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Gas chromatography–mass spectrometry (GC–MS) analysis of ethyl acetate root bark extract of *Strychnos innocua* (Delile)

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Abstract

Background: Majority of phytochemicals have been known to bear valuable therapeutic activities such as insecticidal, antibacterial, antifungal, anticonstipative, spasmolytic, antiplasmodial and antioxidant activities. *Strychnos innocua* is straight-stemmed tree belonging to the family *Loganiaceae* and can grow up to 18 m tall. The plant is used for various pharmacological purposes. The aim of this study was to determine the chemical composition of the ethyl acetate extract of root bark of *S. innocua* using GC–MS analysis. The root bark was collected, air-dried and then crushed to powder. Standard extraction method (maceration) was used to obtain the ethyl acetate extract. The GC–MS was carried out on the extract using GC 7890B, MSD 5977A, Agilent Tech.

Results: Thirty-seven compounds were identified among which dibutyl benzene-1,2-dicarboxylate showed the highest peak area (31.03%) and monomethyl pimelate showed the lowest peak area (0.39%). The major compounds identified were cyclooctane (methoxymethoxy), 2,4-dimethylheptanedioic acid dimethyl ester, azelaic acid, 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester, dibutyl benzene-1,2-dicarboxylate, butyl 8-methylnonyl benzene-1,2-dicarboxylate, 9,15-octadecadienoic acid, methyl ester, cis-vaccenic acid, linoleic acid ethyl ester and ethyl oleate.

Conclusions: In conclusion, these phytoconstituents might be responsible for the medicinal efficacy of the root bark of *S. innocua* and can be used as a source therapeutic drug.

Keywords: GC–MS analysis, *Strychnos innocua*, Chemical composition, *Loganiaceae*

1 Background

About 80% of the world population depend on plant-based medicines as a source of primary health care in rural areas of both developing and developed countries, where modern medicines are mainly used [22]. Majority of phytochemicals have been known to bear valuable therapeutic activities such as insecticidal, antibacterial, antifungal, anticonstipative, spasmolytic, antiplasmodial and antioxidant activities [12]. Antiprotozoal activities of medicinal plants have also been reported [24, 26].

The guidelines for the assessment of herbal medicines have once been issued by WHO, and these guidelines explained basic criteria for the evaluation of quality, safety and efficacy of herbal medicines with the goal of assisting national regulatory authorities, scientific organizations and manufacturers in assessing documentation, submissions and dossiers in respect of such products [25].

Strychnos innocua belongs to the family *Loganiaceae* and is often straight-stemmed tree growing up to 18 m tall. The trunk is usually 7–40 cm in diameter. The leaves are simple, alternate, leathery, subsessile or shortly petiolate, obovate, elliptic or oblong–elliptic, 4–15 × 2 – 9 cm, coriaceous; rounded marginate or subacute at the apex; widely to very narrowly cuneate or rarely rounded at the base; glabrous to pubescent beneath [4, 16]. The root

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decoction is taken as a remedy for gonorrhoea, and the fresh roots are used to treat snakebite [4, 19].

S. innocua was reported to have rich alkaloid in its wood [5]. While studies on nutritional, antinutritional and chemical composition of fruits of *S. innocua* were also reported [1, 6, 20], antitrypanosomal activity of compounds from the leaves of *Strychnos spinosa* was reported [9, 10]. The aim of this study was to determine the chemical composition of the ethyl acetate extract of root bark of *S. innocua* using GC–MS analysis. Ethyl acetate solvent was purposely used for extraction in this study due to its minimum toxicity and medium polarity in extracting both polar and nonpolar phytochemicals.

2 Methods

2.1 Collection of plant sample

The plant *Strychnos innocua* was harvested from forest of Soba Local Government Area, Kaduna State, Nigeria, and was identified by Mallam Namadi Sunusi, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. A voucher specimen number was assigned (V/N 01884).

2.2 Preparation of plant sample

The root bark was rinsed, air-dried for 28 days and crushed to coarse powder.

2.3 Extraction of plant sample

The pulverized plant sample (2000 g) was separately macerated successively in n-hexane, ethyl acetate and methanol according to gradient polarity of the solvents. The maceration technique involved soaking the pulverize plant materials in an aspirator firstly with n-hexane (polarity = 0.009) and allowing to stand at room temperature for a period of 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). The ethyl acetate extract was then used for the gas chromatography–mass spectrometry (GC–MS) analysis.

2.4 GC–MS analysis

The GC–MS analysis of ethyl acetate root bark extract of *Strychnos innocua* was performed using a GC 7890B, MSD 5977A, Agilent Tech and mass detector. Helium was the carrier gas at a flow rate of 1 ml/min, and 1µL of the supernatant of the sample was injected into the GC. The GC oven temperature was programmed from 80 °C, with an increase of 15 °C/min, to 200 °C and then 5 °C/min to 280 °C, ending with a 5-min isothermal at 280 °C. The ion source was set at 230 °C and the ionization voltage at 70 eV.

2.5 Identification of components

Interpretation on GC–MS was conducted using the database of National Institute Standard and Technology (NIST). The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

3 Results

The components identified are presented in Table 1, while the GC chromatogram is presented in Figs. 1 and 2.

4 Discussion

Species of plants in genus *Strychnos* are proving to be promising sources of compounds with important pharmacological properties. The GC–MS analysis of the ethyl acetate extract of root bark of *Strychnos innocua* resulted in the detection of 37 compounds that were identified among which dibutyl benzene-1,2-dicarboxylate showed the highest peak area (31.03%) and monomethyl pimelate showed the lowest peak area (0.39%). The major compounds identified were cyclooctane, (methoxymethoxy), 2,4-dimethylheptanedioic acid dimethyl ester, azelaic acid, 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester, dibutyl benzene-1,2-dicarboxylate, butyl 8-methylnonyl benzene-1,2-dicarboxylate, 9,15-octadecadienoic acid, methyl ester, cis-vaccenic acid, linoleic acid ethyl ester and ethyl oleate.

N-Methyl formamide possesses significant antitumor activities [7]. The azelaic acid is one of the fatty acid compounds that possess several medicinal properties such as antioxidative effects and anti-inflammatory and antimicrobial activities and used in the treatment of many skin problems [15, 18]. Diisooctyl phthalate has been synthesized by catalysis of niobic acid [29]. Ethyl oleate is a fatty acid ester, is safe for oral ingestion [21] and is used as solvent for pharmaceutical drug preparations [17]. β-Elemenone was found in the extract of *Curcuma longa*, and the extract was found to possess antioxidant activity [23]. Linoleic acid ethyl ester has been reported to be effective in the treatment of infantile neuroaxonal dystrophyl [2]. 7-Hexadecenal has been identified among compounds that elicited electroantennogram responses from white-tailed bumblebee [28]. 4-Acetoxy-3-methoxystyrene was identified in the ethanolic extract of stem of *Parthenium hysterophorus* and possesses antimicrobial activity [13]. cis-Vaccenic acid was found as a prominent compound in *Rhodopseudomonas capsulate* and possesses antivirus activity [8]. Cyclooctane (methoxymethoxy) along other compounds was identified in *Michelia champaca* seed extract and possesses antimicrobial, antioxidant and anticancer properties [14]. 9-Octadecenoic acid methyl ester possesses antioxidant and anticancer

Table 1 Phytoconstituents of the ethyl acetate root bark extract of *S. innocua*

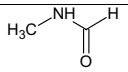
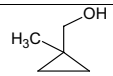
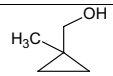
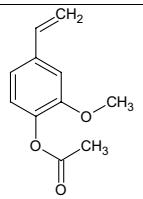
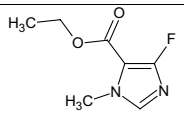
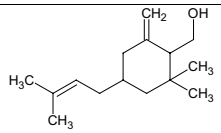
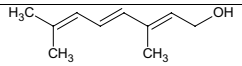
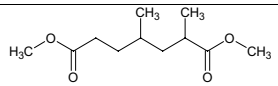
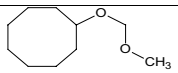
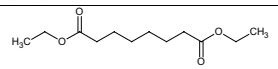
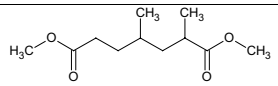
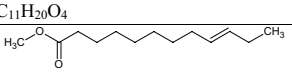
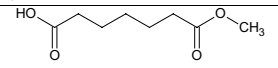
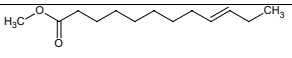
S/No.	RT	COMPOUND NAME	STRUCTURE / FORMULA	MW	PEAK AREA %
1	1.188	Formamide, *N*-methyl	 C_2H_5NO	59	0.89
2	1.914	1-Methylcyclopropanemethanol	 $C_5H_{10}O$	86	0.67
3	1.977	1-Methylcyclopropanemethanol	 $C_5H_{10}O$	86	0.50
4	5.668	4-Acetoxy-3-methoxystyrene	 $C_{11}H_{12}O_3$	192	0.50
5	5.943	4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester)	 $C_7H_9FN_2O_2$	172	0.45
6	6.131	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane	 $C_{15}H_{26}O$	222	0.45
7	6.406	2,4,6-Octatrien-1-ol, 3,7-dimethyl	 $C_{10}H_{16}O$	152	0.78
8	6.601	2,4-Dimethylheptanedioic acid dimethyl ester	 $C_{11}H_{20}O_4$	216	0.39
9	6.847	Cyclooctane, (methoxymethoxy)	 $C_{10}H_{20}O_2$	172	3.74
10	7.236	Diethyl suberate	 $C_{12}H_{22}O_4$	230	1.00
11	7.299	2,4-Dimethylheptanedioic acid dimethyl ester	 $C_{11}H_{20}O_4$	216	2.19
12	7.396	9-Dodecenoic acid, methyl ester	 $C_{13}H_{24}O_2$	212	0.61
13	7.436	Monomethyl pimelate	 $C_8H_{14}O_4$	174	0.39
14	7.510	9-Dodecenoic acid, methyl ester	 $C_{13}H_{24}O_2$	212	1.51

Table 1 (continued)

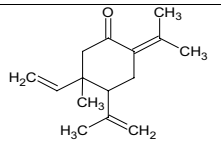
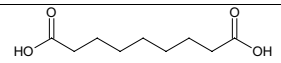
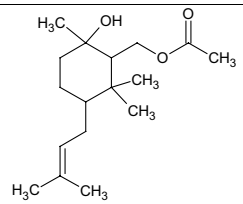
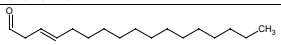
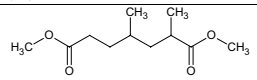
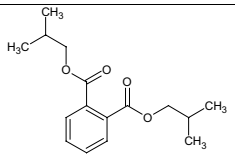
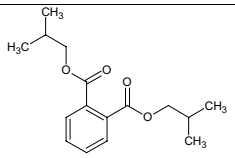
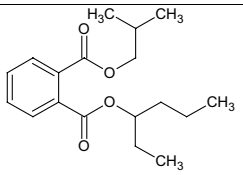
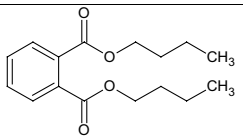
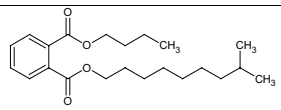
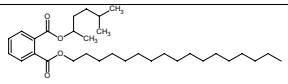
15	7.562	β-Elemenone	 $C_{15}H_{22}O$	218	0.89
16	7.917	Azelaic acid	 $C_8H_{16}O_4$	188	3.93
17	8.34	Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol	 $C_{17}H_{30}O_3$	282	0.40
18	8.695	heptadec-3-enal	 $C_{17}H_{32}O$	252	0.45
19	8.861	2,4-Dimethylheptanedioic acid dimethyl ester	 $C_{11}H_{20}O_4$	216	0.89
20	9.868	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	 $C_{16}H_{22}O_4$	278	1.51
21	10.486	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	 $C_{16}H_{22}O_4$	278	6.47
22	10.743	Hexan-3-yl 2-methylpropyl benzene-1,2-dicarboxylate	 $C_{18}H_{26}O_4$	306	1.00
23	11.184	dibutyl benzene-1,2-dicarboxylate	 $C_{16}H_{22}O_4$	278	31.03
24	11.413	butyl 8-methylnonyl benzene-1,2-dicarboxylate	 $C_{22}H_{34}O_4$	362	6.92
25	11.716	5-methylhexan-2-yl heptadecyl benzene-1,2-dicarboxylate	 $C_{32}H_{54}O_4$	502	0.45

Table 1 (continued)

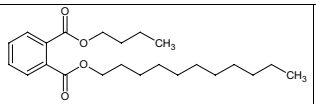
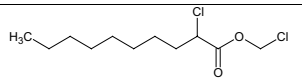
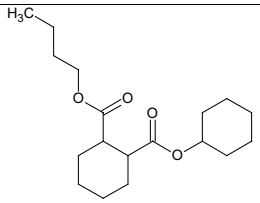
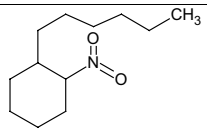
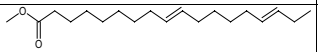
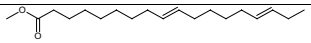
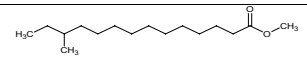
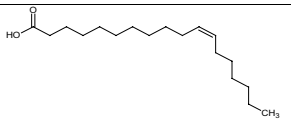
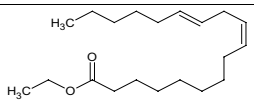
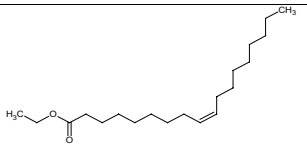
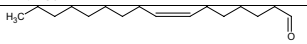
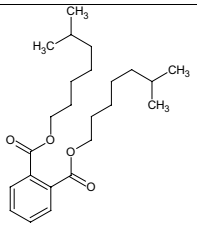
26	11.905	butyl undecyl benzene-1,2-dicarboxylate	 $C_{23}H_{36}O_4$	376	0.698
27	12.202	chloromethyl 2-chlorodecanoate	 $C_{11}H_{20}Cl_2O_2$		0.45
28	12.529	butyl cyclohexyl cyclohexane-1,2-dicarboxylate	 $C_{18}H_{30}O_4$	310	1.12
29	12.82	1-Hexyl-2-nitrocyclohexane	 $C_{12}H_{23}NO_2$	213	0.45
30	12.958	9,15-Octadecadienoic acid, methyl ester	 $C_{19}H_{34}O_2$	284	0.45
31	13.038	9,15-Octadecadienoic acid, methyl ester	 $C_{19}H_{34}O_2$	284	2.51
32	13.416	Tetradecanoic acid, 12-methyl-, methyl ester	 $C_{16}H_{32}O_2$	256	0.89
33	13.816	cis-Vaccenic acid	 $C_{18}H_{34}O_2$	282	6.36
34	14.005	Linoleic acid ethyl ester	 $C_{20}H_{36}O_2$	308	3.57
35	14.093	Ethyl Oleate	 $C_{20}H_{38}O_2$	310	5.94
36	14.474	7-Hexadecenal	 $C_{16}H_{30}O$	238	0.45
37	17.484	Diisooctyl phthalate	 $C_{24}H_{38}O_4$	390	0.67

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

activities [27]. 1-Hexyl-2-nitrocyclohexane was found in *Phormidium autumnale* extract and possesses antibacterial activities against *Bacillus subtilis* and *Shigella sonnei* [3]. Dibutyl benzene-1,2-dicarboxylate was identified among phytocompounds from the roots of *Patrinia scabra* and possesses cytotoxic activity [11]. The presence of various phytocompounds in the root of *S. innocua* might justify the use of the plant in folk medicine for the treatment of various ailments. However, isolation of individual components subjecting it to biological activity is recommended.

5 Conclusions

The present study investigated the phytoconstituents of *S. innocua* root bark that was harvested in the wild of Soba Local Government Area of Yobe State. Nigeria. Thirty-seven compounds were identified (GC–MS analysis) in which bioactivities and or industrial applications of some

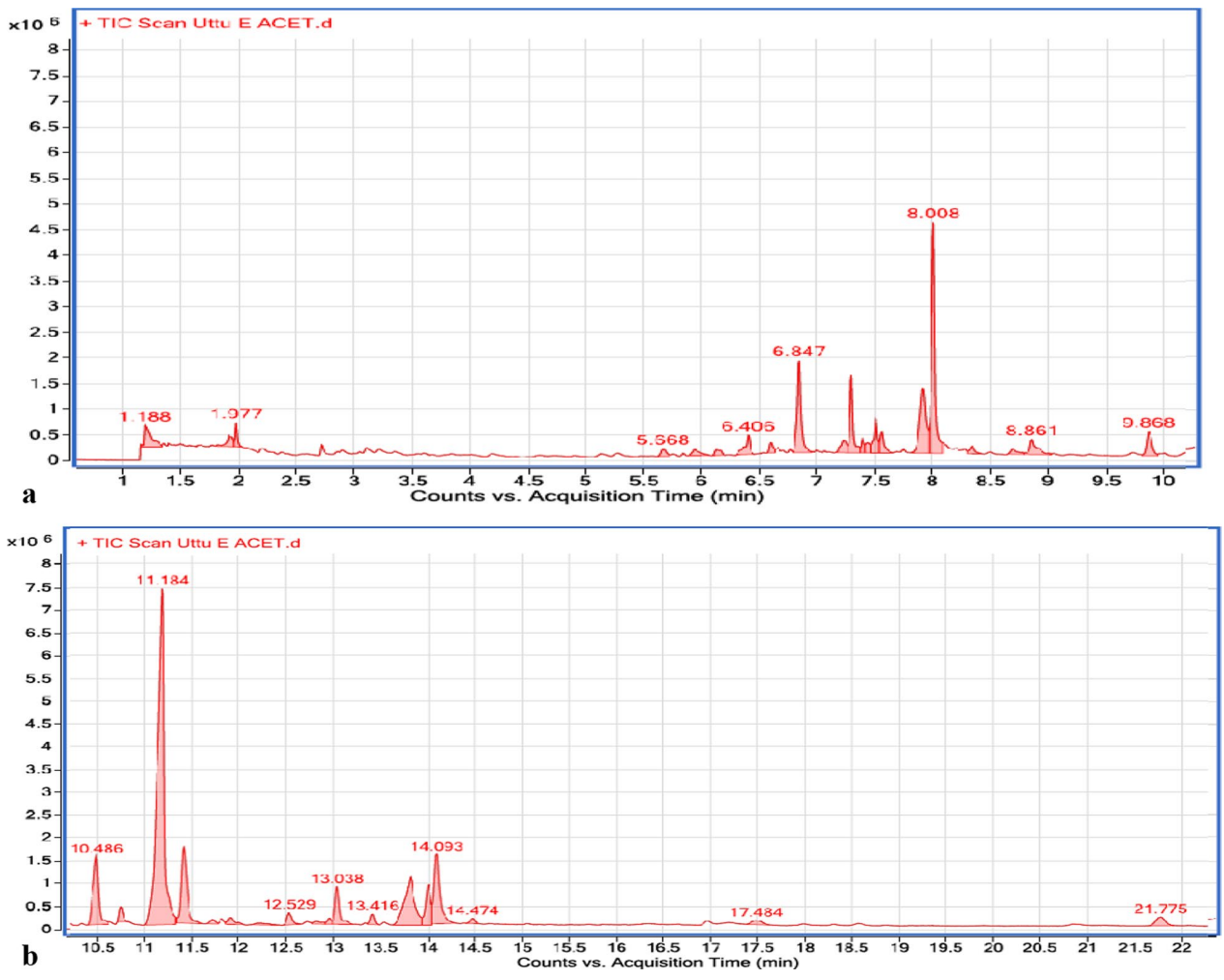


Fig. 2 **a** The chromatogram of phytoconstituents in ethyl acetate root bark extract. **b** The chromatogram of phytoconstituents in ethyl acetate root bark extract

of the compounds have been reported in various studies. The data obtained from the GC–MS analysis might suggest that the root of *S. innocua* may be a good source of therapeutic drugs.

Abbreviations
GC–MS: Gas chromatography–mass spectrometry; V/N: Voucher number; RPM: Rotation per minutes; µL: Microliter; RT: Retention time; MW: Molecular weight; %: Percentage.

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Authors' contributions
HI gave all the procedure/reviewed the manuscript. ORI gave the procedure for GC–MS analysis/reviewed the manuscript. AJU carried out the experiments/wrote the manuscript. MSS assisted in carrying out the experiments/reviewed the manuscript. All authors read and approved the final manuscript.

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