GC-MS chemical profiling and anti-inflammatory effects of dichloromethane fractions from *Dracaena arborea* (Willd.) Link Asparagaceae in mice

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ABSTRACT

Background: This study aimed to investigate the phytochemical composition and anti-inflammatory properties of dichloromethane fractions derived from the leaf, stem, and root of *Dracaena arborea*.

Methods: Plant materials were collected, dried at 40°C, and powdered. The powders were macerated with 70% ethanol for 72 hours, and the extracts were concentrated under reduced pressure. After defatting with n-hexane, the extracts were partitioned with dichloromethane, yielding fractions for leaf (Dal), stem (Das), and root (Dar). Phytochemical analysis was conducted using Gas Chromatography-Mass Spectrometry (GC-MS), and the anti-inflammatory effects were evaluated using egg albumin- and xylene-induced oedema models in mice.

Results: The fractions significantly reduced topical oedema ($p \le 0.05$) in mice compared to dexamethasone (4 mg/kg), a standard anti-inflammatory drug. GC-MS analysis identified bioactive compounds with known anti-inflammatory properties, including 9,12-octadecadienoic acid, 9,12,15-octadecatrienoic acid, n-hexadecanoic acid ethyl ester, oleic acid, and gamma-sitosterol.

Conclusion: The presence of these lipophilic compounds in the leaf, stem, and root of *D. arborea* may account for the observed anti-inflammatory effects, highlighting the plant's potential therapeutic value.

Keywords: GC-MS, Topical, Anti-inflammatory, Dichloromethane and Fractions

1. INTRODUCTION

Dracaena arborea, a boundary tree has been used as sources of fiber and shown to possess aphrodisiac, antidiabetic, fertility-enhancing, anti-inflammatory and analgesic properties [1, 2, 3, 18]. *D. arborea* is found in semi-arid deserts and distributed in the Canary Islands, Madeira, Verde Islands, Morocco, and tropical Africa [1, 5]. Lipophilic constituents of plants are reported to exhibit anti-inflammatory properties and GC-MS as a technique, is shown to be an effective tool in the identification of plants' metabolites [6, 7]. This study was designed to probe the phytochemicals present in the dichloromethane fractions of the leaf, stem and root of *D. arborea* and also challenge these lipophilic fractions to inflammation induced by egg albumin and xylene.

2. MATERIALS AND METHODS

2.1 Materials:

2.1.1 Biological Materials: Albino Wistar mice (25-30 g), Egg albumin

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2.1.2 Chemical and reagents: Dichloromethane (JDH Chemical Bangladesh Limited), Xylene (Merck),

2.1.3 Equipment and Apparatus: Venier caliper, Gas Chromatography Coupled to Mass Spectrophotometer

2.2 Methods

2.2.1 Plant Collection and Identification

The leaf, stem, and root of *D. arborea* were collected from the medicinal plants farm of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo, Akwa Ibom State. The whole plant specimen was identified by a taxonomist and a voucher specimen (UUPHB 30 (1)) was deposited in the herbarium.

2.2.2 Maceration of Plant Materials

The collected plant parts were garbled, washed, air-dried and pulverized. The pulverized plants' materials (1 kg of each of the parts) were cold macerated with 70% ethanol for 72 hours with intermittent agitation at room temperature (21°C) [9]. They were filtered and concentrated using a rotary evaporator (temperature of 40°C, reduced pressure and 250 rpm). The extracts were stored in a refrigerator (-4°C) from where they were used for the studies.

2.2.3 Fractionation of Extracts

Two hundred grams (200 g) each of ethanol leaf, stem and root extracts were first defatted with n-hexane and later partitioned with an adequate volume of dichloromethane [11]. They were concentrated to produce dichloromethane fractions of the leaf (Dal), stem (Das) and root (Dar). One (1 g) of each of the extracts was sent for GC-MS analysis while the remaining parts were used for animal study.

2.2.4 Determination of Dose

The median lethal doses (LD₅₀) as earlier reported by Umoh *et al*, (2022) were adopted for this study [9].

2.2.5 Anti-inflammatory Study

2.2.5.1 Animal Selection/Handling

Adult albino mice (25–30 g) were procured from the Animal House of the Department of Pharmacology and Toxicology, University of Uyo. The animals were kept under standard laboratory conditions and fed a diet of standard pellets. Before the experiments, food was withdrawn 24 hours, while water was provided ad libitum. All procedures adhered to the internationally accepted guidelines for the care and use of laboratory animals (1996), as adopted by the National Institutes of Health and the ethical regulations of the Faculty of Pharmacy, University of Uyo, Nigeria [10].

2.2.5.2 Xylene -- induced Anti-inflammatory Model

The method used here was similar to the one previously described by Umoh *et al*, (2020) with minor modifications [9]. Twenty five (25) albino mice of either sex were randomized and divided into five groups of five mice. The mice were treated thus: group 1, 10 mL/kg of distilled water; group 2, 45 mg/kg of Dal; group 3, 55 mg/kg of Das; group 4, 25 mg/kg of Dar and group 5, 4 mg/kg of dexamethasone. This was followed by induction of oedema by topical application of 50 microliters of xylene to both surfaces of the right ears while the left ears served as control. Mice were sacrificed after 5 hours and both ears were removed. The average weight differences between the ears were taken as a measure of inflammatory response [13].

2.2.5.3 Egg albumin –induced Anti-inflammatory Model

The method here was similar to the one described above for xylene-induced experiment except that the induction of oedema on the sub planter surface of the mice hind paw was done with egg albumin, the measurement of oedema was carried out using a digital vernier caliper before (t=0) and at thirty (30) minutes following the administration of egg albumin for 5 hours and acetyl salicylic acid (ASA 100 mg/kg), as a standard drug for comparison drug [11].

2.2.6 Gas Chromatography-Mass Spectrometry (GC-MS) Analyses

The dichloromethane fractions (Dal, Das and Dar) were subjected to GCMS-QP2010SE at Shimadzu Training Centre for Analytical Instruments (STC, Lagos). The carrier gas was helium and the oven temperature range of 80 - 300° C at time 5 - 15 minutes. The MS was taken at 70 eV with a total run time of 20 minutes for each sample. Identification of compounds was done by comparing them with known compounds in the institute's Library.



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2.3 Data Analysis

Data collected were statistically analyzed using one-way analysis of variance followed by a comparison test and a probability level of ($p \le 0.05$) was considered significant.

3. RESULTS

The result of the various yields of the fractionation of 200-gram weight of ethanol extracts of the leaf, stem and root of *D. arborea* is presented below in Figure 1 while the anti-inflammatory effects of the dichloromethane fractions are represented in Tables 1 and 2.



Figure 1: Percentage yields of dichloromethane fractions

Table 1: Effect of the DCM fractions of *D. arborea* on xylene-induced ear oedema in mice

(mg/kg)	
10 mL/kg	0.060 ± 0.005
45	$0.023 \pm 0.005^{*}$
55	$0.022 \pm 0.003^*$
25	$0.020 \pm 0.003^{*}$
4 mg/kg	$0.017 \pm 0.003^{*}$
	(mg/kg) 10 mL/kg 45 55 25 4 mg/kg

Dal = *D. arborea* leaf, Das = *D. arborea* stem and Dar = *D. arborea* root Values are expressed as Mean \pm S E M, significance relative to control *p \leq 0.05, n=5

Table 2: Effect of the DCM fractions of leaf, stem and root of D. arborea on egg albumin-induced oedema in mice

			Time interval	(minutes)			
Treatments (mg/kg)	0	30	60	120	180	240	300
Distilled water	2.26±0.01	3.78±0.01	3.79±0.01	3.53±0.02	3.08±0.02	2.95±0.02	2.80±0.02
Dal 45	2.21±0.01	3.72±0.01	3.51±0.01	3.43±0.02	2.92±0.01	2.73±0.01	2.72 ± 0.01
Das 55	2.23±0.01	3.70±0.01	3.47±0.02	3.42±0.02	2.73±0.01	2.69 ± 0.01	2.69 ± 0.01
Dar 25	2.24±0.01	3.71±0.01	3.48±0.01	3.37±0.01	2.67±0.01*	2.71±0.01	2.73±0.01
ASA 100	2.27 ± 0.01	3.15±0.01*	$2.29 \pm 0.01 *$	$2.78\pm0.01*$	2.57±0.01*	$2.41\pm0.01*$	2.37±0.01*

Dal = D. arborea leaf, Das = D. arborea stem, Dar = D. arborea root and ASA = Acetylsalicylic acid

Values are expressed as Mean \pm S E M, significance relative to control *p \leq 0.05, n = 5.

The result of the GC/MS analysis of the lipophilic fractions of *D. arborea* (Dal, Das and Dar) are represented in Tables 3, 4 and 5.



Table 3:	Table 3: Phytochemical constituents of dichloromethane leaf (Dal) fractions of D. arborea					
Peak	Retention	Percentage Area	Molecule	Name of Compound		
	Time		Formula			
1	8.632	0.34	$C_6H_8O_4$	2, 3 Dimethylfumaric acid		
2.	9.474	0.43	$C_{28}H_{44}O_3$	4' – Ethoxy – 2 hydroctadecanophenone		
3	9.829	0.32	$C_{13}H_{28}O_3$	Cyclohexanepropanol		
4.	10.286	1.16	$C_{13}H_{22}O_3$	2-Hydroxy – 1,1, 10 – trimethyl – 6,9-		
				epidoxydecalin		
5.	11.208	0.95	$C_{15}H_{28}O$	4aH – Cycloprop(e)azulen – 4a – 01		
6.	11.318	1.24	$C_{13}H_{22}O_3$	2-Hydroxy – 1, 1, 10-trimethyl-6,9-epidoxydecalin		
7.	11.393	0.44	$C_{19}H_{28}O_2$	Ethyl – 14 – methyl – hexadecanoate		
8	12.510	0.57	$C_{20}H_{38}O$	Phytol		
9	12.618	2.43	$C_{20}H_{40}O_4$	Eicosanoic acid		
10	12.797	13.76	$C_{18}H_{36}O_2$	Hexadecanoic acid		
11	13.435	1.87	$C_{29}H_{38}O_2$	Ethyl – 14 – methyl-hexadecanoic		
12	13.577	5.52	$C_{20}H_{40}O$	Phytol		
13	13.756	6.18	$C_{18}H_{32}O_2$	17 – Octadecynoic acid		
14	13.857	9.19	$C_{18}H_{32}O_2$	9, 21 – Octadecatrienonic acid,		
15	13.897	20.45	$C_{20}H_{34}O_2$	9, 21, 15 – Octadecatrienonic acid, ethyl ester		
16	14.043	5.73	$C_{19}H_{38}O_2$	Ethyl – 14 – methyl – hexadecanoic		
17	18.046	16.16	$C_{14}H_{22}O_2$	2 - Isopropenyl - 4 - 4, 7a - trimethyl - 2, 4, 5, 6,		
				7, 7a – hexahydro – benzofuran – 6 – ol		
18	19.218	9.06	$C_{15}H_{26}O$	2H-3, 9a – Methano – 1 – benzoin, octahedron –		
				2, 2, 5a, 9 – tetramethyl		
19	20.218	4.21	$C_{29}H_{50}O$	Gamma –sitosterol		
		100.00				

Table 4: Phytochemical co	onstituents of dichloromethane stem	(Das) fraction of D. arborea
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Peak	Retention Time	Percentage Area	Molecule Formula	Name of Compound
1	3.566	0.07	$C_5H_6N_2O_2$	Thymine
2.	4.147	0.10	$C_6H_8O_4$	4H-Pyran -4 – one, 2, 3 – dihydro -3 , 5 – dihydroxy – 6 – methyl
3.	4.603	0.11	$C_6H_8O_4$	4H – I – Benzopyran – 2 – one, 3, 4 – dihydro – 6, hydroxyl
4.	6.958	0.37	$C_8H_{12}O_4$	Phenol, $2 - 6 - dimethoxy$
5.	7.626	0.08	$C_{10}H_{10}O_4$	Vanillin, acetate
6.	8.360	0.28	$C_9H_9O_3$	2H-I- Benzophyran – 2 – one, 3, 4 – dihydro – 6 – hydroxyl
7	8.680	4.18	$C_{12}H_{18}O_5$	3 – Furamaceticacid, 4-hexyl – 2, 5 dihydro -2, 5- dioxo
8	9.472	0.41	$C_{10}H_{12}O_3$	1, 2, 4 – Cyclopentametrione, 3 – (2-pentenyl)
9	9.910	0.21	$C_{14}H_{28}O$	Tetradecanal
10.	10.344	1.45	$C_6H_6N_2O_4$	2,4,6, (IH, 3H, 5H) – Pyrimidmetrione, 5 – acetyl-Delta, I, alpha – Cyclohexaneacetic acid
11	10.649	0.67	$C_8H_{12}O_2$	Delta I, alpha – Cyclophexameacetic acid
12	11.016	3.25	$C_{10}H_{12}O_3$	4 - (IE) - 3 - Hydroxy - I - propensite) - 2 - methoxyphenyl
13	11.208	0.61	$C_{15}H_{30}O_2$	Pentadecanoic acid
14	11.317	0.71	$C_8H_{12}O_4$	Butanedioic acid, 2 – isopropenyl – 2 – methyl
15	11.929	0.24	$C_{20}H_{40}O_2$	Eicosanoic acid



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16	12.760	10.13	$C_{16}H_{32}O_2$	N- Hexadecanoic acid
17	12.818	5.01	$C_{18}H_{36}O_2$	Hexadecanoic acid, ethyl ester
18	12.859	7.45	C ₈ H ₉ NO ₃	Phenol, 2, $6 - dimethyl - 4 - nitro$
19	13.242	1.67	$C_{18}H_{34}O_2$	Oleic Acid
20	13.360	1.47	$C_{20}H_{38}O_2$	Cis – 10 – Heptadecenoic acid, ethyl ester
21	13.880	36.08	$C_{18}H_{32}O_2$	9, 12 – Octadecadienoic acid ethyl ester
22	13.916	0.45	$C_{20}H_{38}O_2$	(E) - 9 – Octadecenoic acid ethyl ester
23	14.060	0.31	$C_{19}H_{38}O_2$	Ethyl 14 – methyl – hexadecanoic
24	14.571	1.10	$C_{19}H_{36}O_2$	Cis – 10 – Nonadecanoate acid
25	15.048	1.08	$C_{18}H_{32}O_2$	9, 12 – Octadecadienoic acid (ZZ)
26	15.806	2.83	$C_{19}H_{36}O_2$	Hexadecanoic acid, 2 – hydroxyl – I
				(hydroxymethyl ethyl ester
27	16.291	1.32	$C_{20}H_{32}O_2$	5 alpha – Androst – 15 – en – 17 beta – ol, 17 –
				met
28	16.731	1.76	$C_{20}H_{31}NO_2$	5 – Dehydroandrosterone methyl oxime
29	16.683	4.72	$C_{18}H_{34}O$	Z, E – 12, 13 – Octadecadien –I-ol
30	16.815	1.50	$C_{21}H_{42}O_4$	Octadecanoic acid, 2, 3 – hydroxypropyl ester
31	16.985	0.97	$C_{15}H_{32}O$	Benzenemethanol, alpha – 1 (- ethenylpentyl) –
				alphl methyl
32	17.050	1.01	$C_{26}H_{34}O_2$	Sebacic acid, di (2, 6 – methoxyphenyl) ester
33	18.269	4.33	$C_{28}H_{44}O_3$	Pseducosarsasapogenin – 5, 20 – dien methyl
				ester
34	18.374	0.69	$C_{22}H_{42}O$	I – Heptadec – I – ynyl – cylclopentanol
35	18.893	0.57	$C_{29}H_{50}O_2$	Vitamin E
36	19.091	0.93	$C_{27}H_{48}O_2$	Cholestane – 3 beta, 5 beta – diol
37	19.470	0.71	$C_{31}H_{46}O_2$	3 beta-Acetoxystigmasta – 4, 6, 22 - triene
38	20.075	1.19	$C_{31}H_{48}O_2$	Cyclohexanecarboxaldehyde, 3, 3-dimethyl-5-
				CXO-
		100.00		

Table 5: Phytochemical constituents of dichloromethane stem (Das) fraction of D. arborea

Peak	Retention	Percentage	Molecule	Name of Compound
	Time	Area	Formula	
1	6.962	0.28	$C_8H_{10}O_3$	Phenol 2, 6 – dimethoxy
2.	7.644	0.06	$C_{10}H_{10}O_4$	Vamilin, acetate
3.	8.630	0.05	$C_6H_8O_4$	2, 3 – dimethyl fumaric acid
4.	9.337	0.08	$C_{11}H_{19}NO$	Dihydrotecomanine
5.	9.470	0.45	$C_8H_{10}O_3$	3, 5 – Dimethoxyacetophenone
6.	10.317	0.57	$C_9H_{10}O_4$	Benzaldehyde, 4-hydroxyl – 3, 5 – dimethoxy
7	10.680	0.23	$C_{11}H_{14}O_3$	Phenol, $2 - 6 - dimethoxy - 4 - (2 propenyl)$
8	10.934	0.10	$C_{10}H_{12}O_4$	Ethanone, $1 - (4 - hydroxyl - 3, 5 - $
				methoxyphenyl
9	10.984	2.30	$C_{10}H_{10}O_3$	4- (IE) -3 – hydroxyl -1 – propenyl) -2 –
				methoxyphenyl
10.	11.200	0.67	$C_{14}H_{28}O_2$	Tetradecanonic acid
11	11.309	0.19	$C_{27}H_{12}O_2$	Spiro (android – 5 – one – 17, I' cyclobutane – 2 –
				one, 3 – hydroxyl
12	11.392	0.37	$C_{18}H_{36}O_2$	Hexadiecanonic acid, ethyl ester
13	11.631	0.61	$C_{10}H_{12}O_4$	Ethanone, 1-(- hydroxyl – 3, 5 – dimethoxyphenol
14	12.713	12.91	$C_{16}H_{12}O_2$	N – hexadecanoic acid
15	12.810	14.79	$C_{18}H_{36}O_2$	Hexadecanoic acid, ethyl ester
16	13.236	5.84	$C_{16}H_{34}O_3$	Oleic acid
17	13.360	4.54	$C_{18}H_{32}O_2$	Cis – 10 – heptadecenoic acid



18	13.867	23.17	$C_{18}H_{32}O_2$	9, 21 – octadecadienoic acid
19	13.905	12.72	$C_{20}H_{38}O_2$	E-11-Hexadecanoic acid, ethyl ester
20	13.934	0.26	$C_{18}H_{34}O_2$	Cis – 10 – Nonadecenoic acid
21	14.050	9.89	$C_{18}H_{32}O_2$	(E) - 11 - Hexadecenoic acid, ethyl ester
22	14.566	2.47	$C_{19}H_{36}O_2$	Cis – 10 – Nonadecadienoic acid
23	15.600	0.99	$C_{18}H_{32}O_2$	9, 12 – Octadecadienoic acid (ZZ)-
24	15.798	0.87	$C_{16}H_{30}O$	Cis – 9 – Hexadecenal
25	16.628	0.97	$C_{19}H_{36}O_2$	Androst -1 – ene -3 , 11 – dione, (5 alpha)-
26	16.719	1.44	$C_{18}H_{32}O_2$	17 – Octadecynoic acid
27	16.836	1.08	$C_{13}H_{32}O_3$	Methyl 12 – oxo 9 – dodecenoate
28	17.196	0.72	$C_{18}H_{32}O_2$	17-Octadecynoic acid
29	17.843	0.67	$C_{10}H_{14}O_4$	Acetic acid, 2,7 – dioxatricyclo 4,3,1, 0(3, 8 dec –
				5 - yl ester
30	20.223	0.58	C29H50O	Beta-sitosterol
31	20.405	0.15	C27H52O4Si2	9. 12. 15 – Octadecatrienonic acid, 2 –
			-27 52 - 4 - 2	[(trimethysilv]]oxy - 1 - [(trimethyl silv]) oxy
				methyll ethyl ester
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				Gamma-sitosterol

Oleic acid

Figure 2: Structures of some lipophilic anti-inflammatory constituents of *D. arborea*

4. DISCUSSION

The fractionation of the ethanol extracts of *D. arborea* leaf, stem, and root with dichloromethane revealed that the leaf contained the highest concentration of lipophilic constituents, yielding 36 g (18%). The stem and root followed with yields of 27 g (13.5%) and 21 g (10.5%), respectively. Dichloromethane, a moderately non-polar aprotic solvent, effectively dissolved both polar and non-polar compounds. This characteristic enabled it to extract low boiling point components from the ethanol extracts, facilitating both the analgesic study and the subsequent GC-MS analysis [11]. The result of Dal, Das and Dar on xylene-induced oedema and egg albumin-induced oedema models as presented in Tables 1 and 2, revealed that dichloromethane partitioned fractions of *D. arborea* leaf, stem, and root were able to reduce



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rat ear oedema ($p \le 0.05$) like dexamethasone, a standard anti-inflammatory drug. In the egg albumin model, the Dar was only statistically ($p \le 0.05$) active at 3 hours of the experiment. The induction of oedema by xylene is linked to the release of phospholipase A2, therefore the ability of these fractions to inhibit oedema caused by xylene may be due to the blocking of the release of phospholipase $A_2[10, 12]$ GC-MS has been a valuable tool for the study and identification of bioactive compounds in medicinal plants irrespective of the amount in which they are present. The phytoconstituents of the dichloromethane fractions of the leaf, stem and root of D. arborea (Dal, Das and Dar) were studied through GC – MS analysis. Nineteen (19) compounds (Table 3) were identified in dichloromethane leaf fraction (Dal); forty-three (43) compounds (Table 4) in stem fraction (Das) and thirty-one (31) compounds (Table 5) in root fraction. In this study, the result revealed the presence of hexadecanoic acid, ethyl ester, and 9, 12 - octadecadienoic acid as common constituents in all the three fractions while oleic acid, 2, 6 - dimethoxyphenol and cis -10 - heptadecenoic acid were common bioactive constituents of stem bark (Das) and root (Dar). In considering the percentage area of the bioactive components of the three fractions, Dal furnished hexadecanoic acid (13.76%), 9, 12, 15 - octadecatrienoic acid (20.45%), 9, 12 octadecadienoic acid (9.19%) and 2 - isopropenyl - 4, 4, 7a - trimethyl - 2, 4, 5, 6, 7, 7a - hexahydro - benzofuran - 6- ol (16.16%) as the major phytoconstituents. Also, Das has n-hexadecanoic acid (19.13%), 2, 6 - dimethyl - 4 nitrophenol (7.45%), 9, 12 - octadecadienoic acid (13.88%), Z, E, 02, 13 - octadecadien - 1 - ol (4.72%) and pseuduosarsasapogenin -5 - 20 – dien (4.33%) as major constituents while Dar presented 9,12 – octadecadienolic acid (23.17%), (E) - 9 - octadecenoic acid (12.72%). n- hexadecanoic acid (14.79%) and E - 11 - hexadecenoic acid (9.89%)as major constituents. From this study, 9, 12 - octadecadienoic acid, 9, 12, 15 - octadecatrienoic acid, n-hexadecanoic acid, hexadecanoic acid ethyl ester, oleic acid, and gamma - sitosterol are among other bioactive constituents which are known anti-inflammatory agents present in Dal, Das, and Dar and may in part be responsible for their topical antiinflammatory activity [14, 15, 16, 17, 19].

5. CONCLUSION

The result from this study revealed that dichloromethane was able to extract lipophilic constituents from aqueous (70%) ethanol extracts of *D. arborea*, and that, these fractions demonstrated the ability to reduce oedema caused by xylene when compared to distilled water. The use of GC/MS-identified compounds was earlier reported for their involvement in the reduction of inflammation. The data adds to their uses in indigenous medicine practice.

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Contribution of the authors: Uwemedimo F. Umoh supervised the work and proofread the final manuscript, Nsima A. Andy wrote the first draft of the manuscript, Ekikere E. Ubengama assisted in the collection and processing of plant materials, Onojah J. Enema carried out extraction and fractionation, Olubenga Anthony Ojo interpreted the GC/MS result while Imoh Imeh Johnny drew the structures of some of the identified lipophilic compounds.

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