those of the standard mass spectra from National Institute of Standard and Technology (NIST) Library. The microbial susceptibility test was carried out using agar disc diffusion method [8, 9]. The following microorganisms were used, *Escherichia coli Salmonella typhi, Bacillus subtillis, Staphylococcus aureus, Aspergillusniger and Saccharomyces cerevisiae.* These are clinical isolates. The bacteria were cultured and maintained by the methods of Cruicksharea [10]. The essential oil was diluted with hexane to give solutions of 12.25, 25.50 and 100 µgcm^{-1}. Nutrient agar was used for bacteria while Sobourand' sagar was used for fungi. Sterilized filter paper disc were separately soaked in the different solutions of the essential oil and dried. They were placed on plates with different microorganisms and incubated at 37^{0}C for 24h for bacterial and 48h for fungi. After incubation the zone of inhibition was observed in the different plates. For determination of minimal inhibitory concentration (MIC) 50, 25, 12.5, 6.25, 3.13 and 1.75 µgcm^{-1} of the essential oil was placed in different test tubes and 1cm^3 of hexane added to each of them. 4cm^3 of peptone water (Mueller Hinton broth) 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 subsequently incubated at 37 ^{0}C for 48h. After incubation the tubes were observed for clearance or turbidity. The tube with highest degree of clearance was taken as the MIC while the tube preceding the MIC tube is regarded as the minimum bactericidal concentration (MBC) for bacterial or minimum fungicidal concentration (MFC) for fungi. This procedure was separately carried out for the six test microorganisms, namely *Escherichia coli, Salmonella typhi, Bacillus subtilis, Staphylococcus aureus, Aspergillus niger* and *Saccharomyces cerevisiae*.

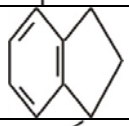
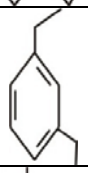
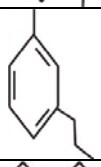
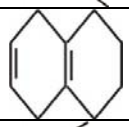
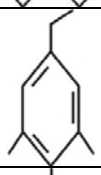
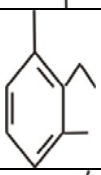
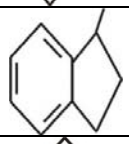
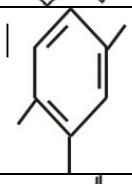
4. Results and discussion

Table 1 show the GC-MS analysis of the essential oil of *Heinsiacrinita* leaf. The oil has fortyconstituents, which are mainly hydrocarbons with some oxygenated compounds. Antibacterial and antifungal sensitivity test results are shown in Table2 while Table3 shows the minimal inhibitory concentrations, minimal bactericidal concentrations and minimal fungicidal concentrations of the essential oil against the test organisms. The oil exhibited antimicrobial activity. Forty natural products were identified in the essential oil of *Heinsiacrinita* leaf. All these compounds are being reported for the first time in *Heinsiacrinita*. 1, 4-Dimethybenzene which accounted for 13.56% of the essential oil is used industrially for the manufacture of polymers like parylene [11]. Phytol (1.45%) is used in the synthesis of vitamin E [12] and has antiradical and antimicrobial activities [13]. It also blocks teratogenic activity of retinol [14]. Identified 1-methyl -3-propylbenzene (1.32%) has antioxidant activity [15] while oleic acid (4.33%) and 9, 12, 15-octadecatrienoic acid -2, 3-dihydroxypropyl ester (0.5%) have anti-inflammatory, hypocholesterolemic, cancer preventive and antihistamine effect [16]. Table 2 shows that the essential oil has inhibitory effect against the fungi (*Aspergillus niger* and *Saccharomyces cervisene),* gram positive (*Bacillus subtilis and Staphylococcus aureus*) and gram negative (*Escherichia coli* and *Salmonella typhi*) bacteria. The growth of all these test organisms was inhibited at the essential oil concentration of 100µgcm$^{-1.}$ *Saccharomyces cervisene* and *Aspergillius niger* have the lowest minimal inhibitory concentration of 1.57 and 3.13 µgcm^{-1} respectively. This shows that the fungi are more sensitive to the essential oil than the bacteria. It was also found that the essential oil is more active against the gram positive *Bacillus subtiles* and *Staphylococcus aureus* than the gram negative *Escherechia coli* and *Salmonella typhi*. The strong antimicrobial activity of the essential oil is due to its chemical constituents such as phytol, hexadecanoic acid and oleic acid etc. Although the 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 [17-20].

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

S/N	Compound name	Retention Time (minutes)	Molecular formula	Chemical structure	Relative Molecular Mass	Percentage Composition
1	Butyl-cyclohexane	5.15	$C_{10}H_{20}$		140	1.09
2	1,2,4-trimethyl- cyclohexane	5.629	C_9H_{18}		126	0.59
3	1,3,5-trimethyl- cyclohexane	5.700	C_9H_{18}		126	1.21
4	Ethylbenzene	6.092	C_8H_{10}		106	5.40

5	1,4-dimethylbenzene	6.556	C_8H_{10}		106	13.56
6	1-ethyl-2-methyl-cyclohexane	7.161	C_9H_{18}		126	2.76
7	1,3-dimethylbenzene	7.381	C_8H_{10}		106	5.75
8	Dodecane	7.938	$C_{12}H_{26}$		170	5.02
9	2-methyloctan-hydropentalene	8.308	C_9H_{16}		124	1.25
10	Propylcyclohexane	8.865	C_9H_{18}		126	1.13
11	5-methyl-2-iso-propylcyclohexanol	9.070	$C_{10}H_{20}O$		156	0.72
12	2,6-dimethyl- octane	9.274	$C_{10}H_{22}$		124	0.82
13	Propylbenzene	9.863	C_9H_{12}		120	1.78
14	1,2-diprophy- cyclopentane	10.114	$C_{10}H_{22}$		154	0.56
15	1-ethyl-3-methyl-benzene	10.303	C_9H_{12}		120	5.82
16	1,2,3-trimethyl- benezene	10.625	C_9H_{12}		120	3.69
17	1-ethyl-2-methyl- benzene	10.971	C_9H_{12}		120	1.35
18	1-methyl-3-(1-methyl ethyl) cyclohexane		$C_{10}H_{20}$		140	0.59

19	1,2,3-trimethyl- benzene	11.630	C_9H_{12}		120	8.08
20	2-(1-hydroxypropyl) – 1,3-dimethyl- cyclopentane	11.803	$C_{10}H_{20}O$		156	0.43
21	4-methyldecane	12.982	$C_{11}H_{24}$		156	0.56
22	Cyclopentano- benzene	13.107	C_8H_{10}		106	1.56
23	1,3-diethyl- benezene	13.830	$C_{10}H_{14}$		134	0.91
24	1-methyl-3- propylbenzene	13.916	$C_{10}H_{14}$		134	1.32
25	1,2,3,4,5,8- hexahydro-naphthalene	14.073	$C_{10}H_{14}$		134	1.04
26	1-ethyl-3,5-dimethylbenzene	14.246	$C_{10}H_{14}$		134	1.73
27	1-methyl-4-prophyl-benzene	14.466	$C_{10}H_{14}$		134	0.42
28	2-ethyl-1,3-dimethylbenzene	14.977	$C_{10}H_{14}$		134	1.86
29	1-methylindane	15.150	$C_{10}H_{12}$		132	0.41
30	1,2,4,5-tetra- methylbenzene	16.391	$C_{10}H_{12}$		132	0.78
31	1-methyl-2(2-propenyl) – benzene	17.137	$C_{10}H_{12}$		132	0.77

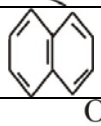
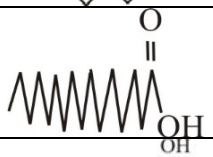
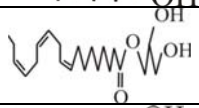
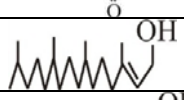
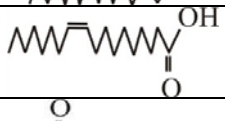
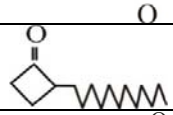
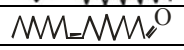
32	1-ethenyl- 4-ethyl- benzene	17.514	$C_{10}H_{12}$		132	0.94
33	Naphthalene	18.724	$C_{10}H_8$		128	0.45
35	n-hexadecanoic acid	39.847	$C_{16}H_{32}O_2$		256	1.31
36	9,12,15-Octadecatrienoic – 2,3-dihydroxypropyl ester	39.925	$C_{21}H_{36}O_4$		352	0.52
37	Phytol	40.020	$C_{20}H_{40}O$		296	1.45
38	Oleic acid	40.310	$C_{18}H_{34}O_2$		282	4.53
39	2-dodecyl- cyclobutanone	40.962	$C_{16}H_{30}O$		238	1.28
40	9-Octadecanal	42.023	$C_{18}H_{34}O$		266	4.17

Table 2: Antimicrobial Sensitivity Test of *Heinsiacrinita* leaf Essential Oil

Isolates	100µg/ml	50µg/ml	25µg/ml	12.5µg/ml	Control
Escherichia coli	26mm	6mm	6mm	6mm	6mm
Salmonella typhi	29mm	6mm	6mm	6mm	6mm
Staphylococcus aureus	22mm	6mm	6mm	6mm	6mm
Bacillus subtilis	27mm	6mm	6mm	6mm	6mm
Aspergillusniger	28mm	10mm	6mm	6mm	11mm
Saccharomyces cereviseae	31mm	18mm	8mm	7mm	7mm

Table 3: Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal or Fungicidal Concentration of *Heinsiacrinita* Essential Oil

Isolates	MIC	MBC
Escherichia coli	25µg/ml	50µg/ml
Salmonella typhi	12.5µg/ml	25µg/ml
Bacillus subtilis	3.13µg/ml	12.5µg/ml
Staphylococcus aureus	6.25µg/ml	25.5µg/ml
Aspergillusniger	3.13µg/ml	6.25µg/ml
Saccharomyces cerevisene	1.57µg/ml	3.13µg/ml

5. Conclusion

Heinsia crinita leaf essential oil contains forty chemical compounds which are being identified for the first time in *Heinsia crinita.* This essential oil has antifungal as well as antibacterial activity against test gram positive and gram negative bacteria. A good number of these identified compounds will serve as useful drugs and important industrial raw materials.

Conflict of interest

The authors declare no conflict of interest.

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