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Investigation of the Unsaponifiable and Saponifiable Matters of *Higginsia* sp. Sponge by GC/MS

Hanaa B. Elkhouly[1*], Eman Z. Attia[1], Amgad I. M. Khedr[2], Mamdouh N. Samy[1], Mostafa A. Fouad[1]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Minia University, 61519 Minia, Egypt.
[2]Department of Pharmacognosy, Faculty of Pharmacy, Port Said University, 42526 Port Said, Egypt.

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Abstract

Sponges (phylum Porifera) are sessile marine filter feeders that have developed efficient defense mechanisms against foreign attackers such as viruses, bacteria, or eukaryotic organisms. Marine sponges considered to be a great source of pharmacologically of pharmacologically-active chemicals from marine organisms. *Higginsia sp.* (Family Desmoxyidae) was collected from the Red Sea and possesses a great number of secondary metabolites. The present study showed that, the unsaponifiable matter of *Higginsia sp.* contains various compounds identified as hydrocarbons (23.07%), fatty alcohols (32.32%) as well as other oxygenated compounds (21.62%). While, the saponifiable matter contains various compounds identified as methyl and ethyl esters of saturated fatty acids (34.54%) in addition to unsaturated fatty acids (35.37%) while other compounds (30.09%) couldn't be identified. The identification of the compounds depends on the retention time and mass spectrum.

Keywords

Higginsia sp., Desmoxyidae, Unsaponifiable Matter, Saponifiable Matter, GC/MS.

Introduction

Marine sponges are an important component throughout the world, regarding their potential to influence benthic or pelagic processes. The family Desmoxyidae (Demospongiae: Halichondrida) consists of 19 genera and about 100 species. Desmoxyids are widly distributed and live mainly in the shallow water [1]. Sponges belong to order Halichondrida show a great diversity of fatty acids which have been found in various species [2]. Sponges (phylum Porifera) are among the oldest multicellular animals (Metazoa). They grow slowly and the growth rate depends on the species and the methods of culture conditions. Besides, about 15,000 sponges, most occur in marine environment. Only about 1% of the species inhabits freshwater. Furthermore, they are distributed widely in the world's oceans from tropical to the arctic. The marine sponges are recognized as a rich source of biologically active secondary metabolites with interesting chemical diversity, which possess antibacterial activity, antifouling activity and cytotoxic activity [3, 4] . The South African coast therefore represents a potentially resourceful environment for the exploration of novel bioactive compounds. The genus *Higginsia* belong to the class Demospongiae was first described by Higginin 1877,with several species such as, *H. bidentifera*, *H. tethyoides*, *H. mixta*, *H. arborea*, *H. lamella* and *H. palmate* [5]. Besides, *H. papillosa*, *H. petrosioides*, *H. pumila* from the Red Sea [6]. The genus Higginsia possess growth forms such as erect, lamellate, massive, vasiform or lobate; surface conulose, papillose, often silt covered or membraneous. Skeletal structure ranges from halichondroid with a partially compressed, reticulate axis, and an irregularly plumo-reticulate extra-axial region [7] A great number of secondary metabolites with interesting biological activities have been provided from the genus Higginsia such as tricyclic diterpenes and alkaloids. These metabolites were recently shown to possess a range of biological activities such as cytotoxic activity against lung (A549), human colon (HT29), and breast (MDA-MB-231) cancer cell lines [8]. Hence, the present attempt is to investigate the bioactive compounds of Higginsia sp. sponge using GC-MS technique.

2.Experimental

2.1. Biological material

The marine sponge *Higginsia sp.* was collected by diving in the Red Sea at depth 1.5 m, GPS: N 27 22 39.98, E 33 40 58.95 in May 2018 and identified by Mohammed A. Abu El-Regal (Professor of Biological Oceanography, Marine Biology Department, Faculty of marine Science, King Abdulaziz University, Jeddah, Saudi Arabia). The sample was transferred to the laboratory in plastic container containing sea water, cut into small pieces and left to dry. A voucher specimen (HS-10) was deposited in Pharmacognosy Department, Faculty of Pharmacy, Minia University (**Figure 1**).

2.2. Preparation of samples

2.2.1. Preparation of the ethanolic extract

The air-dried powdered sponge (270 g) of *Higginsia sp.* was extracted by maceration with 95% ethanol (1x3), and then concentrated under reduced pressure to give (15.10 g) of viscous

**** Correspondence:*** *Hanaa B. Elkhouly*
Tel.: *+201001616758*
Email Address: *hanaa.bahaaelkhouly@yahoo.com*

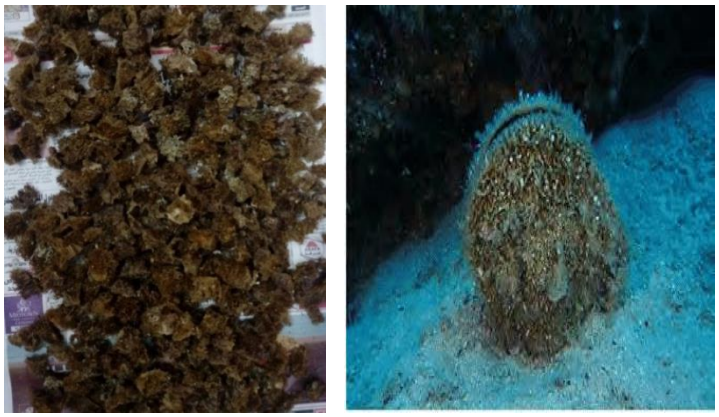

Figure 1: A voucher specimen of Higginsia sp.

residue of the ethanol extract. Then, the ethanol extract (15.10g) was suspended in 500 ml of 70% methanol, and then transferred to a separating funnel and partitioned with successive portions of petroleum ether (300 ml each). The combined petroleum ether fractions were concentrated under reduced pressure to yield (5.5 g). The remaining mother liquor was concentrated under reduced pressure and suspended in 500 ml of distilled water, also transferred to a separating funnel and partitioned with successive portions of dichloromethane (300 ml each), ethyl acetate (300 ml each). These fractions were concentrated under reduced pressure to afford 1.2 g, 260 mg and mother liqour, respectively.

2.2.2. Preparation of the fatty acids

The alkaline aqueous solution (soap) after removal of the unsaponifiable matter was acidified with sulphuric acid (10 %). The liberated fatty acids were extracted with successive portions of chloroform. The combined chloroform extracts were washed with distilled water till the washings were neutral to litmus. The chloroform was distilled off and the residue of the total fatty acids was semisolid and brownish in color [9].

2.2.3. Preparation of the fatty acids methyl esters

The residue of the total fatty acids was refluxed with 50 ml of methanol in presence of 1.5 ml of sulphuric acid for two hours on a boiling water bath. The major part of the alcohol present was distilled off, and the liquid left was diluted with twice its volume of distilled water, extracted with several portions of chloroform until exhaustion. The combined chloroform extracts were washed with distilled water till the washings were neutral to litmus. The chloroform was evaporated and the obtained brownish yellow semisolid residue of fatty acid methyl esters (135 mg) was dried over anhydrous sodium sulphate and kept for further investigation [10].

2.2.4. Preparation of the unsaponifiable matter

The obtained dried petroleum ether extract (3g) was subjected to alkali hydrolysis by refluxing with 50 ml of N/2 alcoholic potassium hydroxide for eight hours on a boiling water bath. The major part of the alcohol was distilled off, and the liquid left was diluted with twice its volume of distilled water, extracted with several portions of chloroform until exhaustion. The combined chloroform extracts were washed with sodium hydroxide (T.S), then with distilled water until the washings were free from any alkalinity. The chloroform extracts were dehydrated over anhydrous sodium sulphate and then the chloroform was distilled off. The obtained residue (0.7 g) was dark brown in color. This residue was reserved for further investigation [9] .

2.3. GC-MS analysis

This analysis was carried out using Trace GC – TSQ mass spectroscopy (thermo Scientific) connected to FINNIGAN SSQ 7000 mass spectrometer. A capillary column (30 m x 0.25µm film thickness) packed with TG-5MS (5% -diphenyl/ 95% dimethyl polysiloxane) and flame ionization detector were used. The column oven temperature was initially held at 50°C and increased at 5°C/min to 250°C (hold for 2min) and then increased to the final temperature 300°C by 30°C/min and hold for 2min. The injector and MS transfer line tempratures were set at 260 and 270°C, respectively. Helium was used as a carrier gas at a constant rate flow of 1ml/min. The solvent delay was 4min and 1µl of the diluted samples were injected automatically using an auto sampler AS1300 coupled with GC in the split mode. Electron impact mass (EI) spectra were recorded at 70 ev ionization voltage in a full scan mode over a range of 50- 500 *m/z*, with the ion source temperature was set at 200 °C. Compounds were identified by comparing their mass spectra with those available in a number of databases, including Wiley library 09, NIST, Mainlib and Replib.

3. Results and Discussion

3.1. The Unsaponifiable Matter

The results of GC/MS analysis of the unsaponifiable matter of *Higginsia sp.* sponge shown in Figure 2 and Table 1 revealed the presence of 44 compounds from which 33 (80.49%) were identified and eleven compounds (19.49%) couldn't identified. The identified compounds were classified as Hydrocarbon (23.07%), saturated and unsaturated fatty alcohol (32.32%), minor fatty acid methyl esters(3.5%) and other oxygenated compounds such as ethers and amines (21.62%). Furthermore, 2-Butyl-1-octanol was the major identified saturated fatty alcohol (14.64%) followed by 5- Undecene was the major identified unsaturated alkene (9.16%) and then, Trans-2-Undecen-1-ol was the major identified unsaturated fatty alcohol (6.44%).

3.2 The saponifiable matter (Fatty acids)

The results of GC/MS analysis of the saponifiable matter of *Higginsia sp.* sponge shown in Figure 3 and Table 2 revealed the presence of 106 compounds from which 46 compounds (69.91%) were identified as methyl/ethyl esters of saturated/unsaturated fatty acids, while 60 compounds (30.09%) couldn't be identified. The unsaturated fatty acids represented (35.37%) of the fatty acid fraction were greater than saturated ones (34.54 %) of the fatty acids fraction. Furthermore, the oleic acid methyl ester was the major identified fatty acid methyl ester (32.90%) followed by hexadecanoic acid methyl ester (palmitic acid methyl ester) (30.3%), octadecanioc acid, methyl ester (Methyl octanoate) (2.16%) and 7-hexadecenoic acid, methyl ester, (Z)-(methyl (7E) hexadecenoate) (1.33%). While, the remaining esters of fatty acids were present in minor amounts.

4. Conclusion

The present study that includes the investigation of the unsaponifiable and saponifiable matters of *Higginsia sp*. sponge by GC/MS could be helpful to identify valuable compounds such as hydrocabons, fatty alcohols and saturated and unsaturated esters of fatty acid, that urging further analysis of this sponge using other techniques, which may lead to the development of drug formulation.

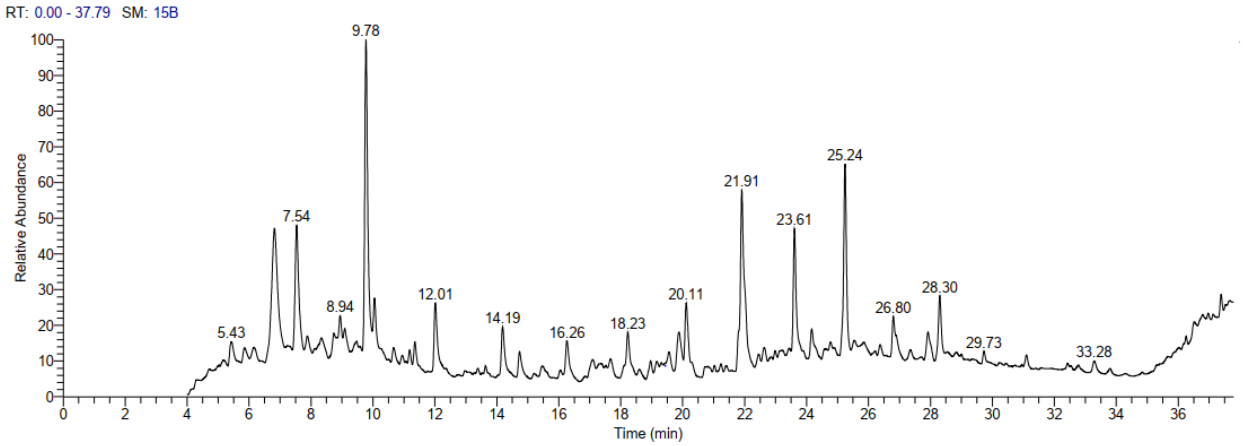

Figure 2: Gas chromatography-mass spectrometry chromatogram of unsaponifiable matters of *Higginsia sp.*

Table 1: Results of GC/MS analysis of unsaponifiable matters of *Higginsia sp.*

No.	Compound Name	Molecular Formula	Molecular Weight	RT	RRT	Area %	Base Peak	Charcteristic signals	Library
1	(E)-2-Decen-1-ol	$C_{10}H_{20}O$	156	5.43	0.55	1.36	57	95 81 70 57(100%) 43 41	Wiley Regi stry8e
2	4-Trifluoroacetoxypentadecane	$C_{17}H_{31}F_3O_2$	324	5.85	0.59	0.83	55	97 83 69 55(100%) 43 41	Mainlib
3	1,10-Decandiol	$C_{10}H_{22}O_2$	174	6.16	0.63	0.90	55	95 82 68 55(100%) 41	Wiley Regi stry8e
4	7,8-Dioxabicyclo[3.2.1]oct-2-ene.	$C_6H_8O_2$	112	6.81	0.69	7.07	81	112 83 81(100%) 55 53	Wiley Regi stry8e and mainlib
5	Trans-2-Undecen-1-ol	$C_{11}H_{22}O$	170	7.53	0.77	6.44	57	82 67 57(100%) 43 41	Replib
6	(E)- 2-Tridecen-1-ol	$C_{13}H_{26}O$	198	7.88	0.80	0.82	57	152 96 82 57(100%) 43 41	Mainlib
7	1,12-Tridecadiene	$C_{13}H_{24}$	180	8.34	0.85	1.59	55	109 95 81 67 55(100%) 41	mainlib

No.	Compound Name	Molecular Formula	Molecular Weight	RT	RRT	Area %	Base Peak	Charcteristic signals	Library
8	Unidentified	--	--	8.73	0.89	0.83	55	147 119 110 95 82 68 55(100%)	--
9	1-(ethenyloxy)- Octadecane	$C_{20}H_{40}O$	296	8.94	0.91	1.04	43	97 83 69 57 43(100%) 41	Replib
10	Z-10-Pentadecen-1-ol	$C_{15}H_{30}O$	226	9.09	0.93	0.72	55	82 81 67 55(100%) 41	Mainlib
11	2-Butyl- 1-Octanol	$C_{12}H_{26}O$	186	9.77	1.00	14.64	57	125 111 85 69 57(100%) 43 41	replib or mainlib
12	1-Decanol	$C_{10}H_{22}O$	158	10.05	1.02	1.76	55	112 83 70 55(100%)n 43 41(100%)	Wiley Regi stry8e Nist-ms
13	3-Butoxy-1- Cyclooctene	$C_{12}H_{22}O$	182	10.66	1.09	0.80	83	108 83(100%) 82 57 55 41	Wiley Regi stry8e and mainlib
14	1-Decene	$C_{10}H_{20}$	140	11.18	1.14	0.65	56	97 83 70 56(100%) 55 43 41	Wiley Regi stry8e
15	8-Methylene-Pentadecane	$C_{16}H_{32}$	224	11.35	1.16	1.10	56	125 97 84 69 57 56(100%) 55 41	Wiley Regi stry8e & mainlib
16	1-Nonanol	$C_9H_{20}O$	144	12.01	1.22	3.22	56	98 83 70 69 56(100%) 55 43	Wiley Regi stry8e
17	Hydroxylamine, O-decyl (O-Decylhydroxylamine)	$C_{10}H_{23}NO$	173	14.18	1.45	2.30	43	140 112 85 71 57 43(100%)	Mainlib

No.	Compound Name	Molecular Formula	Molecular Weight	RT	RRT	Area %	Base Peak	Charcteristic signals	Library
18	Z,Z,Z-1,4,6,9-Nonadecatetraene	$C_{19}H_{32}$	260	14.73	1.50	1.25	41	133 119 105 91 79 43 41(100%)	Mainlib
19	1-Tetradecanol	$C_{14}H_{30}O$	214	16.26	1.66	1.65	43	168 112 97 83 70 57 43(100%)	Wiley Regi stry8e
20	Z,Z,Z-4,6,9-Nonadecatriene ((4Z,6Z,9Z)-4,6,9-Nonadecatriene)	$C_{19}H_{34}$	262	17.09	1.74	1.09	41	147 105 93 91 79 43 41(100%)	Mainlib
21	5,7-Dodecadiyn-1,12-diol	$C_{12}H_{18}O_2$	194	17.68	1.80	0.73	91	115 91(100%) 79 77 55 41	Mainlib
22	5-Methyl-1-decene	$C_{11}H_{22}$	154	18.23	1.86	1.63	55	97 83 69 56 55(100%) 41	Wiley Regi stry8e
23	5,6-Dipropyldecane	$C_{16}H_{34}$	226	18.96	1.94	0.84	57	169 112 85 71 57(100%) 43 41	mainlib
24	Unidentified	--	--	19.56	2.00	0.82	89	161 118 105 89(100%) 69 55	--
25	Unidentified	--	--	19.87	2.03	2.38	67	161 122 106 93 85 67(100%) 55	--
26	(Z)- 5-Tridecene	$C_{13}H_{26}$	182	20.11	2.05	3.19	55	111 98 83 69 55(100%) 43 41	Mainlib
27	13 Oxabicyclo[10.1.0]tridecane. (1,2-Epoxy Cyclododecane)	$C_{12}H_{22}O$	182	21.79	2.23	0.37	55	121 111 96 82 67 55(100%) 41	Mainlib

No.	Compound Name	Molecular Formula	Molecular Weight	RT	RRT	Area %	Base Peak	Charcteristic signals	Library
28	5-Undecene	$C_{11}H_{22}$	154	21.91	2.24	9.16	55	126 111 97 83 69 55(100%) 41	Wiley Regi stry8e
29	Unidentified	--	--	22.45	2.29	0.54	74	105 95 91 85 68 55(100%)	--
30	Unidentified	--	--	22.63	2.31	0.86	56	171 125 105 90 81 70 56(100%)	--
31	1,1'-oxybis- Decane (Decyl ether)	$C_{20}H_{42}O$	298	23.61	2.41	5.67	57	140 112 97 85 71 57(100%) 43 41	Replib
32	Hexane, 1-(hexyloxy)-5-methyl. (1-(Hexyloxy)-5-methylhexane)	$C_{13}H_{28}O$	200	24.17	2.47	2.13	57	97 85 70 57(100%) 56 43	Wiley Regi stry8e
33	Unidentified	--	--	24.77	2.53	0.53	57	147 95 81 69 57(100%)	--
34	Unidentified	--	--	25.24	2.58	9.25	55	157 126 99 87 69 55(100%)	--
35	Unidentified	--	--	26.38	2.70	0.61	55	128 97 83 68 55(100%)	--
36	Unidentified	--	--	26.80	2.74	2.68	57	127 96 83 69 57(100%)	--
37	Oxacyclotetradecan-2-one	$C_{13}H_{24}O_2$	212	27.92	2.85	1.99	41	110 96 82 69 55(100%) 41	Replib
38	10-Methyl-E-11-tridecen-1-ol propionate	$C_{17}H_{32}O_2$	268	28.30	2.89	2.85	57	157 124 96 82 68 57(100%) 55	Mainlib

No.	Compound Name	Molecular Formula	Molecular Weight	RT	RRT	Area %	Base Peak	Charcteristic signals	Library
39	Tridecanal	$C_{13}H_{26}O$	198	29.73	3.04	0.55	57	154 124 110 96 82 68 57(100%) 55	Wiley Regi stry8e
40	Unidentified	--	--	31.10	3.18	0.62	55	148 96 84 68 57 56(100%) 55	--
41	Cyclopropanenonanoic acid, 2-[(2-butylcyclopropyl)methyl]-, methyl ester	$C_{21}H_{38}O_2$	322	33.28	3.40	0.65	55	238 149 95 81 67 55(100%) 41	Mainlib
42	Unidentified	--	--	36.25	3.71	0.37	55	129 96 80 68 56 55(100%)	--
43	(9E,12E)-9,12-Octadecadienoyl chloride **(Linoleoyl chloride)**	$C_{18}H_{31}ClO$	298	36.50	3.73	0.71	55	165 123 109 95 81 67 55(100%) 41	Wiley Regi stry8e
44	Cis,trans-5,9-Cyclododecadiene-cis-1 ,2-diol ((1R*,2S*,5Z,9E)- 5,9-Cyclododecadiene-1,2-diol	$C_{12}H_{20}O_2$	196	37.38	3.82	0.81	54	109 97 81 67 54(100%) 41	Mainlib
Total identified hydrocarbons									23.07%
Total identified fatty alcohols(saturated and unsaturated)									32.32%
Other total identified oxygenated compounds									21.62%
Minor fatty acid methyl esters									3.5%
Total unidentified compounds									19.49%

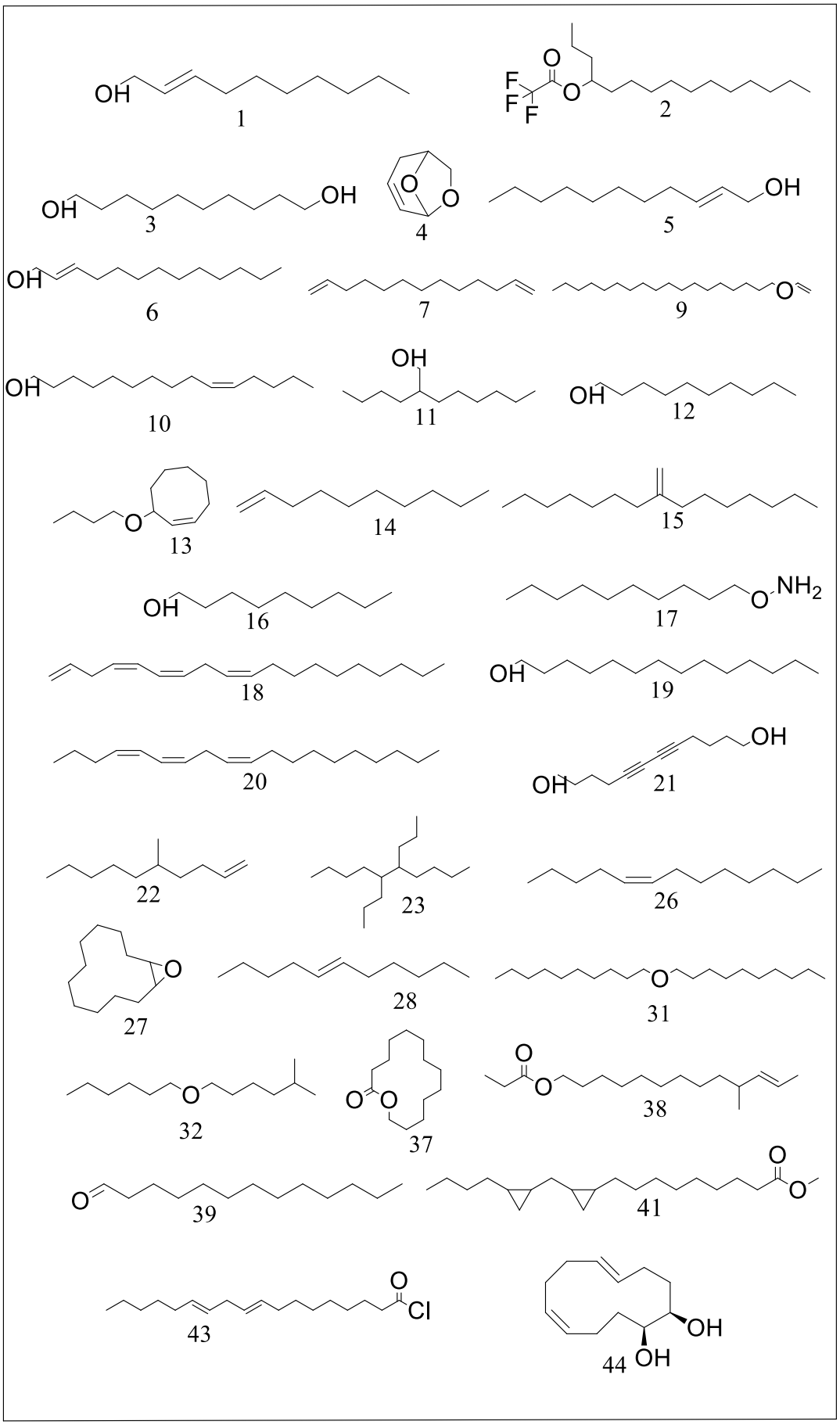

Figure 3: Structures of compounds identified from GC/MS analysis of unsaponifiable matters of *Higginsia* sp.

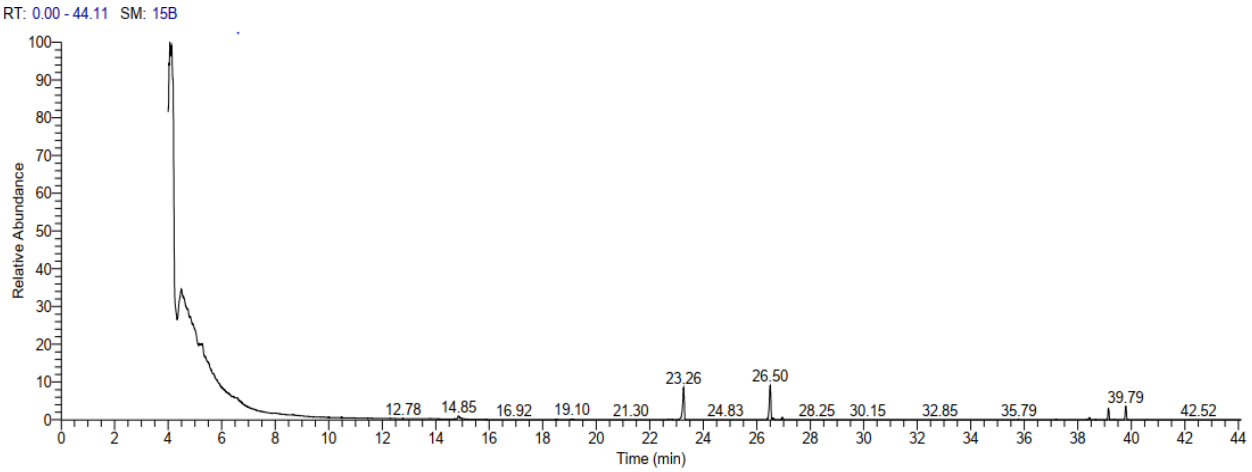

Figure 4: Gas chromatography-mass spectrometry of the saponifiable matters of *Higginsia sp.*

Table 2: Results of GC/MS analysis of saponifiable matters of *Higginsia sp.*

No.	Compound Name	Molecular Formula	Molecular Weight	RT	RRT	Area %	Base Peak	Charcteristic signals	Library
1	Unidentified	--	--	14.85	0.56	3.24	57	206 191 163 115 83 57(100%)	--
2	Benzeneacetic acid, cyclohexyl ester (Cyclohexyl phenylacetate)	$C_{14}H_{18}O_2$	218	15.72	0.59	0.02	83	137 91 83(100%) 55	Mainlib
3	(E)-2-Isopropyl-5-methylphenyl 2-methylbut-2-enoate	$C_{15}H_{20}O_2$	232	15.92	0.60	0.02	83	149 135 105 91 83 (100%) 55	Mainlib
4	Cis-3-Hexenyl isovalerate. Isovaleric acid cis-3-hexenyl ester	$C_{11}H_{20}O_2$	184	16.21	0.61	0.05	82	103 85 82(100%) 67 57 41	Mainlib
5	Unidentified	--	--	16.35	0.62	0.03	85	188 173 141 105 85(100%) 55	--
6	Unidentified	--	--	16.57	0.625	0.01	83	207 190 131 104 91 83(100%) 67	--
7	Unidentified	--	--	16.65	0.628	0.02	83	218 191 156 107 83(100%) 55	--
8	2-(tert-Butylperoxy)-2-ethylbutyl propionate	$C_{13}H_{26}O_4$	246	16.94	0.639	0.06	57	157 139 83 73 57(100%) 43	Mainlib

No.	Compound Name	Molecular Formula	Molecular Weight	RT	RRT	Area %	Base Peak	Charcteristic signals	Library
9	Unidentified	--	--	17.03	0.642	0.03	83	173 129 105 85 83(100%) 71	--
10	Unidentified	--	--	17.21	0.649	0.03	85	225 177 133 104 85(100%) 71	--
11	5-Isopropenyl-2-methyl-2-cyclohexen-1-yl pivalate (Limonen-6-ol, pivalate)	$C_{15}H_{24}O_2$	236	17.28	0.651	0.05	57	109 93 57(100%) 43 41	Mainlib
12	Unidentified	--	--	17.49	0.659	0.02	83	164 134 117 83(100%) 70 55	Mainlib
13	5,5- Dimethyl-3-oxo-hexanoic acid, ethyl ester	$C_{10}H_{18}O_3$	186	17.61	0.664	0.06	43	129 101 83 57 43(100%)	WileyRe gi stry8e
14	Unidentified	--	--	17.78	0.670	0.04	83	183 145 120 83(100%) 57	--
15	9-Octadecenoic acid (Z)-, phenylmethyl ester (Benzyl oleate)	$C_{25}H_{40}O_2$	372	17.91	0.675	0.09	91	151 127 108 91(100%) 43 41	Mainlib
16	Cyclooctane carboxylic acid, 1-ethyl-, methyl ester (Methyl-1- ethyl cyclooctane carboxylate)	$C_{12}H_{22}O_2$	198	17.97	0.677	0.01	55	139 115 97 83 55(100%) 41	WileyRe gi stry8e Mainlib
17	Methoxyacetic acid, 2-tridecyl ester (1-Methyldodecyl methoxyacetate)	$C_{16}H_{32}O_3$	272	18.07	0.681	0.07	57	85 71 57(100%) 45 43	mainlib
18	Unidentified	--	--	18.25	0.688	0.02	56	230 183 91 83 70 56(100%)	--
19	Unidentified	--	--	18.51	0.698	0.23	85	280 169 138 99 85(100%) 71	--
20	Pentanoic acid, 1,1-dimethylpropyl ester (tert-Pentyl pentanoate)	$C_{10}H_{20}O_2$	172	18.67	0.704	0.01	85	85(100%) 71 57 43	mainlib
21	Methoxyacetic acid, 2-pentadecyl ester (1-Methyltetradecyl methoxyacetate)	$C_{18}H_{36}O_3$	300	18.72	0.706	0.03	45	91 71 45(100%) 43	Mainlib

No.	Compound Name	Molecular Formula	Molecular Weight	RT	RRT	Area %	Base Peak	Charcteristic signals	Library
22	Unidentified	--	--	18.80	0.709	0.01	82	178 115 100 82 71	--
23	Eicosanoic acid, methyl ester (Arachidic acid methyl ester)	$C_{21}H_{42}O_2$	326	19.10	0.720	0.62	74	199 185 143 87 74(100%) 43	WileyRegi stry8e
24	Unidentified	--	--	19.41	0.732	0.11	85	177 132 102 85(100%) 70 55	--
25	Unidentified	--	--	19.57	0.738	0.04	84	158 144 121 107 84(100%) 71	--
26	Unidentified	--	--	19.71	0.743	0.02	85	165 127 98 85(100%) 55	--
27	Unidentified	--	--	19.92	0.751	0.03	112	149 112(100%) 99 85 55	--
28	Unidentified	--	--	20.07	0.757	0.13	82.8	180 154 119 84 82.8(100%) 69	--
29	Unidentified	--	--	20.21	0.762	0.04	83	218 147 129 107 83(100%) 55	--
30	Unidentified	--	--	20.33	0.766	0.01	83	198 163 133 95 85 83(100%) 71	--
31	Unidentified	--	--	20.38	0.768	0.02	80	186 173 119 91 80(100%) 79	--
32	14-Pentadecynoic acid, methyl ester	$C_{16}H_{28}O_2$	252	20.47	0.772	0.05	74	178 125 109 95 87 74(100%) 55 41	WileyRegi stry8e

No.	Compound Name	Molecular Formula	Molecular Weight	RT	RRT	Area %	Base Peak	Charcteristic signals	Library
33	Unidentified	--	--	20.61	0.777	0.13	85	239 135 115 85(100%) 55	--
34	Unidentified	--	--	20.90	0.788	0.04	73	209 183 138 85 73(100%)	--
35	Pentanoic acid, 2-ethylhexyl ester (Valeric acid, 2-ethylhexyl ester)	$C_{13}H_{26}O_2$	214	21.00	0.792	0.02	85	112 85(100%) 70 57	Mainlib
36	Malonic acid, 2-decyl heptyl ester	$C_{20}H_{38}O_4$	342	21.10	0.795	0.03	105	203 185 105(100%) 57	Mainlib
37	5- heptanoic acid,7-[(tetrahydro-2H-pyran-2-yl)oxy]-	$C_{12}H_{18}O_4$	226	21.15	0.797	0.03	85	125 101 85(100%) 79 55	WileyRegistry8e
38	Dichloroacetic acid, 4-tetradecyl ester	$C_{16}H_{30}CL_2O_2$	324	21.30	0.803	0.04	55	183 97 83 55(100%) 41	Mainlib
39	Unidentified	--	--	21.38	0.806	0.03	83	182 138 83(100%) 55	--
40	Unidentified	--	--	21.61	0.815	0.03	129	228 185 129(100%) 99 85	--
41	Ethyl 2- methyl-3-oxohexanoate	$C_9H_{16}O_3$	172	21.71	0.818	0.01	83	102 85 83(100%) 71 47	WileyRegistry8e
42	Unidentified	--	--	21.90	0.826	0.03	128	128(100%) 110 83 43	--
43	Valeric acid, 2-pentadecyl ester (1-Methyltetradecyl pentanoate)	$C_{20}H_{40}O_2$	312	22.16	0.835	0.10	41	129 103 85 57 41(100%)	Mainlib
44	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans	$C_{19}H_{36}O_3$	312	22.30	0.841	0.02	55	199 143 87 74 55(100%) 41	Mainlib
45	Unidentified	--	--	22.46	0.847	0.03	83	163 132 97 83(100%) 71	--
46	2-Butynoic acid, 4-[(tetrahydro-2H-pyran-2-yl)oxy]-, methyl ester	$C_{10}H_{14}O_4$	198	22.59	0.852	0.01	83	99 83(100%) 55	Mainlib

No.	Compound Name	Molecular Formula	Molecular Weight	RT	RRT	Area %	Base Peak	Charcteristic signals	Library
47	7-Hexadecenoic acid, methyl ester, (Z)- (Methyl (7E)-7-hexadecenoate)	$C_{17}H_{32}O_2$	268	22.70	0.856	0.32	55	98 74 69 55(100%) 41	Mainlib
48	Unidentified	--	--	22.81	0.860	0.25	71	211 152 126 85 71(100%)	--
49	Decanoic acid, didecyl ester	$C_{30}H_{58}O_4$	482	22.92	0.864	0.01	57	97 85 71 57(100%) 43	WileyRegistry8e
50	(Palmitic acid, methyl ester) (Methyl, hexadecanoate) Hexanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	23.27	0.877	30.3	74	143 87 74(100%) 55 43	WileyRegistry8e and replib
51	Pentadecanoic acid, 14-methyl-, methyl ester (Methyl 14-methylpentadecanoate)	$C_{17}H_{34}O_2$	270	23.41	0.883	0.04	74	143 129 87 74(100%) 43	Mainlib WileyRegistry8e
52	Unidentified	--	--	23.57	0.889	0.05	71	167 126 112 84 75 71(100%)	--
53	7-Octenioc acid, methyl ester (Methyl-7-octenoate)	$C_9H_{16}O_2$	156	23.67	0.892	0.04	55	125 82 74 55(100%) 43	Mainlib
54	Unidentified	--	--	24.07	0.907	0.01	55	153 127 105 85 55(100%)	--
55	3-Chlropropionic acid, nonyl ester (Nonyl 3-chloropropanoate)	$C_{12}H_{23}CLO_2$	234	24.11	0.909	0.01	56	126 109 91 70 56(100%) 55	Mainlib
56	Unidentified	--	--	24.15	0.910	0.04	82.9	193 154 123 97 82.9(100%)	--
57	Undecanoic acid, ethyl ester	$C_{13}H_{26}O_2$	214	24.39	0.920	0.08	88	169 157 101 88(100%)	
58	Unidentified	--	--	24.74	0.933	0.05	82	181 151 111 82(100%) 69	--
59	(11E)-10,13,13-Trimethyl-11-tetradecenyl acetate	$C_{19}H_{36}O_2$	296	24.81	0.935	0.08	57	166 95 83 57(100%)	Mainlib
60	Unidentified	--	--	24.97	0.941	0.04	60	101 87 73 60(100%) 43	--

No.	Compound Name	Molecular Formula	Molecular Weight	RT	RRT	Area %	Base Peak	Charcteristic signals	Library
61	Unidentified	--	--	25.10	0.946	0.02	60	129 73 60(100%) 57 43	--
62	Unidentified	--	--	25.24	0.952	0.13	56	163 124 87 73 55(100%)	--
63	Succinic acid, 2,2-dimethylpent-3-yl ethyl ester	$C_{13}H_{24}O_4$	244	25.70	0.969	0.02	129	129(100%) 115 101 57	Mainlib
64	5-Chloropentanoic acid, 2-octyl ester (1-Methylheptyl 5-chloropentanoate)	$C_{13}H_{25}CLO_2$	248	25.95	0.978	0.02	55	119(100%) 56 55 41	Mainlib
65	17-Octadecynoic acid, methyl ester	$C_{19}H_{34}O_2$	294	26.23	0.989	0.04	74	95 87 81 74(100%) 55	Mainlib
66	Oleic acid, methyl ester	$C_{19}H_{36}O_2$	296	26.51	1.00	32.90	55	87 83 74 55(100%) 41	Replib
67	7-Hexadecenoic acid, methyl ester, (Z)-(Methyl (7E) hexadecenoate)	$C_{17}H_{32}O_2$	268	26.61	1.003	1.33	55	96 74 69 55(100%) 41	Replib
68	Octadecanioc acid, methyl ester (Methyl octanoate)	$C_{19}H_{38}O_2$	298	26.96	1.016	2.16	74	255 143 87 74(100%) 43	WileyRegistry8e
69	Unidentified	--	--	27.12	1.023	0.01	43	143 129 85 73 60 43(100%)	--
70	Unidentified	--	--	27.16	1.024	0.01	106	227 125 106(100%) 75	--
71	Unidentified	--	--	27.27	1.028	0.06	69	267 158 99 69(100%)	--
72	Unidentified	--	--	28.01	1.056	2.53	55	222 97 79 69 55(100%)	--
73	Unidentified	--	--	28.20	1.063	0.12	55	67 57 55(100%) 43 41	--
74	Unidentified	--	--	28.26	1.066	0.25	56	289 173 126 84 55(100%)	--

No.	Compound Name	Molecular Formula	Molecular Weight	RT	RRT	Area %	Base Peak	Charcteristic signals	Library
75	Unidentified	--	--	28.59	1.078	0.17	73	199 129 73(100%) 60 43	--
76	Unidentified	--	--	28.98	1.093	0.02	81	179 155 105 81(100%) 55	--
77	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (Linoleic acid, methyl ester)	$C_{19}H_{34}O_2$	294	29.12	1.098	0.02	67	263 109 95 81 67(100%) 55	Mainlib
78	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	29.25	1.10	0.02	55	264 222 69 55(100%) 41	Mainlib
79	Unidentified	--	--	32.84	1.23	0.05	55	264 111 97 83 69 55(100%)	--
80	Unidentified	--	--	33.68	1.27	0.01	43	284 241 185 73 60 55 43(100%) 41	--
81	Unidentified	--	--	34.35	1.29	0.13	56	141 85 71 56(100%)	--
82	Oxalic acid, isobutyl hexadecyl ester	$C_{22}H_{42}O_4$	370	35.80	1.35	0.33	57	83 71 57(100%) 43 41	Mainlib
83	Unidentified	--	--	37.19	1.40	0.28	55	111 97 83 69 55(100%)	--
84	Hexadecanoic acid, 2,3-dihydroxypropyl ester	$C_{19}H_{38}O_4$	330	38.26	1.44	0.08	55	213 129 98 57(100%) 55 41	WileyRegistry8e
85	Unidentified	--	--	38.44	1.450	1.89	57	85 71 57(100%)	--
86	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{40}O_4$	356	38.56	1.454	0.01	55	98 81 69 55(100%) 41	Mainlib
87	Unidentified	--	--	38.68	1.459	0.21	79	105 91 79(100%) 67 41	--

No.	Compound Name	Molecular Formula	Molecular Weight	RT	RRT	Area %	Base Peak	Charcteristic signals	Library
88	Oleic acid, 3-(octadecyloxy)propyl ester. (3-(Octadecyloxy)propyl (9E)-9-octadecenoate).	$C_{39}H_{76}O_3$	592	38.85	1.466	0.11	57	322 83 69 57 43	Mainlib WileyRegistry8e
89	Unidentified	--	--	38.97	1.47	0.01	55	155 131 81 55(100%)	--
90	Unidentified	--	--	39.15	1.476	8.29	57	85 71 57(100%)	--
91	Unidentified	--	--	39.36	1.484	0.25	41	98 83 69 55 41(100%)	--
92	Oleic acid, eicosyl ester (9-Octadecenoic acid (Z)-, eicosyl ester)	$C_{38}H_{74}O_2$	562	39.53	1.491	0.08	57	274 97 83 69 57(100%)	WileyRegistry8e
93	Undec-10-ynoic acid, isobutyl ester	$C_{15}H_{26}O_2$	238	39.63	1.494	0.07	56	95 81 69 56(100%) 55 41	Mainlib
94	Unidentified	--	--	39.79	1.500	10.63	55	133 105 85 71 55(100%)	--
95	Unidentified	--	--	39.90	1.505	0.08	57	170 152 116 67 55(100%)	--
96	Unidentified	--	--	40.02	1.509	0.04	55	278 229 71 69 55(100%)	--
97	Unidentified	--	--	40.13	1.513	0.03	69	281 241 151 69(100%) 55	--
98	Unidentified	--	--	40.26	1.518	0.04	54	206 159 133 87 74	--
99	Unidentified	--	--	40.37	1.520	0.07	69	295 202 168 144 104 69(100%)	--
100	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	40.56	1.529	0.28	67	95 81 67(100%) 55 41	WileyRegistry8e
101	Unidentified	--	--	40.84	1.540	0.02	55	294 135 107 74 55(100%)	--

No.	Compound Name	Molecular Formula	Molecular Weight	RT	RRT	Area %	Base Peak	Charcteristic signals	Library
102	Unidentified	--	--	40.95	1.544	0.01	81	236 108 135 109 81(100%)	--
103	Unidentified	--	--	41.07	1.549	0.03	99	232 165 99(100%) 76	--
104	Hexanoic acid, 3,5,5-trimethyl-, tridec-4-yl ester	$C_{22}H_{44}O_2$	340	41.17	1.552	0.14	57	182 141 71 57(100%) 43	Mainlib
105	Unidentified		--	41.46	1.563	0.09	57	239 296 105 84 57(100%)	--
106	Glutaric acid, 2-methylpent-3-yl dec-9-enyl ester	$C_{21}H_{38}O_4$	354	42.51	1.603	0.02	115	253 158 115(100%) 85 55	Mainlib
Total identified saturated long chain fatty acids									34.54%
Total identified unsaturated long chain fatty acids									35.37%
Total unidentified compounds									30.09%

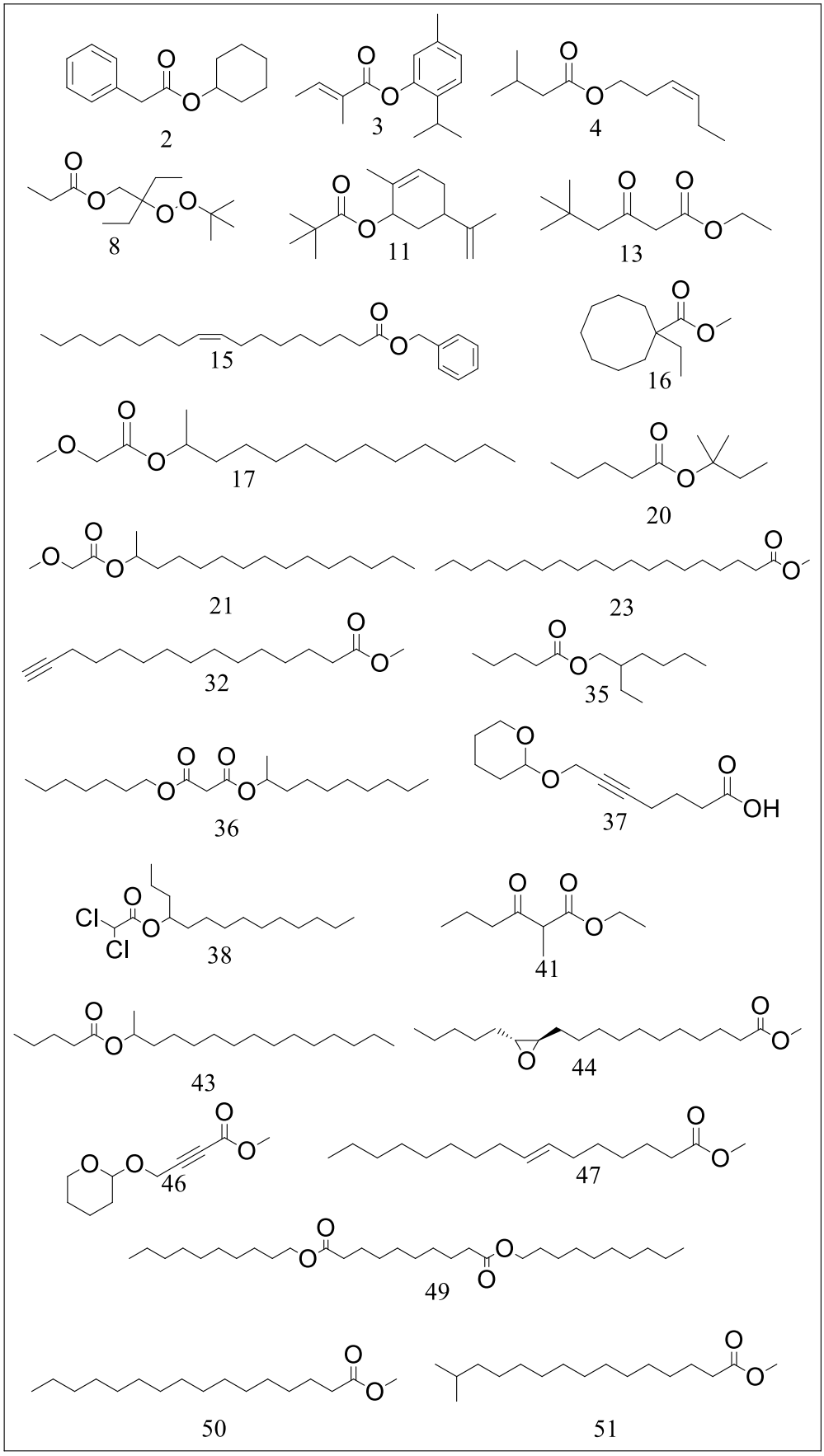

Figure 5: Structures of compounds identified from GC/MS analysis of saponifiable matters of *Higginsia* sp.

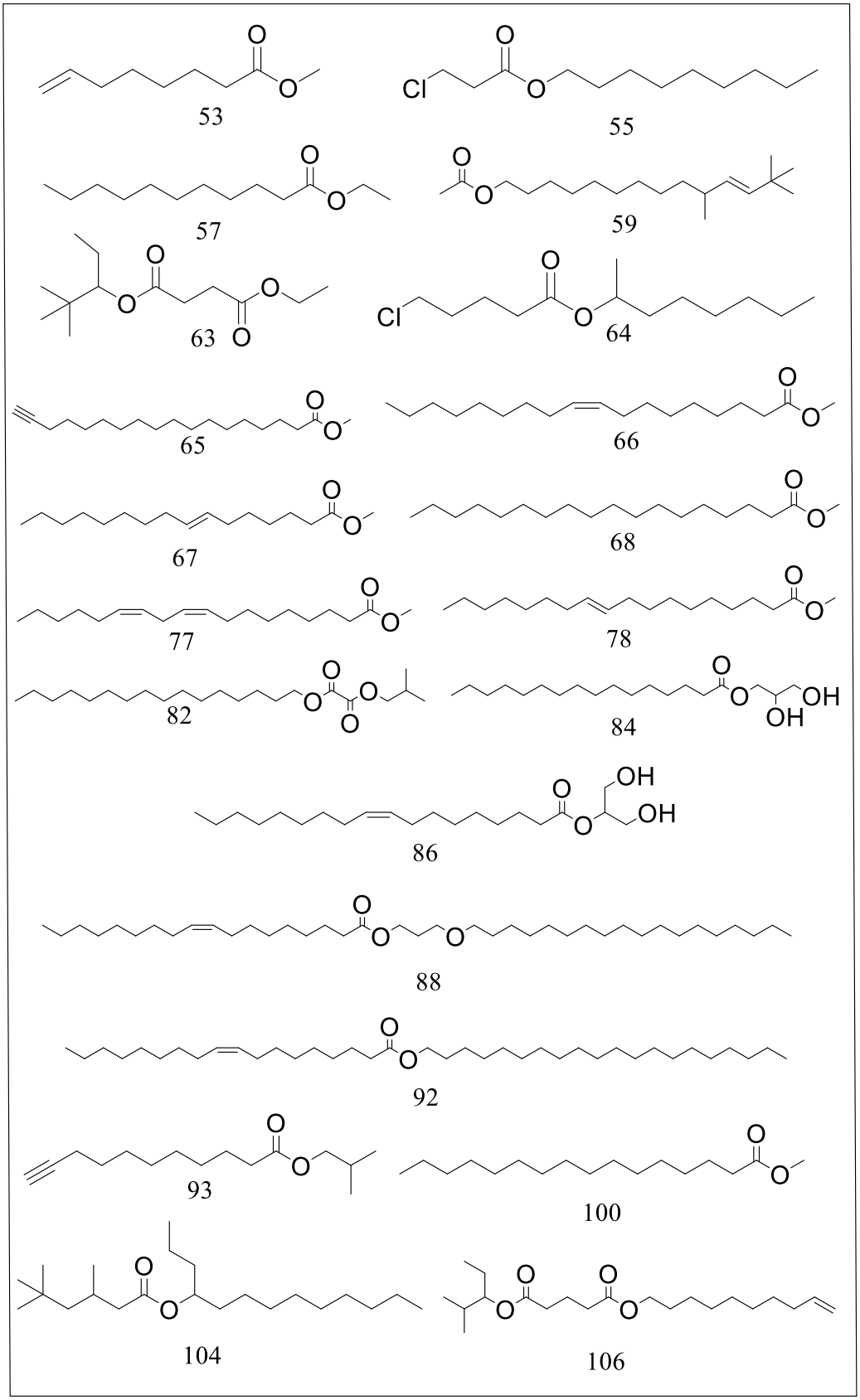

Figure 5: Continued.

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