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GC-MS Analysis of Methanol Extract of *Strychnos Innocua* (Delile) Root Bark

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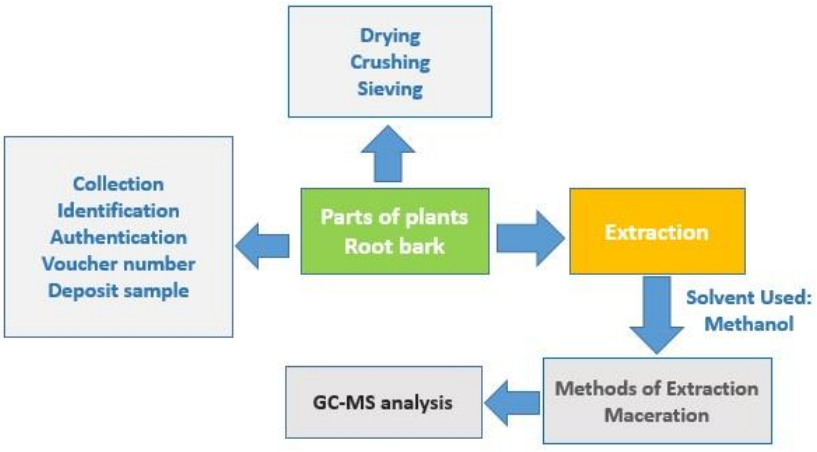
ABSTRACT

The use of plant products as medicines could be traced as far back as the beginning of human civilization. The medicinal plant "*Strychnos innocua* (a *Loganiaceae* plant)" is a deciduous shrub growing 2-12 m tall. This study aimed at using GC-MS to determine the chemical composition and antifungal activity of a root bark methanol extract against *Candida krusei*. The plant sample was collected and identified at Ahmadu Bello University, Zaria. The maceration extraction method was used to obtain the methanol root bark extract. The chromatogram from the GC-MS analysis revealed sixty-four (64) peaks corresponding to fifty-five phytoconstituents from various organic groups. Among the significant compounds discovered are 9,12-Octadecadienoic acid (5.48%), Benzofuran, 6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenyl-, trans- (6.72%), Tetradecanoic acid, 12-methyl-, methyl ester (6.87%), Ethyl Oleate (8.29%), 1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester (9.10%), Linoleic acid ethyl ester (10.31%) with 6-Octadecenoic acid, methyl ester and peak area of 11.42% being considered the highest peak area. In contrast, Bicyclo[13.1.0]hexadecan-2-one with a peak of 0.03% is considered the lowest peak area. It can be concluded that the phytocompounds discovered in the root bark methanol extract of *Strychnos innocua* could be valuable in treating some human ailments.

GRAPHICAL ABSTRACT



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Introduction

Herbs have been used for pharmacological therapy of disease for a long time. Herbs were extensively used in traditional folk healing methods throughout the world [1]. Since the dawn of civilization, plant-based medications have been used as medicines [2]. Researchers studying medicinal plants have looked at various low-cost targets such as prototypic drug discovery and therapeutic compounds development [3].

Secondary metabolites such as flavonoids, alkaloids, triterpenes, glycosides, saponins, tannins, carbohydrates, and essential oils, among others, contain a variety of complex chemical substances that contribute to medicinal plants' therapeutic properties [4, 5]. Various antimicrobial activity investigations of herbal plant extract have shown potential antibacterial efficacy against bacterial and fungal pathogens [25]

Strychnos innocua is a member of the *Loganiaceae* family. The plant is a deciduous shrub with numerous branches that grows between 2 m and 12 m tall and has a smooth, green, or yellow-white powdery bark. However, some plant specimens can grow to be 18 m tall. The 7–40 cm diameter straight trunk is commonly branched from low down [6]. The root decoction is taken as a remedy for gonorrhea, and the fresh roots are used to treat snakebite [6, 7].

Strychnos innocua fruits have been studied for their nutritional, antinutritional, and chemical composition [8-10]. This study aimed to use GC-MS to determine a root bark methanol extract's chemical composition and antifungal activity against *Candida krusei*.

Figure 1. *Strychnos innocua*, showing the leaves and branches

Methods

Collection and Identification

The plant materials were collected from the wild, Soba Local Government Area, Kaduna State, Nigeria. It was then authenticated by Mallam Namadi Sunusi of the herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria – Nigeria. A voucher specimen number (V/N – 01884).

Preparation and extraction of plant material

Then, the root bark was rinsed, air-dried for 28 days, and crushed to a coarse powder. The pulverize plant sample (2000g) was separately macerated successively in n-hexane solvent, ethyl acetate solvent, and methanol solvent according to gradient polarity. The maceration technique involved soaking the pulverized plant materials in an aspirator first with n-hexane (polarity = 0.009) and standing at room temperature for 3 days with frequent agitation. After exhaustive extraction with n-hexane, the procedure was repeated for ethyl acetate (polarity = 0.228) and methanol (polarity = 0.762).

GC-MS Analysis

GC-MS analysis of *S. innocua* methanol root bark extract was performed using GC 7890B, MSD 5977A, Agilent Tech.

The sample was dissolved in ethanol and votex mixed for 1 min followed by centrifuging at 3000 rpm for 10 min. The GC was injected with supernatant (1 µL) and ran for 30 min.

The volatile compounds contained in the sample were analyzed by the GC/MS on an Agilent 7890B gas chromatography (GC) directly coupled to the mass spectrometer system (MS) of an Agilent 5977 A. The ionization voltage and ion source were set at 70 eV and 230 °C, respectively. The oven temperature of the GC was programmed from 80 °C, increased at 15 °C/min to 200 °C, then 5 °C/min to 280 °C, finally end with a 5 min isothermal at 280 °C. Helium was the carrier gas used at 1 mL/min flow rate.

Antifungal Activity

The antifungal was tested against *Candida krusei* and *Aspergillus nigre*. The growth medium was Sabouraud dextrose agar (SDA), and the screening method was the agar well diffusion method. In addition, 0.1 mL of the fungi's standard inoculum was seeded into freshly produced SDA. A sterile cork borer with a diameter of 6 mm was then used to punch a well in the center of the medium. After that, 0.1 mL of the appropriate extract concentration solution was added to the well. The SDA plates were incubated at 30 °C for 7 days. The growth inhibition zone was then seen on the media plates. The zone was measured with a clear ruler, and the result was recorded in millimeters (mm). Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) were determined using broth dilution [26, 27].

Results and Discussion

The combined gas chromatography and mass spectrometry (GC-MS) are used to identify distinct volatile compounds in a sample. The GC-MS chromatogram revealed the presence of fifty-four (54) peaks corresponding to forty-five (45) phytoconstituents from various organic groups. The chromatograms in Figure 1a and Figure 1b revealed the peaks based on RT and area %. The phytoconstituents identified in the extract of methanol root bark of *Strychnos innocua* are presented in Table 1.

Table 1. Phytoconstituents of the extract of methanol root bark of *Strychnos innocua*

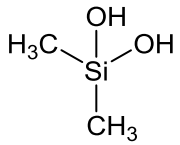
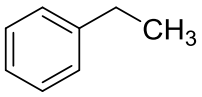
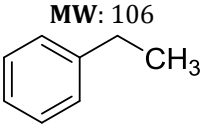
Peaks	Rt	Name of Compounds	Chemical Structure Molecular Formula	Peak Area %
1	2.006	Silanediol, dimethyl	 $C_2H_8O_2Si$ **MW**: 92	0.45
2	2.183	Ethylbenzene	 C_8H_{10} **MW**: 106	0.51
3	2.361	Ethylbenzene	 C_8H_{10} **MW**: 104	0.30

Table 1. Continued

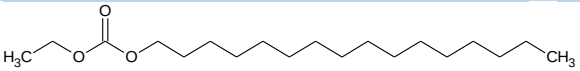
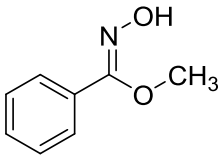
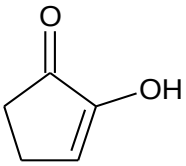
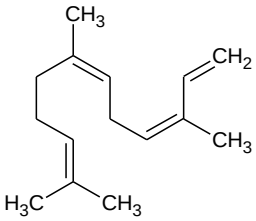
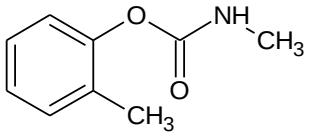
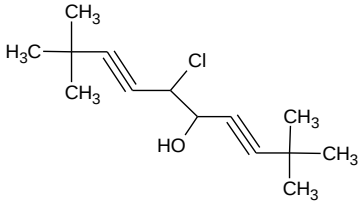
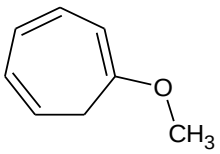
Peaks	Rt	Name of Compounds	Chemical Structure Molecular Formula	Peak Area %
4	2.567	Carbonic acid, ethyl hexadecyl ester	 $C_{19}H_{38}O_3$ **MW**: 314	0.20
5	2.732	Oxime-, methoxy-phenyl-	 $C_8H_9NO_2$ **MW**: 151	0.25
6	2.973	2-Cyclopenten-1-one, 2-hydroxy-	 $C_5H_6O_2$ **MW**: 98	0.13
7	3.465	(Z,Z)-α-Farnesene	 $C_{15}H_{24}$ **MW**: 204	0.15
8	4.014	2-Methylphenyl-N-methylcarbamate	 $C_9H_{11}NO_2$ **MW**: 165	0.35
9	4.192	6-Chloro-2,2,9,9-tetramethyl-3,7-decadiyn-5-ol	 $C_{14}H_{21}ClO$ **MW**: 204	0.10
10	4.518	1,3,5-Cycloheptatriene, 1-methoxy-	 $C_8H_{10}O$ **MW**: 122	0.68

Table 1. Continued

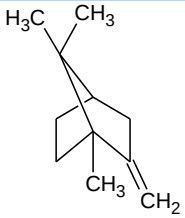
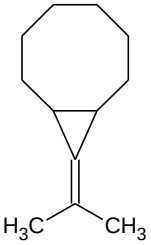
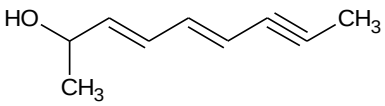
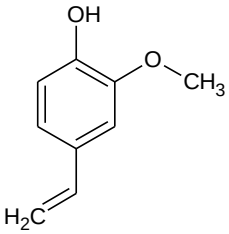
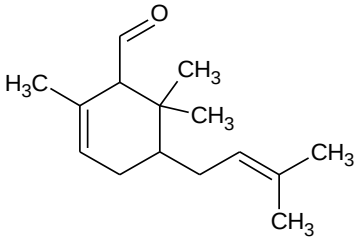
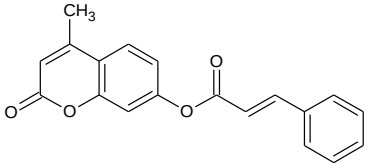
Peaks	Rt	Name of Compounds	Chemical Structure Molecular Formula	Peak Area %
11	4.735	2-Methylenebornane	 $C_{11}H_{18}$ **MW**: 150	0.53
12	4.775	Bicyclo[6.1.0]nonane, 9-(1-methylethylidene)-	 $C_{12}H_{20}$ **MW**: 164	2.27
13	5.136	3,5-Nonadien-7-yn-2-ol, (E,E)-	 $C_9H_{12}O$ **MW**: 136	0.81
14	5.679	2-Methoxy-4-vinylphenol	 $C_9H_{10}O_2$ **MW**: 150	0.61
15	5.937	1-Formyl-2,2,6-trimethyl-3-cis-(3-methylbut-2-enyl)-5-cyclohexene	 $C_{15}H_{24}O$ **MW**: 220	0.30
16	6.126	4-Methylcoumarine-7-cinnamate	 $C_{19}H_{14}O_4$ **MW**: 306	1.18

Table 1. Continued

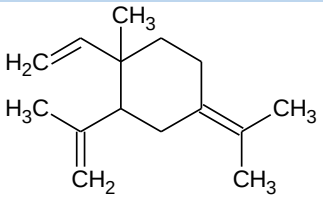
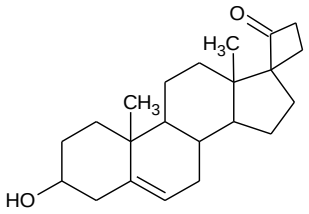
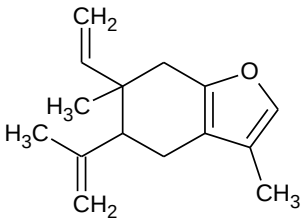
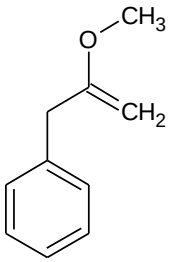
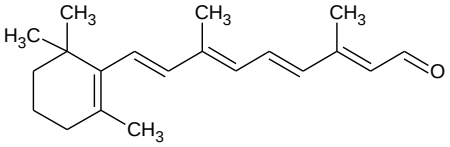
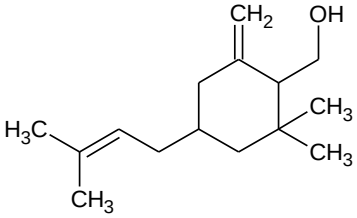
Peaks	Rt	Name of Compounds	Chemical Structure Molecular Formula	Peak Area %
17	6.412	γ-Elemene	 $C_{15}H_{24}$ **MW**: 204	1.06
18	6.606	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3β,17β)-	 $C_{22}H_{32}O_2$ **MW**: 328	0.81
19	6.847	Benzofuran, 6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenyl-, trans-	 $C_{15}H_{20}O$ **MW**: 216	6.72
20	6.915	Benzene, (2-methoxy-2-propenyl)-	 $C_{10}H_{12}O$ **MW**: 148	0.91
21	7.196	Retinal	 $C_{20}H_{28}O$ **MW**: 284	0.15
22	7.487	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane	 $C_{15}H_{26}O$ **MW**: 222	0.38

Table 1. Continued

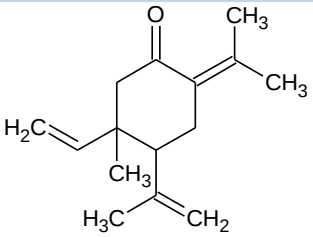
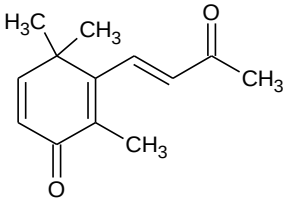
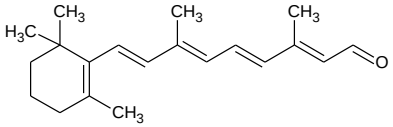
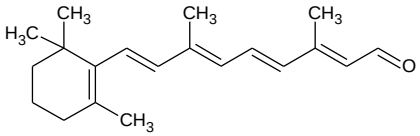
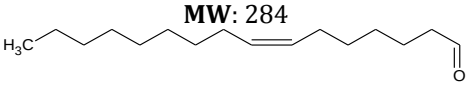
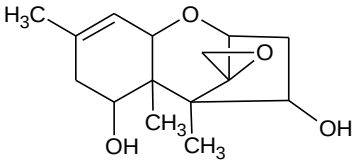
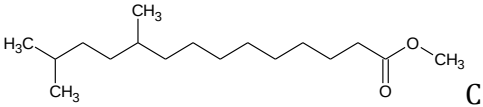
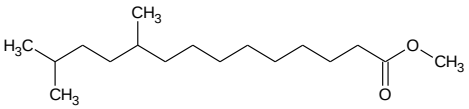
Peaks	Rt	Name of Compounds	Chemical Structure Molecular Formula	Peak Area %
23	7.562	β-Elemenone	 $C_{15}H_{22}O$ **MW**: 218	0.86
24	7.751	2,3-Dehydro-4-oxo-β-ionone	 $C_{13}H_{16}O_2$ **MW**: 204	0.30
25	7.854	Retinal	 $C_{20}H_{28}O$ **MW**: 284	0.81
26	8.020	Retinal	 $C_{20}H_{28}O$ **MW**: 284	1.97
27	8.117	Hexadec-7-enal	 $C_{16}H_{30}O$ **MW**: 238	1.06
28	8.220	7-Hydroxyisotrichodermol	 $C_{15}H_{22}O_4$ **MW**: 266	1.11
29	8.277	Tetradecanoic acid, 10,13-dimethyl-, methyl ester	 $C_{17}H_{34}O_2$ **MW**: 270	0.35
30	8.334	Tetradecanoic acid, 10,13-dimethyl-, methyl ester	 $C_{17}H_{34}O_2$ **MW**: 270	0.60

Table 1. Continued

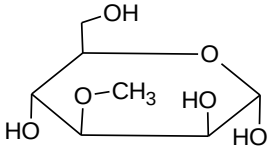
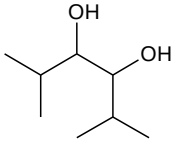
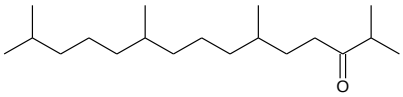
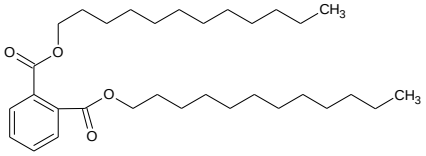
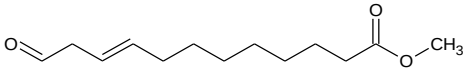
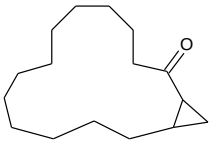
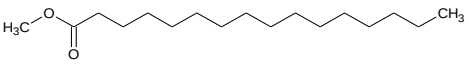
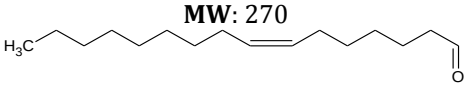
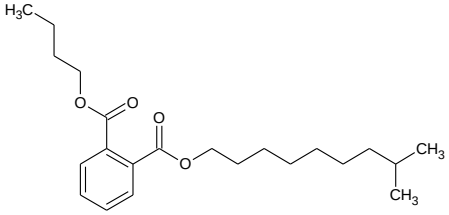
Peaks	Rt	Name of Compounds	Chemical Structure Molecular Formula	Peak Area %
31	8.861	3-Methylmannoside	 $C_7H_{14}O_6$ **MW**:194	1.61
32	9.210	Meso-2,5-Dimethyl-3,4-hexanediol	 $C_8H_{18}O_2$ **MW**: 146	0.30
33	9.736	2,6,10,14-Tetramethylpentadecan-3-one	 $C_{19}H_{38}O$ **MW**: 282	0.10
34	9.868	Didodecyl benzene-1,2-dicarboxylate	 $C_{32}H_{54}O_4$ **MW**: 504	0.19
35	10.137	Methyl 12-oxo-9-dodecenoate	 $C_{13}H_{22}O_3$ **MW**: 280	0.10
36	10.188	Bicyclo[13.1.0]hexadecan-2-one	 $C_{16}H_{28}O$ **MW**: 236	0.23
37	10.451	Hexadecanoic acid, methyl ester	 $C_{17}H_{34}O_2$ **MW**: 270	4.65
38	10.829	Hexadec-7-enal	 $C_{16}H_{30}O$ **MW**: 238	0.40
39	11.144	1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester	 $C_{22}H_{34}O_4$ **MW**: 362	9.10

Table 1. Continued

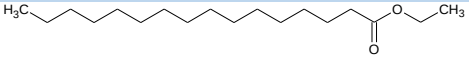
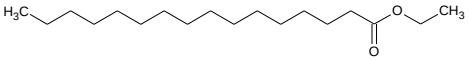
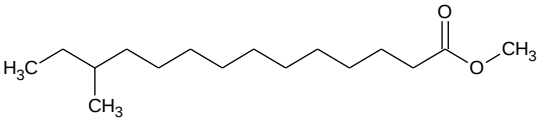
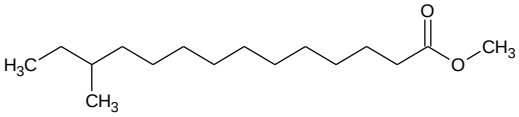
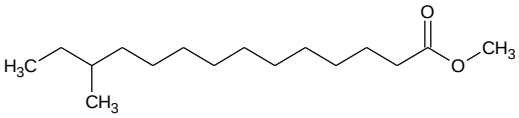
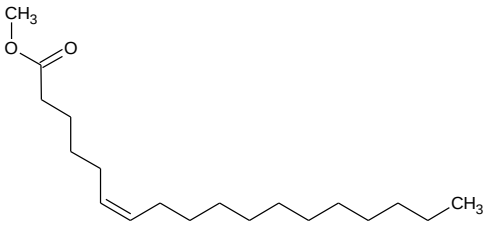
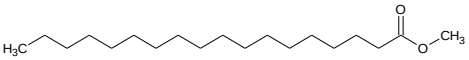
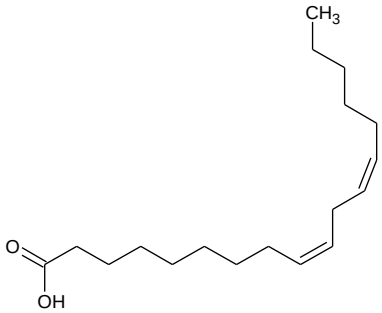
Peaks	Rt	Name of Compounds	Chemical Structure Molecular Formula	Peak Area %
40	11.396	Hexadecanoic acid, ethyl ester	 $C_{18}H_{36}O_2$ **MW**: 284	3.79
41	11.550	Hexadecanoic acid, ethyl ester	 $C_{18}H_{36}O_2$ **MW**: 284	0.30
42	11.870	Tetradecanoic acid, 12-methyl-, methyl ester	 $C_{16}H_{32}O_2$ **MW**: 256	0.30
43	12.546	Tetradecanoic acid, 12-methyl-, methyl ester	 $C_{16}H_{32}O_2$ **MW**: 256	0.30
44	12.963	Tetradecanoic acid, 12-methyl-, methyl ester	 $C_{16}H_{32}O_2$ **MW**: 256	6.87
45	13.055	6-Octadecenoic acid, methyl ester	 $C_{19}H_{36}O_2$ **MW**: 296	11.42
46	13.415	Methyl stearate	 $C_{19}H_{38}O_2$ **MW**: 298	1.67
47	13.804	9,12-Octadecadienoic acid	 $C_{18}H_{32}O_2$ **MW**: 280	5.48

Table 1. Continued

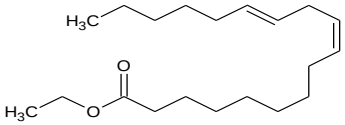
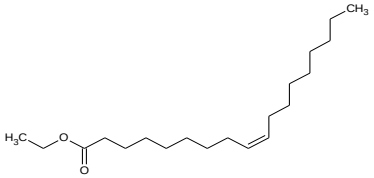
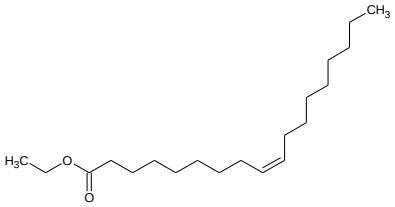
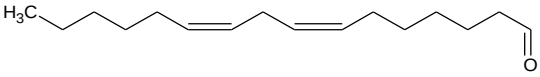
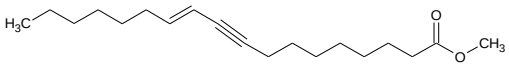
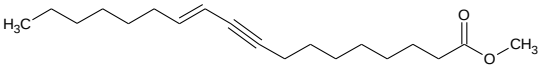
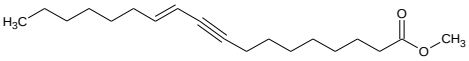
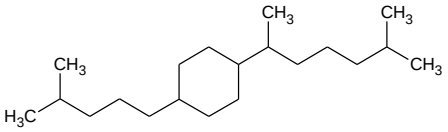
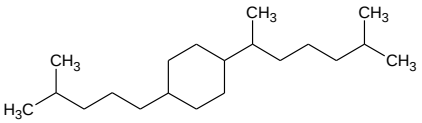
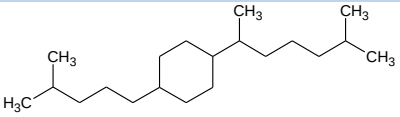
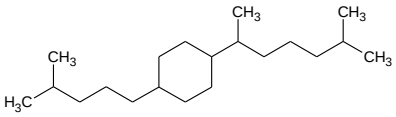
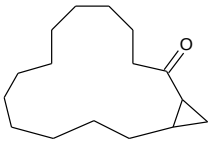
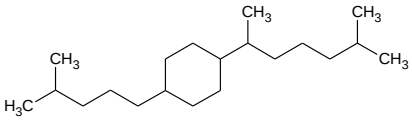
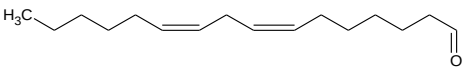
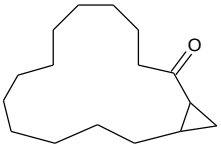
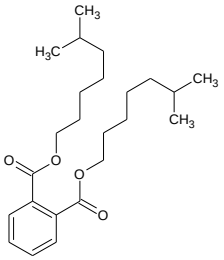
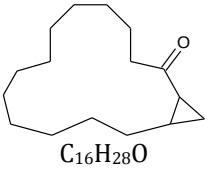
Peaks	Rt	Name of Compounds	Chemical Structure Molecular Formula	Peak Area %
48	14.016	Linoleic acid ethyl ester	 $C_{20}H_{36}O_2$ **MW**: 308	8.29
49	14.102	Ethyl Oleate	 $C_{20}H_{38}O_2$ **MW**: 310	10.31
50	14.480	Ethyl Oleate	 $C_{20}H_{38}O_2$ **MW**: 310	1.01
51	14.686	Hexadeca-7,10-dienal	 $C_{16}H_{28}O$ **MW**: 236	0.15
52	15.006	Methyl octadeca-9-yn-11-trans-enoate	 $C_{19}H_{32}O_2$ **MW**: 292	1.52
53	15.544	Methyl octadeca-9-yn-11-trans-enoate	 $C_{19}H_{32}O_2$ **MW**: 292	0.20
54	16.070	Methyl octadeca-9-yn-11-trans-enoate	 $C_{19}H_{32}O_2$ **MW**:292	2.35
55	16.231	1-(6-Methylheptan-2-yl)-4-(4-methylpentyl)cyclohexane	 $C_{20}H_{40}$ **MW**: 280	0.30
56	16.374	1-(6-Methylheptan-2-yl)-4-(4-methylpentyl)cyclohexane	 $C_{20}H_{40}$ **MW**: 280	0.20

Table 1. Continued

Peaks	Rt	Name of Compounds	Chemical Structure Molecular Formula	Peak Area %
57	16.631	1-(6-Methylheptan-2-yl)-4-(4-methylpentyl)cyclohexane	 $C_{20}H_{40}$ **MW**:	0.38
58	17.009	1-(6-Methylheptan-2-yl)-4-(4-methylpentyl)cyclohexane	 $C_{20}H_{40}$ **MW**: 280	0.15
59	17.415	Bicyclo[13.1.0]hexadecan-2-one	 $C_{16}H_{28}O$ **MW**: 236	0.25
60	17.570	1-(6-Methylheptan-2-yl)-4-(4-methylpentyl)cyclohexane	 $C_{20}H_{40}$ **MW**:	0.15
61	19.143	hexadeca-7,10-dienal	 $C_{16}H_{28}O$ **MW**:236	0.10
62	20.820	Bicyclo[13.1.0]hexadecan-2-one	 $C_{16}H_{28}O$ **MW**: 236	0.18
63	21.769	Bis(6-methylheptyl) benzene-1,2-dicarboxylate	 $C_{24}H_{38}O_4$ **MW**: 390	1.62
64	27.200	Bicyclo[13.1.0]hexadecan-2-one	 $C_{16}H_{28}O$ **MW**: 236	0.03

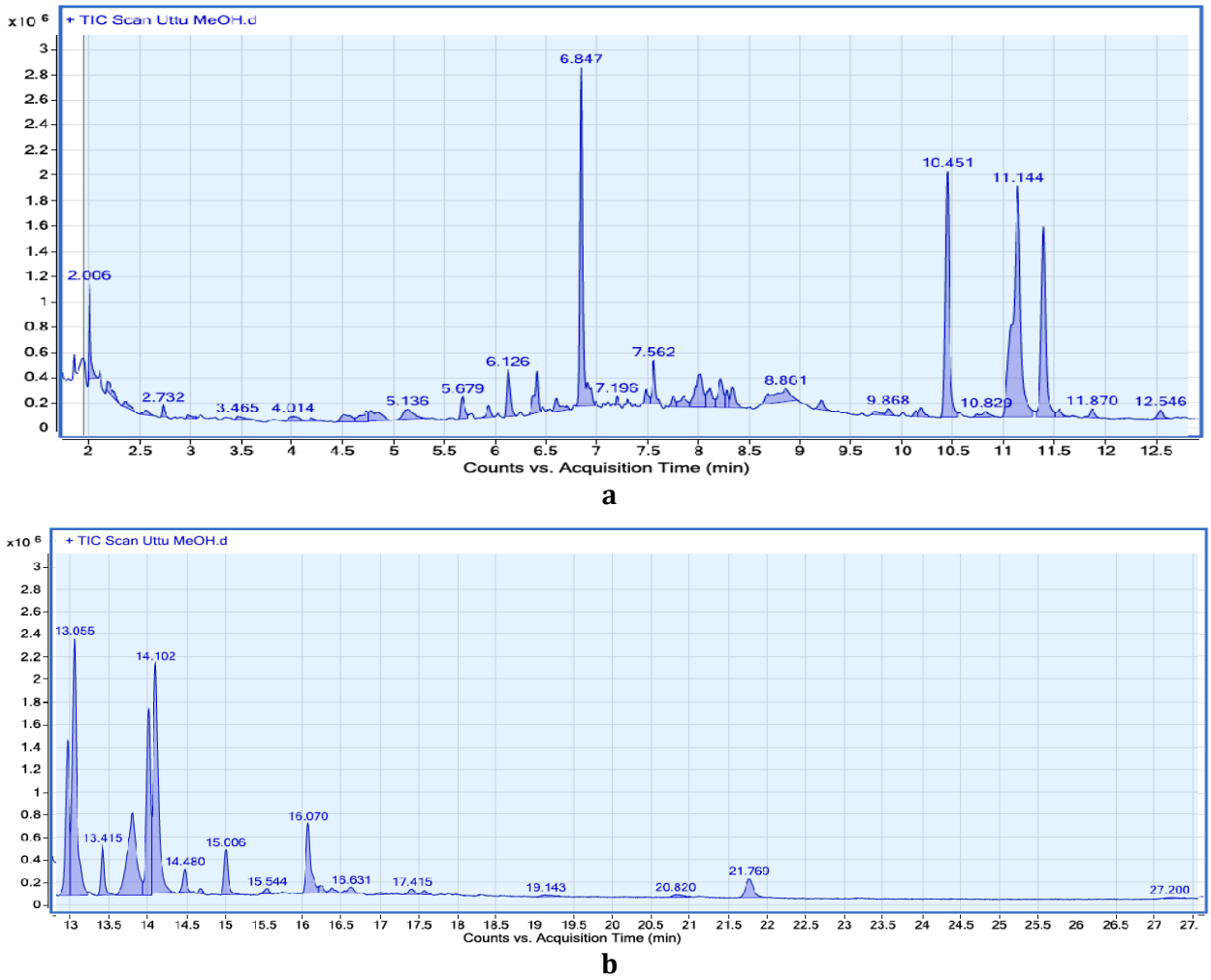


Figure 2. The chromatogram of phytoconstituents in methanol root bark extract, 1-10 min (a), 10-27 min (b)

Table 2. Antifungal activity of the methanol root bark extracts of *S. innocua*

S/N	Test Organisms	Zone of Inhibition (mm)	MIC (mg/mL)	MFC (mg/mL)
1	*Candida krusei*	34.00	5.00	10.00
2	*Aspergillus nigre*	0.00	–	–

Table 2 shows the zone of inhibition (mm), MIC, and MFC of methanol extract from the root bark of *S. innocua*.

Silanediol dimethyl was found in citrus limon and papaya after an investigation of microbial concentration [11]. Ethylbenzene production for commercialization has been reported in industries using the benzene alkylation process [12]. One of the primary compounds found in *Pseudomonas aeruginosa* with antimicrobial activity is oxime-, methoxy-phenyl [13]. Hexadec-7-enal has been identified among compounds that elicited electroantennogram responses from white-tailed bumblebees [14]. Ethyl oleate is a fatty acid ester and safe for oral ingestion [15] and is used as a solvent for pharmaceutical drug preparations [16]. Linoleic acid and ethyl ester have been reported to effectively treat infantile neuroaxonal dystrophyl [17]. 9,12-octadecadienoic acid (Z, Z) was also discovered in *Gossypium* seeds and had antimicrobial activity [18]. 6-octadecenoic acid, methyl ester was found

in *Staphylococcus* and is highly effective in suppressing the growth of *Aspergillus terreus* [19]. Hexadecanoic acid methyl ester, known as palmitic acid methyl ester, was reported to contribute as paracrine perivascular relaxing factors to the regulation of vascular contraction [20]. Bicyclo[13.1.0]hexadecan-2-one was discovered in methanol extracts of *Diadema setosum* [21], *Thaumatococcus danielli* seed [22], and *Catharanthus roseus* leaf extract [23]. γ-Elemene, along with other compounds found in *Tridax procumbens*, has anticancer activity [24].

The antifungal activity of the methanol extract was recorded against *C. krusei* and *A. nigre,* which are of variable values. However, the zone of inhibition was presented in Table 2, which showed excellent activity against *C. krusei,* a nosocomial pathogen found immunocompromised with natural resistance fluconazole [28] and causative of Candidaemia infection. For *C. krusei* and *A. nigre*, the zone of inhibitory activity is 34.00 mm and 0.00 mm, respectively. There was no activity against *A. nigre*. The antifungal properties of this extract could be attributed to the synergistic therapeutic effects of the phytoconstituents shown in Table 1, which may have contributed to the antifungal activity against *C. krusei* [29]. The concentration of extract that inhibits *C. krusei* growth (MIC) is 5 mg/mL. In comparison, the concentration of extract that kills *C. krusei* (MFC) is 10 mg/mL, indicating the extract's potency against *C. krusei*.

Conclusions

GC-MS analysis of root bark methanol extract of *Strychnos innocua* showed a highly composite profile of phytoconstituents which include 6-Octadecenoic acid, methyl ester at the highest peak area (11.42%), and bicyclo[13.1.0]hexadecan-2-one at the lowest peak area (0.03%). Hence, it is concluded that the revealed phytocompounds in root bark methanol extract of *Strychnos innocua* might be highly valuable in medicinal usage to remedy some human ailments.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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