Pharmacogn J. 2023; 15(3): 321-333
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

Metabolite Profiling of Compounds from *Sargassum polycystum* using UPLC-QToF-MS/MS

Pramudita Riwanti[1], Intan Kris Prasetyanti[2], Burhan Ma'arif[3,*]

ABSTRACT

Background: There are many types of seaweed that have high economic value. Brown seaweed (*Sargassum polycystum*) can be used as a raw material in the industry and as a medicinal plant. Maintaining the quality of a compound requires an analytical method that can identify the diversity of metabolome profiles. **Objective:** This investigation seeks to discover the metabolite profile of S. *polycystum* from Sumenep, Madura Island, Indonesia, utilizing the UPLC-QToF MS/MS equipment. **Materials and Methods:** The extract was further fractioned using n-hexane, ethyl acetate, and water. The metabolite profiling of extract and fractions used the UPLC-QToF-MS/MS instrument. It was produced with SPE and then introduced into the MS Xevo G2-S QToF detector of the ACQUITY UPLC® H-Class System. The findings of the UPLC-QToF-MS/MS analysis were processed with the MassLynx 4.1 software to obtain chromatogram data and m/z spectra of each observed peak, which were then validated using the ChemSpider and MassBank databases. **Results:** Based on the results of metabolite profiling using UPLC-QToF-MS/MS, the 96 % ethanol extract of *S. polycystum* indicated a total of 61 compounds, the n-hexane fraction indicated a total of 55 compounds, the ethyl acetate fraction indicated a total of 67 compounds, and the water fraction indicated a total of 49 compounds. **Conclusion:** There are 232 compounds in the extract and a fraction of *S. polycystum* consisting of 168 known compounds and 64 unknown compounds.

Key words: Metabolite profiling, *Sargassum polycystum*, Seaweed, UPLC-QToF-MS/MS.

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History

- Submission Date: 21-03-2023;
- Review completed: 25-04-2023;
- Accepted Date: 28-04-2023.

DOI : 10.5530/pj.2023.15.82

Article Available online

http://www.phcogj.com/v15/i6

INTRODUCTION

In Indonesia, there are many types of seaweed that have high economic value. One of them is brown seaweed (*Sargassum polycystum*). *S. polycystum* has the potential to be used as a raw material in industry and as a medicinal plant. It has secondary metabolites like alkaloids, glycosides, tannins, and steroids that are good for your health and are widely used in medicine and the pharmaceutical industry.[1] They also contain bioactive compounds such as fucoxanthin, steroids, phlorotannins, flavonoids, and saponins.[2-4]

The development of research on medicinal plants is increasing to get the benefits of the medicinal content of these plants for health. Therefore, it is important to ensure the quality of medicinal plants meets the requirements. The factors that can affect differences in chemical composition and quantity of a compound in plants are growing environmental conditions such as climate, growing media, the altitude where it grows, and metabolic processes (anabolism and catabolism) and their biosynthetic pathways.[5] Thus, an analytical method is needed that can identify the diversity of metabolome profiles (total metabolites present in the sample). One method that can be used in determining the diversity of metabolite profiles is the metabolomic approach. Metabolomics is the study of metabolite profiles in isolated biological samples, tissues, and cells. The aim is to identify all analytes, their concentrations, and metabolite profiles in plants.[6] Metabolite profiling analysis can use several techniques, namely a combination of chromatography and spectrophotometry. These techniques can provide detailed chromatographic profile of the detected sample.[7]

Using the UPLC-QToF MS/MS equipment, this study seeks to determine the metabolite profile of brown seaweed (*Sargassum polycystum*) from Sumenep, Madura Island, Indonesia. Using the Solid Phase Extraction (SPE) technique, extracts and fractions were obtained. The samples were subsequently placed within the ACQUITY UPLC® H-Class System's MS Xevo G2-S QToF detector (Waters, USA). The samples were separated on an ACQUITY BEH C18 column (1.7 m x 2.1 x 50 mm) with acetonitrile + 0.05% formic acid and water + 0.05% formic acid as mobile phases, with a flow rate of 0.2 ml/min. The results of the UPLC-QToF-MS/MS analysis were processed using MassLynx 4.1 software to obtain chromatogram data and m/z spectra of each detected peak. The detected compounds were further confirmed using the ChemSpider and MassBank online databases.

MATERIALS AND METHODS

Plant material

Brown seaweed (*Sargassum polycystum*) is harvested from Sumenep, Madura Island, Indonesia. The plants were then identified at the Fish Health and Aquatic Environment Management Service Unit, Faculty of Fisheries and Marine Affairs, Universitas Airlangga, Surabaya, with a determining key of *S. polycystum* and an identification letter number of No.015/ULMKILP/UA.FPK/10/2018.

Cite this article: Riwanti P, Prasetyanti IK, Ma'arif B. Metabolite Profiling of Compounds from *Sargassum polycystum* using UPLC-QToF-MS/MS. Pharmacogn J. 2023;15(3): 321-333.

Chemical material

The 96% ethanol, acetonitrile, formic acid, n-hexane, and ethyl acetate for use as the solvent and mobile phase in UPLC-QToF-MS/MS were purchased from Merck (Darmstadt, Germany).

Extraction and fractionation

*S. polycystum*was extracted with 96% ethanol using an ultrasonic method (Soltec Sonica 5300EP S3, Italy) for 3x5 minutes. Then it was filtered, and the filtrate was evaporated with a rotary evaporator Heidolph G3 at a temperature of 50°C with a rotation speed of 70 rpm. The ethanol extract then suspended in water at a 1:10 ratio. In a separating funnel, the water suspension (aqueous phase) was combined in a 1:1 ratio with n-hexane for liquid-liquid fractionation. The shaking procedure was repeated multiple times. After separating the n-hexane phase from the water phase, the n-hexane fraction of seaweed is evaporated using a rotary evaporator. The extracted and separated aqueous phase is then combined with ethyl acetate in a 1:1 ratio in a separating funnel for liquid-liquid fractionation using the same processes. The same procedures were followed to create the seaweed water fraction.

Metabolite profiling

Utilizing the UPLC-QToF-MS/MS technology, the metabolite profiling procedure was carried out at the Forensic Laboratory Center of the Indonesian National Police Criminal Investigation Agency. The SPE method was utilized throughout the process of extract and fraction preparation. Following this step, the samples were introduced into the ACQUITY UPLC® H-Class System's MS Xevo G2-S QToF detector (Waters, USA). The samples were separated using an ACQUITY BEH C18 column that was 2.1 m long and 50mm wide diameter and had a flow rate of 0.2 ml/min. The mobile phases consisted of acetonitrile + 0.05 % formic acid and water + 0.05 % formic acid. The findings of the UPLC-QToF-MS/MS analysis were processed with the MassLynx 4.1 software in order to get chromatogram data as well as the m/z spectra of each observed peak. Using the internet databases ChemSpider and MassBank, further verification of the chemicals that were found was carried out.

RESULTS AND DISCUSSION

Metabolite profiling was carried out to predict the content of compounds contained in extracts and fractions of *S. polycystum*.[8] Metabolite profiling was carried out with the UPLC-QToF-MS/MS instrument. Previously, extracts and fractions from *S. polycystum* had been prepared using the Solid Phase Extraction (SPE) method. The advantage of sample preparation with SPE is that it can separate impurities from the sample to produce higher spectral sensitivity.[9]

Metabolite profiling of extracts and fractions *S. polycystum* was carried out with the UPLC-QToF-MS/MS instrument, which had previously been prepared using the Solid Phase Extraction (SPE) method. Analysis of the blank total ion chromatogram (TIC) was determined before determining the TIC of the compound in the sample so as not to cause bias when identifying the sample. A mass spectral analysis of each TIC peak was performed using MassLynx 4.1 software and confirmed with ChemSpider and MassBank online databases.

The results of metabolite profiling of extracts and fractions of *S. polycystum* using the UPLC-QToF-MS/MS instrument in the form of a total ion chromatogram (TIC) in (Figure 1, Figure 2, Figure 3, and Figure 4), while the values of retention time (RT), %Area, m/z, molecular formulas, compound names, and their activities based on literature studies in (Table 1, Table 2, Table 3, and Table 4).

According to the findings of metabolite profiling carried out with UPLC-QToF-MS/MS, the extract and a fraction of *S. polycystum* include a total of 232 compounds. Of these compounds, 168 are known compounds, while the remaining 64 are unknown compounds. In the process of metabolite profiling, it is not possible to identify all of the peaks in the TIC based on the sum of all of the discovered chemicals. This is demonstrated by the presence of chemicals with uncertain identities in each extract and fraction. Compounds that are unable to be recognized in the database are referred to as unknown compounds. These compounds may be impurities or breakdown products that are still picked up by the instrument, or they may be new compounds that aren't in the database yet, particularly unknown compounds with high levels.[10,11] Either way, the instrument may still be able to detect them.

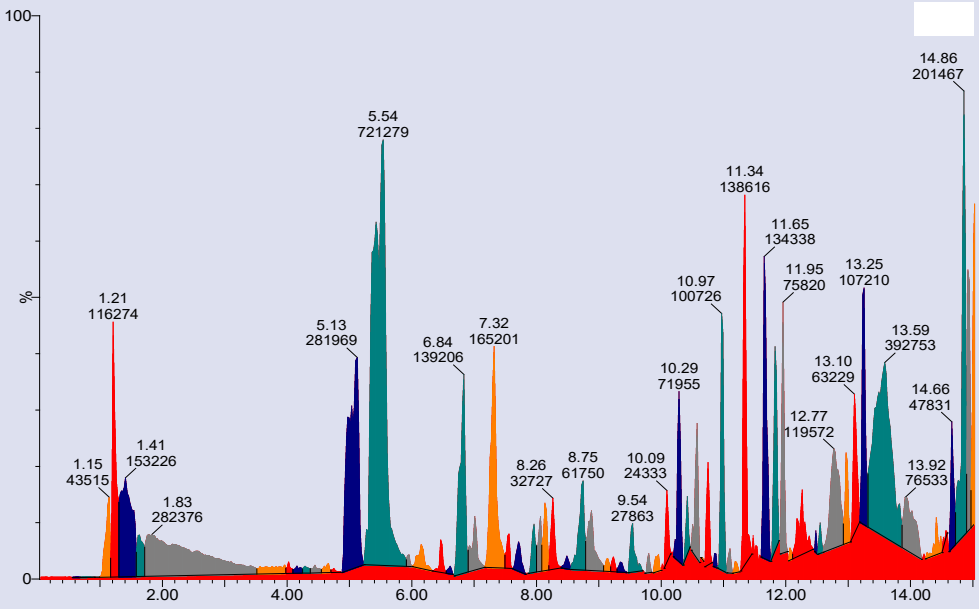

Figure 1: Total ion chromatogram (TIC) in 96% ethanol extract

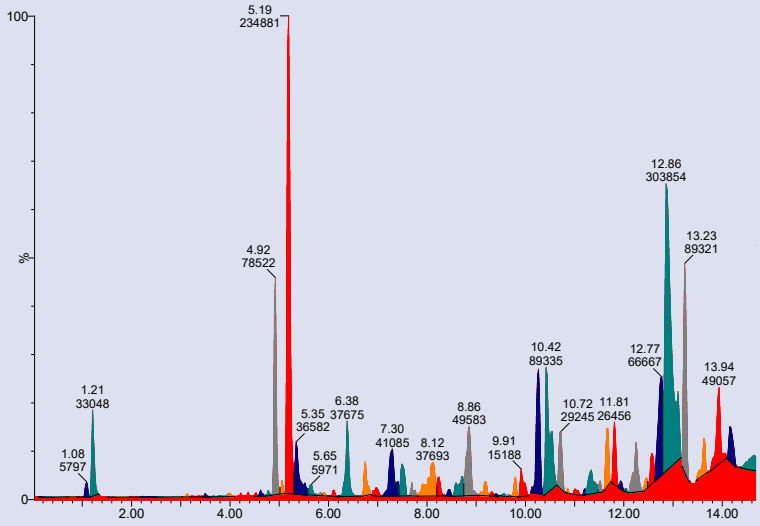

Figure 2: Total ion chromatogram (TIC) in the n-hexane fraction

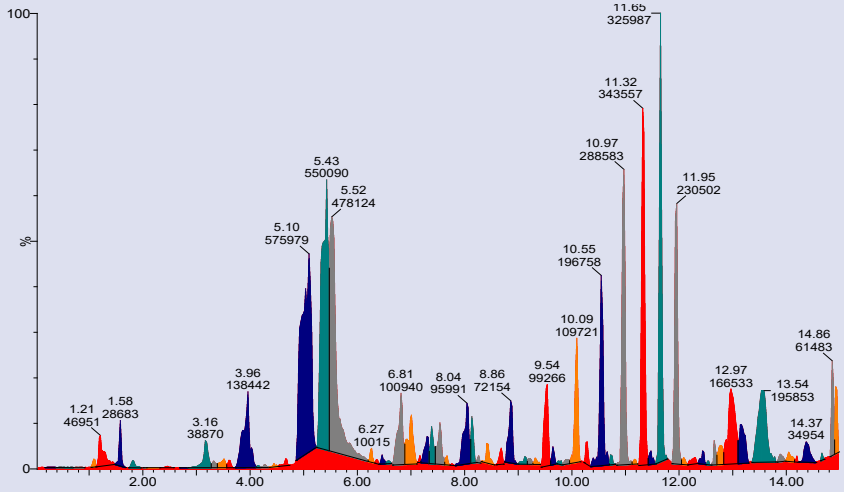

Figure 3: Total ion chromatogram (TIC) in the ethyl acetate fraction

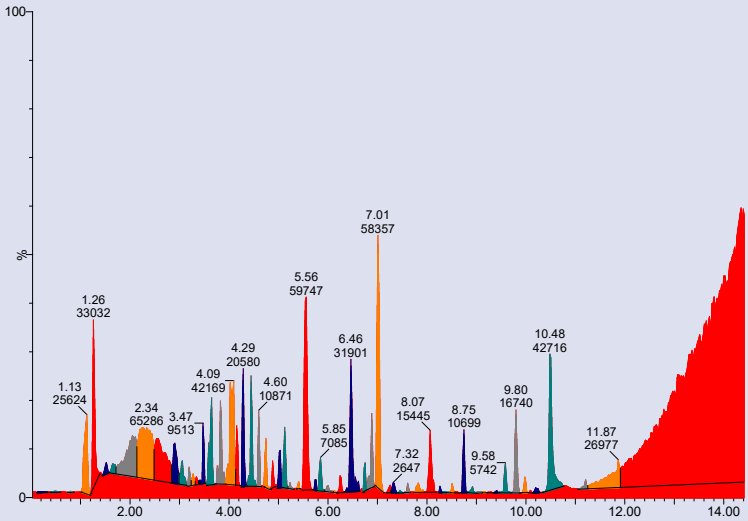

Figure 4: Total ion chromatogram (TIC) in the water fraction

Table 1: Prediction of compounds in 96% ethanol extract.

No	Retention Time	% Area	Measured Mass	Molecular Formula	Compounds Name	Activity Based on References
1	0.641	0.0060%	390.2764	$C_{19}H_{39}N_4O_2Cl$	3-{2-[(1-Amino-2,4-dimethyl-2-pentanyl) amino]-2-oxoethyl}-N-isobutyl-1-piperidinecarboxamide hydrochloride (1:1)	-
2	0.775	0.0075%	390.2755	$C_{13}H_{34}N_{12}S$	Unknown	-
3	0.951	0.0022%	390.2765	$C_{24}H_{38}O_4$	Bis(2-ethylhexyl) phthalate	Antimicrobial, antimutagenic. [12, 13]
4	1.148	0.9990%	150.0280	$C_3H_6N_2O_5$	Urea oxalate	-
5	1.830	13.4375%	142.0742	$C_6H_{10}N_2O_2$	Ectoine	Natural cell protectant treats inflammatory irritation.[14,15]
6	3.960	0.4770%	194.0808	$C_8H_{10}N_4O_2$	Caffeine	Antibacterial, antimicrobial, anticancer.[16, 17, 18]
7	4.023	0.1127%	653.2613	$C_{35}H_{31}N_{11}O_3$	Unknown	-
8	4.157	0.1209%	653.2613	$C_{14}H_{12}S$	4,6-DMDBT	-
9	4.663	0.1246%	175.0999	$C_{11}H_{13}NO$	Benzalamide	-
10	4.747	0.0372%	519.3272	$C_{23}H_{45}N_5O_8$	2-Methyl-2-propanyl 4-(20-azido-3,6,9,12,15,18-hexaoxaicos-1-yl)-1-piperazinecarboxylate	-
11	5.542	23.0318%	196.1103	$C_{11}H_{16}O_3$	2-Methyl-2-(3-oxobutyl)-1,3-cyclohexanedione	-
12	5.956	0.0946%	387.2789	$C_{10}H_{29}N_{17}$	Unknown	-
13	6.153	0.4444%	276.0096	$C_5H_4N_6O_8$	Methyl 5-(trinitromethyl)-2H-1,2,3-triazole-4-carboxylate	-
14	6.463	0.3034%	452.3368	$C_{24}H_{44}N_4O_4$	1,8,15,22-Tetraazacyclooctacosane-2,7,16,21-tetrone	-
15	6.617	0.0721%	359.2091	$C_{16}H_{30}N_5O_2Cl$	[4-(3-Aminopropoxy)-1-piperidinyl][1-(3-methylbutyl)-1H-1,2,3-triazol-4-yl]methanone hydrochloride (1:1)	-
16	6.835	3.1958%	236.1417	$C_{14}H_{20}O_3$	Heptylparaben	Antimicrobial. [19]
17	7.011	0.8334%	379.1842	$C_{16}H_{29}NO_9$	6-Deoxy-3-O-(4,6-dideoxy-4-formamido-3-C-methyl-2-O-methyl-α-L-mannopyranosyl)-2-O-methyl-D-mannose	-
18	7.320	3.7926%	236.1425	$C_7H_{20}N_6OS$	Unknown	-
19	7.559	0.3401%	264.1365	$C_{15}H_{20}O_4$	Abscisic acid	Managing glucose homeostasis. [20]
20	7.714	0.4232%	507.3188	$C_{25}H_{37}N_{11}O$	Unknown	-
21	7.961	0.5280%	227.1208	$C_9H_{17}N_5S$	Ametryn	-
22	8.065	0.5168%	465.2216	$C_{13}H_{35}N_7O_9S$	Unknown	-
23	8.136	0.7528%	188.1204	$C_{13}H_{16}O$	Cyclohexyl phenyl ketone	-
24	8.262	0.7513%	262.1575	$C_9H_{22}N_6OS$	1-(2-{Methyl[2-(methylsulfanyl)ethyl] amino}ethyl)-1,2,3-triazolidine-4-carbohydrazide	-
25	8.488	0.1409%	260.1406	$C_{11}H_{21}N_4OCl$	N2-[(1-Ethyl-3,5-dimethyl-1H-pyrazol-4-yl)methyl]-N-methylglycinamide hydrochloride (1:1)	-
26	8.748	1.4176%	180.1154	$C_{11}H_{16}O_2$	3-BHA	Antioxidants. [21]
27	8.881	1.2493%	282.1472	$C_{15}H_{22}O_5$	Artemisinin	Anti-malarial, anticancer, antiviral, etc. [22]
28	9.141	0.1523%	407.2146	$C_{23}H_{29}N_5S$	4-Benzyl-1-[(1-cyclopentyl-1H-tetrazol-5-yl)(2-thienyl)methyl] piperidine	-
29	9.233	0.1405%	290.1873	$C_{13}H_{27}N_4OCl$	N,2,2-Trimethyl-N-{[3-(2-methyl-2-propanyl)-1,2,4-oxadiazol-5-yl] methyl}-1,3-propanediamine hydrochloride (1:1)	-
30	9.346	0.1225%	290.1878	$C_{18}H_{26}O_3$	Octinoxate	UV filter. [23]
31	9.542	0.6396%	637.2965	$C_{22}H_{43}N_{11}O_9S$	Unknown	-
32	9.802	0.1317%	342.2880	$C_{19}H_{38}N_2O_3$	3-Lauroylamidopropyl βine	-

33	9.957	0.1991%	493.2533	$C_{23}H_{35}N_5O_7$	Lysyl-α-glutamylalanylphenylalanine	-
34	10.091	0.5586%	723.3318	$C_{32}H_{53}NO_{17}$	2-(Adamantan-2-yl)-N-(2-{[α-D-galactopyranosyl-(1->4)-β-D-galactopyranosyl-(1->4)-β-D-glucopyranosyl]oxy}ethyl)acetamide	-
35	10.288	2.5007%	254.1671	$C_{18}H_{22}O$	3-(4-Methylbenzylidene)camphor	Uterotrophic activity.[24]
36	10.421	0.3990%	213.2453	$C_{14}H_{31}N$	Lauryldimethylamine	-
37	10.576	1.2111%	809.3698	$C_{37}H_{55}N_5O_{15}$	Unknown	-
38	10.639	0.0061%	220.1464	$C_{14}H_{20}O_2$	2,6-Bis(2-methyl-2-propanyl)-1,4-benzoquinone	-
39	10.857	0.0906%	533.2479	$C_{15}H_{27}N_{21}S$	Unknown	-
40	10.970	2.3124%	895.4052	$C_{40}H_{65}NO_{21}$	Unknown	-
41	11.103	0.1888%	312.0998	$C_{18}H_{16}O_5$	3',4',5'-Trimethoxyflavone	-
42	11.187	0.0996%	274.1928	$C_{18}H_{26}O_2$	Nandrolone	Anabolic steroids to treat some illnesses and injuries.[26]
43	11.342	3.1822%	981.4389	$C_{40}H_{67}N_7O_{21}$	1,1'-(2-Chloro-1,3-propanediyl)bis(4-methylpiperazine)	-
44	11.652	3.0840%	1067.4810	$C_{50}H_{69}N_9O_{17}$	Unknown	-
45	11.827	1.9039%	303.2926	$C_{21}H_{37}N$	2-Hexyl-3,5-dipentylpyridine	-
46	11.953	1.7406%	1153.5175	$C_{54}H_{75}N_9O_{19}$	Unknown	-
47	12.066	0.0774%	306.1986	$C_{22}H_{26}O$	2,8-Dibenzylcyclooctanone	Hypolipidemic activity.[27]
48	12.263	1.1729%	519.3327	$C_{29}H_{41}N_7O_2$	3-[(4-Cyclohexyl-1-piperazinyl)(1-cyclohexyl-1H-tetrazol-5-yl)methyl]-6-ethoxy-2(1H)-quinolinone	-
49	12.480	0.0903%	276.2093	$C_{18}H_{28}O_2$	Octadecatetraenoic acid	Anti-inflammatory,cancer preventive, hepatoprotective, antihistaminic, etc.[28]
50	12.551	0.2015%	471.3550	$C_{23}H_{41}N_{11}$	Unknown	-
51	12.769	2.7451%	573.3167	$C_{29}H_{43}N_5O_7$	6-[9-sec-Butyl-6-(4-methoxybenzyl)-1,4,7,10-tetraoxotetradecahydropyrrolo[1,2-a][1,4,7,10]tetraazacyclododecin-3-yl]-N-hydroxyhexanamide	-
52	12.966	0.8484%	497.3715	$C_{28}H_{51}NO_6$	(2R)-2-{(4R,5R)-2,2-Dimethyl-5-[(1E)-1-tetradecen-1-yl]-1,3-dioxolan-4-yl}-2-({[(2-methyl-2-propanyl)oxy]carbonyl}amino)ethyl acetate	-
53	13.100	1.4516%	521.3532	$C_{22}H_{47}N_7O_7$	Unknown	-
54	13.255	2.4613%	481.3529	$C_{27}H_{43}N_7O$	N-{(1S)-3-[4-(3-Isopropyl-5-methyl-4H-1,2,4-triazol-4-yl)-1-piperidinyl]-1-phenylbutyl}-4-methyl-1-piperazinecarboxamide	-
55	13.585	9.0165%	332.2059	$C_{14}H_{28}N_4O_5$	Seryllysylvaline	-
56	13.916	1.7570%	499.3872	$C_{28}H_{53}NO_6$	Ethyl (3R)-3-(6-methoxy-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)-3-(pentadecylamino)propanoate	-
57	14.414	0.3924%	378.2772	$C_{23}H_{38}O_4$	2-Arachidonoylglycerol	Anti-inflammatory.[29]
58	14.569	0.0616%	394.2723	$C_{23}H_{38}O_5$	Methyl (2E,11α,13E,15R)-11,15-dihydroxy-16,16-dimethyl-9-oxoprosta-2,13-dien-1-oate	-
59	14.661	1.0981%	462.3005	$C_{13}H_{34}N_{16}O_3$	Unknown	-
60	14.857	4.6251%	608.2670	$C_{40}H_{36}N_2O_4$	2,2'-Bis[4-(1-piperidinyl)phenyl]-1H,1'H-2,2'-biindene-1,1',3,3'(2H,2'H)-tetrone	-
61	14.920	2.1247%	624.2614	$C_{24}H_{36}N_{10}O_{10}$	1,4-di(7-guanosinyl)butane	-

Table 2: Prediction of compounds in n-hexane fraction.

No	Retention Time	% Area	Measured Mass	Molecular Formula	Compounds Name	Activity Based on References
1	1.085	0.3682%	150.0280	$C_3H_6N_2O_5$	Urea oxalate	-
2	1.211	2.0987%	142.0739	$C_6H_{10}N_2O_2$	Ectoine	Natural cell protectant treats inflammatory irritation.[14,15]
3	1.541	0.0082%	122.0482	$C_6H_6N_2O$	Nicotinamide	Anti-inflammatory effects, lightening effect, anti-microbial effect, barrier protective effect, photoprotective effect.[30]
4	2.223	0.2416%	390.2769	$C_{24}H_{38}O_4$	Bis(2-ethylhexyl) phthalate	Anti-microbial, antimutagenic. [12,13]
5	2.905	0.1484%	390.2763	$C_{19}H_{39}N_4O_2Cl$	3-{2-[(1-Amino-2,4-dimethyl-2-pentanyl)amino]-2-oxoethyl}-N-isobutyl-1-piperidinecarboxamide hydrochloride (1:1)	-
6	3.123	0.0612%	246.1727	$C_{15}H_{22}N_2O$	Sophocarpine	Analgesic and anti-inflammatory. [31]
7	3.496	0.0614%	196.1094	$C_6H_{17}N_4OCl$	1-[2-(Dimethylamino)ethyl]-2-hydroxy-3-methylguanidine hydrochloride (1:1)	-
8	3.960	0.0547%	370.2579	$C_{18}H_{34}N_4O_4$	2,3,16,17-Octadecanetetraone tetraoxime	-
9	4.002	0.0434%	182.0944	$C_{10}H_{14}O_3$	Mephenesin	Treating depression, muscle relaxant. [32]
10	4.621	0.1262%	211.1100	$C_5H_{17}N_5O_2S$	Unknown	-
11	4.776	0.0998%	452.3358	$C_{24}H_{44}N_4O_4$	1,8,15,22-Tetraazacyclooctacosane-2,7,16,21-tetrone	-
12	4.923	7.3096%	196.1104	$C_{11}H_{16}O_3$	2-Methyl-2-(3-oxobutyl)-1,3-cyclohexanedione	-
13	5.056	0.2470%	411.2971	$C_{18}H_{42}N_5O_3Cl$	Unknown	-
14	5.190	14.9160%	196.1109	$C_4H_{16}N_6OS$	Unknown	-
15	5.655	0.4505%	274.0105	$C_{13}H_{11}N_2Br$	1-[4-(Bromomethyl)phenyl]-2-phenyldiazene	-
16	5.851	0.0642%	410.3968	Unknown	Unknown	-
17	6.111	0.1480%	412.2790	$C_{18}H_{40}N_2O_8$	Bis[2-hydroxy-N-(2-hydroxyethyl) ethanaminium] sebacate	-
18	6.224	0.0107%	390.2755	$C_{13}H_{34}N_{12}S$	Unknown	-
19	6.379	2.3925%	452.3354	$C_{23}H_{48}O_8$	(4S,7S,10S,13S,16S,19S,22S)-4,7,10,13,16,19-Hexamethyl-2,5,8,11,14,17,20-heptaoxatetracosan-22-ol	-
20	6.969	0.3526%	264.1366	$C_{15}H_{20}O_4$	Abscisic acid	Managing glucose homeostasis. [20]
21	7.299	3.5806%	236.1414	$C_{14}H_{20}O_3$	Heptylparaben	Antimicrobial. [19]
22	7.496	1.3363%	264.1356	$C_{10}H_{21}N_4O_2Cl$	2-[4-(Ethoxycarbonyl)-1-piperazinyl] imidazolidin-1-ium chloride	-
23	7.693	0.3182%	507.3186	$C_{25}H_{37}N_{11}O$	Unknown	-
24	8.115	2.3937%	188.1205	$C_{13}H_{16}O$	Cyclohexyl phenyl ketone	-
25	8.241	0.5872%	262.1568	$C_{16}H_{22}O_3$	Homosalate	Exhibits estrogenic and antiandrogenic activity. [33]
26	8.438	0.1799%	260.1423	$C_9H_{20}N_6OS$	1-(1-Imidazolidinyl)-2-[(1-propyl-5-tetrazolidinyl)sulfanyl]ethanone	-
27	8.706	1.0802%	180.1162	$C_4H_{16}N_6S$	Unknown	-
28	8.860	3.1488%	282.1473	$C_{16}H_{26}S_2$	1,2-Bis[(2,2-dimethylpropyl)sulfanyl] benzene	-
29	9.191	0.4936%	278.0862	$C_8H_{14}N_4O_7$	Diazolidinyl urea	Antimicrobial preservatives. [34]
30	9.317	0.1008%	290.1883	$C_{18}H_{26}O_3$	Octinoxate	UV filter. [23]
31	9.409	0.0576%	299.1723	$C_{10}H_{26}N_5O_3Cl$	Unknown	-
32	9.563	0.0670%	278.1512	$C_{11}H_{23}N_4O_2Cl$	1-Boc-4-[(Aminoiminomethyl) amino]-piperidine monohydrochloride	-

33	9.668	0.0365%	332.1613	$C_{14}H_{25}N_4O_3Cl$	1-(2-Hydroxy-3-{[(5-methoxy-1,3-dimethyl-1H-pyrazol-4-yl)methyl] amino}propyl)-2-pyrrolidinone hydrochloride (1:1)	-
34	9.802	0.4518%	342.2883	$C_{19}H_{38}N_2O_3$	3-Lauroylamidopropyl βine	-
35	9.915	0.9645%	284.2836	Unknown	Unknown	-
36	10.266	6.4701%	254.1675	$C_{18}H_{22}O$	3-(4-Methylbenzylidene)camphor	Uterotrophic activity. [24]
37	10.421	5.6732%	213.2466	Unknown	Unknown	-
38	10.857	0.0604%	691.3794	$C_{34}H_{53}N_5O_{10}$	4-(4-{[3-(β-D-Glucopyranosyloxy)-5-isopropyl-1H-pyrazol-4-yl] methyl}-3-methylphenoxy)-N-{1-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-1-oxo-2-propanyl}butanamide	-
39	11.011	0.2035%	489.2918	$C_{28}H_{43}NO_4S$	N-(4-{3-[4-(2-Hydroxy-3,3-dimethylbutoxy)-3-methylphenyl]-3-pentanyl}-2-methylbenzyl) ethanesulfonamide	-
40	11.208	0.0963%	370.3180	Unknown	Unknown	-
41	11.342	1.3174%	545.2881	$C_{24}H_{43}N_5O_7S$	L-Methionyl-L-valyl-L-leucyl-L-seryl-L-proline	-
42	11.518	0.2361%	517.3178	$C_7H_{35}N_{25}OS$	Unknown	-
43	11.673	1.7162%	506.2234	$C_{21}H_{30}N_8O_7$	α-Aspartylhistidylhistidylvaline	-
44	11.806	1.6801%	493.3186	$C_{15}H_{43}N_9O_9$	Unknown	-
45	11.932	0.1902%	303.2930	$C_{21}H_{37}N$	2-Hexyl-3,5-dipentylpyridine	-
46	12.045	0.0703%	306.1976	$C_{14}H_{30}N_2O_3S$	N-[2-(Diisopropylamino) ethyl]-N,2-dimethyltetrahydro-3-furansulfonamide	-
47	12.242	2.0315%	519.3334	$C_{22}H_{41}N_{13}S$	Unknown	-
48	12.459	0.2576%	294.2198	$C_{18}H_{30}O_3$	13-Oxooctadecadienoic acid	Anti-inflammatory. [25]
49	12.572	1.0559%	456.3913	Unknown	Unknown	-
50	12.769	4.2337%	573.3181	$C_{30}H_{39}N_9O_3$	L-Arginyl-N-[2-(1H-indol-3-yl)ethyl] glycyl-N2-[2-(1H-indol-3-yl)ethyl] glycinamide	-
51	12.861	19.2961%	332.2063	$C_{14}H_{28}N_4O_5$	Seryllysylvaline	-
52	13.233	5.6723%	481.3523	$C_{19}H_{47}N_9O_3S$	Unknown	-
53	13.627	1.1378%	473.3706	$C_{23}H_{43}N_{11}$	Unknown	-
54	13.937	3.1153%	578.2178	$C_{34}H_{26}N_8O_2$	2,2'-[1,3-Phenylenebis(iminomethyly lidene)]bis(1-hydroxy-3-methyl-1,2-dihydropyrido[1,2-a]benzimidazole-4-carbonitrile)	-
55	14.154	1.4868%	360.2387	$C_{20}H_{41}Br$	1-Bromoicosane	-

Table 3: Prediction of compounds in the ethyl acetate fraction.

No	Retention Time	% Area	Measured Mass	Molecular Formula	Compounds Name	Activity Based on References
1	0.311	0.0080%	390.2761	$C_{19}H_{39}N_4O_2Cl$	3-{2-[(1-Amino-2,4-dimethyl-2-pentanyl)amino]-2-oxoethyl}-N-isobutyl-1-piperidinecarboxamide hydrochloride (1:1)	-
2	0.600	0.0224%	390.2774	$C_{24}H_{38}O_4$	Bis(2-ethylhexyl) phthalate	Antimicrobial, antimutagenic. [12, 13]
3	0.859	0.0114%	390.2781	$C_{25}H_{34}N_4$	[Methylenebis(1H-indole-3,5-diyl)] bis(N,N,N-trimethylmethanaminium)	-
4	1.085	0.1202%	150.0282	$C_3H_6N_2O_5$	Urea oxalate	-
5	1.211	0.9731%	142.0737	$CH_{11}N_6Cl$	Unknown	-
6	1.583	0.5945%	122.0479	$C_6H_6N_2O$	Nicotinamide	Anti-inflammatory effects, lightening effect, anti-microbial effect, barrier protective effect, photoprotective effect. [30]
7	1.809	0.1577%	180.0899	$C_9H_{12}N_2O_2$	Dulcin	Artificial sweeteners. [35]
8	2.462	0.0269%	164.0951	$C_9H_{12}N_2O$	Fenuron	-
9	2.751	0.0050%	390.2755	$C_{13}H_{34}N_{12}S$	Unknown	-
10	3.165	0.8056%	246.1740	$H_{18}N_{14}O_2$	Unknown	-

11	3.320	0.1516%	220.1203	$C_7H_{17}N_6Cl$	N-1-Piperidinylimidodicarbonimidic diamide hydrochloride (1:1)	-
12	3.516	0.2788%	196.1108	$C_4H_{16}N_6OS$	Unknown	-
13	3.608	0.1377%	145.0528	C_9H_7NO	2-Quinolinol	-
14	3.960	2.8694%	194.0808	$C_8H_{10}N_4O_2$	Caffeine	Antibacterial, antimicrobial, anticancer. [16, 17, 18]
15	4.157	0.0248%	129.1512	Unknown	Unknown	-
16	4.445	0.0499%	431.2713	$C_{15}H_{33}N_{11}O_4$	Unknown	-
17	4.663	0.1253%	182.0858	$C_{11}H_{15}Cl$	2,3,5,6-Tetramethylbenzyl chloride	-
18	5.098	33.2489%	196.1096	$C_{11}H_{16}O_3$	2-Methyl-2-(3-oxobutyl)-1,3-cyclohexanedione	-
19	6.266	0.2076%	262.1207	$C_{15}H_{18}O_4$	Austricin	Anti-inflammatory. [36]
20	6.358	0.0529%	290.1882	$C_{18}H_{26}O_3$	Octinoxate	UV filter. [23]
21	6.463	0.1311%	452.3373	$C_{25}H_{40}N_8$	N2-Cycloheptyl-N4-[4-(dimethylamino)phenyl]-N6-[(1-ethyl-2-pyrrolidinyl)methyl]-1,3,5-triazine-2,4,6-triamine	-
22	6.575	0.0517%	308.2206	$C_{16}H_{36}OS_2$	Unknown	-
23	6.814	2.7793%	236.1414	$C_{14}H_{20}O_3$	Heptylparaben	Antimicrobial. [19]
24	7.011	1.6061%	379.1844	$C_{20}H_{30}N_3SCl$	N-(3-Chloro-2-methylphenyl)-2-[2-(1-piperidinyl)ethyl]-1-piperidinecarbothioamide	-
25	7.166	0.0573%	414.2142	$C_{18}H_{31}N_6O_3Cl$	N-(2-Aminoethyl)-3-(3-butyl-7-isobutyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)propanamide hydrochloride (1:1)	-
26	7.383	0.7487%	246.1264	$C_8H_{18}N_6OS$	N-(1-Ethyl-5-tetrazolidinyl)-2-(1,3-thiazolidin-5-yl)acetamide	-
27	7.538	1.0215%	264.1383	$CH_{12}N_{16}O$	Unknown	-
28	7.672	0.1236%	255.1269	$C_{10}H_{23}N_3Cl_2$	3-(2,6-Dimethyl-piperidin-1-yl)-propionamidine 2HCl	-
29	7.764	0.0156%	582.3732	$C_{27}H_{54}N_2O_{11}$	Unknown	-
30	8.044	1.9895%	465.2206	$C_{33}H_{27}N_3$	(3Z)-N-(4-Methylphenyl)-1,2,4,4-tetraphenyl-1,2-diazetidin-3-imine	-
31	8.136	0.9414%	188.1204	$C_{13}H_{16}O$	Cyclohexyl phenyl ketone	-
32	8.262	0.0755%	262.1572	$C_{16}H_{22}O_3$	Homosalate	Exhibits estrogenic and antiandrogenic activity. [33]
33	8.417	0.3860%	396.2048	$C_{23}H_{28}N_2O_4$	Paynantheine	Antinociception. [37]
34	8.860	1.4955%	551.2586	$C_{15}H_{29}N_{21}OS$	Unknown	-
35	9.120	0.1777%	407.2165	$C_{19}H_{29}N_5O_5$	Lysylasparaginylphenylalanine	-
36	9.212	0.1319%	499.3714	$C_{14}H_{45}N_{17}OS$	Unknown	-
37	9.317	0.1059%	292.2026	$C_{13}H_{29}N_4OCl$	Propanoyl chloride - 1,4,8,11-tetraazacyclotetradecane (1:1)	-
38	9.542	2.0574%	637.2951	$C_{34}H_{39}N_9O_2S$	2-({3-[4-(4-Benzyl-1-piperazinyl) phenyl]-3H-[1,2,3]triazolo[4,5-d] pyrimidin-5-yl}amino)-N-cyclohexyl-N-methylbenzenesulfonamide	-
39	9.647	0.2713%	306.2181	Unknown	Unknown	-
40	9.802	0.0650%	342.2877	Unknown	Unknown	-
41	9.936	0.1256%	493.2521	$C_{35}H_{31}N_3$	(3Z)-1,2-Bis(3-methylphenyl)-N-(4-methylphenyl)-4,4-diphenyl-1,2-diazetidin-3-imine	-
42	10.091	2.2741%	723.3341	$C_{34}H_{45}N_9O_9$	2-(Ethyl{4-[(E)-(4-nitrophenyl) diazenyl]phenyl}amino)ethyl 4-[(3R,4R)-3,4-bis({[(2S,4R)-4-hydroxy-2-pyrrolidinyl]carbonyl} amino)-1-pyrrolidinyl]-4-oxobutanoate	-
43	10.287	0.3606%	254.1673	$C_{18}H_{22}O$	3-(4-Methylbenzylidene)camphor	Uterotrophic activity. [24]
44	10.547	4.0781%	809.3723	$C_{39}H_{47}N_{13}O_7$	Unknown	-
45	10.723	0.1449%	254.1662	$C_{10}H_{26}N_2O_3S$	N,N,N-Trimethylmethanaminium 3-(isopropylamino)-1-propanesulfonate	-

46	10.970	5.9812%	895.4044	$C_{53}H_{57}N_3O_{10}$	Methyl [6a-(allyloxy)-4-[(benzyloxy)imino]-1,2-bis(4-hydroxybutyl)-10-(3-nitrophenoxy)-1,2,4,5,6,6a,11b,11c-octahydrobenzo[kl]xanthen-6-yl](1-naphthylmethyl)carbamate	-
47	11.166	0.0769%	451.2594	$C_{18}H_{33}N_{11}OS$	Unknown	-
48	11.321	7.1206%	981.4477	$C_{43}H_{67}N_9O_{15}S$	L-Valyl-L-isoleucyl-L-isoleucyl-L-alanyl-L-α-aspartyl-L-cysteinylglycyl-L-α-glutamyl-L-tyrosine	-
49	11.476	0.2713%	549.3884	$C_{11}H_{39}N_{27}$	Unknown	-
50	11.652	6.7565%	1067.4767	$C_{45}H_{69}N_{11}O_{19}$	L-Seryl-L-asparaginyl-L-glutaminyl-L-α-glutamyl-L-tyrosyl-L-leucyl-L-α-aspartyl-L-leucyl-L-serine	-
51	11.953	4.7774%	1153.5145	$C_{40}H_{63}N_{31}O_9S$	Unknown	-
52	12.087	0.1256%	877.3958	$C_{41}H_{59}N_5O_{16}$	Unknown	-
53	12.305	0.1965%	531.3422	$C_{28}H_{45}N_5O_5$	3-Cyclohexyl-1-({(2R,3R)-5-[(2R)-1-hydroxy-2-propanyl]-9-[(isopropylcarbamoyl)amino]-3-methyl-6-oxo-2,3,4,5,6,7-hexahydro-1,5-benzoxazonin-2-yl}methyl)-1-methylurea	-
54	12.439	0.2358%	671.3084	$C_{31}H_{49}N_3O_{11}S$	(2S,3R,4E)-2-Azido-1-[(6-O-sulfo-β-D-galactopyranosyl)oxy]-4-octadecen-3-yl benzoate	-
55	12.530	0.0431%	471.3564	$C_{26}H_{49}NO_6$	3-(Nonyloxy)propyl 2,3-O-isopropylidene-5-O-[2-(1-pyrrolidinyl)ethyl]-α-D-lyxofuranoside	-
56	12.656	0.3294%	714.3308	$C_{29}H_{42}N_{14}O_8$	N,N,N',N'-Tetrakis[2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl]-1,5-pentanediamine	-
57	12.790	0.5837%	573.3217	$C_{35}H_{39}N_7O$	3-{(1-Cyclohexyl-1H-tetrazol-5-yl)[4-(diphenylmethyl)-1-piperazinyl]methyl}-6-methyl-2(1H)-quinolinone	-
58	12.966	3.4516%	332.2066	$C_{15}H_{32}N_4S_2$	1,5-Pentanediyl bis(N'-butylcarbamimidothioate)	-
59	13.163	1.4307%	331.3239	$C_{23}H_{41}N$	Benzyldioctylamine	-
60	13.535	4.0593%	677.4734	$C_{21}H_{59}N_{17}O_8$	Unknown	-
61	13.887	0.1754%	499.3865	$C_{25}H_{45}N_{11}$	Unknown	-
62	14.041	0.2392%	535.3728	$C_{10}H_{37}N_{27}$	Unknown	-
63	14.196	0.0538%	484.2828	$C_{29}H_{40}O_6$	Cortisone 21-cyclopentanepropionate	-
64	14.372	0.7245%	360.2361	$C_{11}H_{33}N_8O_3Cl$	Unknown	-
65	14.661	0.0620%	462.2986	$C_{27}H_{42}O_6$	Trihexyl trimellitate	-
66	14.857	1.2743%	608.2634	$C_{35}H_{36}N_4O_6$	2-[4-(Diphenylmethyl)-1-piperazinyl]ethyl methyl 2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate	-
67	14.920	0.9441%	624.2569	$C_{47}H_{32}N_2$	(E,E)-N,N'-(9H-Fluorene-9,9-diyldi-4,1-phenylene)bis[1-(1-naphthyl)methanimine]	-

Table 4: Prediction of compounds in the water fraction.

No	Retention Time	% Area	Measured Mass	Molecular Formula	Compounds Name	Activity Based on References
1	0.311	0.1307%	390.2766	$C_{24}H_{38}O_4$	Bis(2-ethylhexyl) phthalate	Antimicrobial, antimutagenic. [12, 13]
2	1.127	3.6449%	150.0277	$C_3H_6N_2O_5$	Urea oxalate	-
3	2.336	26.6756%	142.0738	$C_6H_{10}N_2O_2$	Ectoine	Natural cell protectant treats inflammatory irritation. [14, 15]
4	2.905	2.2484%	187.0997	$C_{12}H_{13}NO$	Naphthalene acetamide	-
5	3.060	0.9949%	207.1253	$C_7H_{18}N_5Cl$	N-(N'-Carbamimidoylcarbamimidoyl)-1-pentanaminium chloride	-

6	3.194	0.4447%	246.1725	$C_{15}H_{22}N_2O$	Sophocarpine	Analgesic and anti-inflammatory. [31]
7	3.278	0.3379%	248.1889	$C_{15}H_{24}N_2O$	Matrine	Anticancer, anti-inflammatory, antiviral, analgesic, anti-fibrotic, insecticidal, antimicrobial, etc. [38]
8	3.341	0.1993%	247.1212	$C_{14}H_{17}NO_3$	Ethyl 2-ethoxy-1(2H)-quinolinecarboxylate	-
9	3.475	1.3531%	276.0982	$C_4H_{12}N_{12}OS$	Unknown	-
10	3.650	2.9426%	237.1743	Unknown	Unknown	-
11	3.826	2.5859%	320.1239	$C_{21}H_{20}OS$	4-(5-n-Butylthiophen-2-yl) benzophenone	-
12	4.094	5.9982%	364.1493	$C_{15}H_{20}N_6O_5$	N-Allyl-6-diazo-5-oxonorleucyl-6-diazo-5-oxonorleucine	-
13	4.157	1.3529%	228.0404	$C_7H_{13}O_6Cl$	2,4,4-Trimethyl-5,6-dihydro-4H-1,3-dioxin-1-ium perchlorate	-
14	4.291	2.9273%	408.1749	$C_{16}H_{28}N_2O_{10}$	2-Acetamido-3-O-(3-acetamido-3,6-dideoxy-β-D-glucopyranosyl)-2-deoxy-D-galactose	-
15	4.445	2.7686%	452.2026	$C_{20}H_{32}N_6O_2S_2$	N,N'-(1,8-Octanediyldi-1,3,4-thiadiazole-5,2-diyl)bis(2-methylpropanamide)	-
16	4.600	1.5463%	496.2292	$C_{22}H_{36}N_6O_3S_2$	2-({4-Amino-5-[3-(diethylsulfamoyl) phenyl]-4H-1,2,4-triazol-3-yl} sulfanyl)-N,N-diisobutylacetamide	-
17	4.747	1.1279%	540.2553	$C_{24}H_{40}N_6O_4S_2$	Diethyl 2,2'-{1,4-butanediylbis[(4-butyl-4H-1,2,4-triazole-5,3-diyl) sulfanediyl]}diacetate	-
18	4.881	0.5101%	584.2815	$C_{26}H_{44}N_6O_5S_2$	Unknown	-
19	5.036	1.1319%	238.0616	$C_{10}H_{19}S_2Cl$	4-(Chloromethyl)-2-hexyl-1,3-dithiolane	-
20	5.127	1.7193%	196.1105	$C_{11}H_{16O3}$	2-Methyl-2-(3-oxobutyl)-1,3-cyclohexanedione	-
21	5.232	0.1284%	411.2973	$C_5H_{29}N_{23}$	Unknown	-
22	5.408	0.1226%	300.0973	$C_{21}H_{16}S$	2-Methyl-4,6-diphenyl-1-benzothiophene	-
23	5.563	8.4985%	314.0770	$C_{21}H_{14}OS$	1-Phenyl-2-(9H-thioxanthen-9-ylidene)ethanone	-
24	5.851	1.0078%	678.5013	$C_{32}H_{62}N_{12}O_4$	Unknown	-
25	6.132	0.0349%	142.0730	$CH_{11}N_6Cl$	Unknown	-
26	6.245	0.4810%	215.1525	$C_{11}H_{21}NO_3$	Hexaminolevulinate	-
27	6.463	4.5376%	452.3373	$C_{25}H_{40}N_8$	N2-Cycloheptyl-N4-[4-(dimethylamino)phenyl]-N6-[(1-ethyl-2-pyrrolidinyl)methyl]-1,3,5-triazine-2,4,6-triamine	-
28	6.751	0.6979%	419.2878	$C_{21}H_{41}NO_7$	Unknown	-
29	6.885	1.9138%	501.2937	$C_{25}H_{43}NO_9$	N-{[6-(2,3-Dimethoxypropyl)-5,5-dimethyl-4-oxotetrahydro-2H-pyran-2-yl](methoxy)methyl}-2-hydroxy-2-(2-methoxy-5,6-dimethyl-4-methylenetetrahydro-2H-pyran-2-yl) acetamide	-
30	7.011	8.3007%	400.1121	$C_{20}H_{21}N_4OSCl$	6-Chloro-N-({4-[(2E)-3-phenyl-2-propen-1-yl]-1-piperazinyl} carbonothioyl)nicotinamide	-
31	7.258	0.2038%	447.2822	$C_{22}H_{46}N_3OBr$	4-(Diethylcarbamoyl)-1-dodecyl-1-methylpiperazin-1-ium bromide	-
32	7.320	0.3764%	236.1412	$C_{14}H_{20}O_3$	Heptylparaben	Antimicrobial. [19]
33	7.454	0.0513%	527.3438	$C_{32}H_{49}NO_3S$	(1R,3R,5E)-5-[(2Z)-2-{(1R,7aR)-1-[(2S,6R)-6-(Hydroxymethyl)-7-methyl-6-(1,3-thiazol-2-yl)-2-octanyl]-7a-methyloctahydro-4H-inden-4-ylidene}ethylidene]-4-methylene-1,3-cyclohexanediol	-

34	7.609	0.1819%	366.1791	$C_{18}H_{26}N_2O_6$	Methylatropine nitrate	Spasmolytic. [39]
35	8.065	2.1969%	529.3239	$C_{32}H_{43}N_5S$	N-Isopropyl-4-{2-[(1R,5S)-3-(2-methyl-1H-benzimidazol-1-yl)-8-azabicyclo[3.2.1]oct-8-yl]ethyl}-4-phenyl-1-piperidinecarbothioamide	-
36	8.262	0.1085%	262.1561	$C_{11}H_{23}N_4OCl$	N-[(5-Methoxy-1,3-dimethyl-1H-pyrazol-4-yl)methyl]-N,N'-dimethyl-1,2-ethanediamine hydrochloride (1:1)	-
37	8.333	0.1071%	390.2781	$C_{25}H_{34}N_4$	[Methylenebis(1H-indole-3,5-diyl)] bis(N,N,N-trimethylmethanaminium)	-
38	8.509	0.1900%	529.3277	$C_{29}H_{39}N_9O$	(2R)-1-{Methyl[1-(6-{[9-(trans-4-methylcyclohexyl)-9H-pyrido[4',3':4,5]pyrrolo[2,3-d] pyrimidin-2-yl]amino}-3-pyridazinyl)-4-piperidinyl]amino}-2-propanol	-
39	8.747	1.9238%	529.3249	$C_{27}H_{47}NO_9$	(5R)-5-(11-{2-[2,2-Dihydroxy-2-(1-piperidinyl)ethoxy]ethoxy}-10,10-dihydroxy-8-undecyn-1-yl)-3-(2-hydroxypropyl)dihydro-2(3H)-furanone	-
40	8.923	0.1963%	372.1193	$C_{17}H_{20}N_6S_2$	2-{[(3-Cyclopropyl-1H-1,2,4-triazol-5-yl)sulfanyl]methyl}-6,7,8,9-tetrahydro-5H-cyclohepta[4,5] thieno[2,3-d]pyrimidin-4-amine	-
41	9.015	0.1143%	390.2759	$C_{19}H_{39}N_4O_2Cl$	3-{2-[(1-Amino-2,4-dimethyl-2-pentanyl)amino]-2-oxoethyl}-N-isobutyl-1-piperidinecarboxamide hydrochloride (1:1)	-
42	9.409	0.0423%	469.3400	$C_{26}H_{47}NO_6$	2-Hydroxy-3-(stearoyloxy)propyl 5-oxo-L-prolinate	-
43	9.584	0.8168%	342.1080	$C_{23}H_{18}OS$	4-[Phenyl(phenylsulfanyl)methyl]-1-naphthol	-
44	9.802	2.3812%	342.2884	$C_{19}H_{38}N_2O_3$	3-Lauroylamidopropyl βine	-
45	9.978	0.3875%	344.1263	$C_{19}H_{20}O_6$	4-Hydroxy-2',3,4',6'-tetramethoxychalcone	-
46	10.091	0.0402%	366.3242	$C_{22}H_{42}N_2O_2$	2-Allyl-N,N'-diheptyl-N,N'-dimethylmalonamide	-
47	10.196	0.2050%	327.9977	$C_5H_{13}N_2O_{10}SCl$	Unknown	-
48	11.208	0.2759%	370.3163	Unknown	Unknown	-
49	11.869	3.8373%	303.2920	Unknown	Unknown	-

The results of metabolite profiling performed on 96% ethanol extract showed a total of 61 compounds, 46 of which were known compounds and 15 of which were unknown compounds; the n-hexane fraction showed a total of 55 compounds, consisting of 38 known compounds and 17 unknown compounds; the ethyl acetate fraction showed a total of 67 compounds, consisting of 45 known compounds and 22 unknown compounds; and the water fraction showed a total of 49 The interpretation of these metabolites reveals that several dominant compounds or major compounds have higher levels (indicated by percent area) when compared to the levels of other compounds found in the sample. This is the case because these levels are higher than the levels of the other compounds. In the 96% ethanol extract, the major components were 2-methyl-2-(3-oxobutyl)-1,3-cyclohexanedione with a percent area of 27.6748%; in the n-hexane fraction, the major components were seryllysylvaline with a percent area of 29.8551%; in the ethyl acetate fraction, the major components were 2-methyl-2-(3-oxobutyl)-1,3-cyclohexanedione with a percent area of 41.4148 %; and in water fraction, the major components were ectoin with a percent area of 29.9702 %.

CONCLUSION

The 96 % ethanol extract of *S. polycystum* indicated a total of 61 compounds, including 46 known compounds and 15 unknown compounds; the n-hexane fraction indicated a total of 55 compounds, including 38 known compounds and 17 unknown compounds; the ethyl acetate fraction indicated a total of 67 compounds, including 45 known compounds and 22 unknown compounds; and the water fraction indicated a total of 49 compounds, including 39 known compounds and 4 unknown compounds.

ACKNOWLEDGEMENT

None declared.

CONFLICTS OF INTEREST

There are no conflicts of interest with the parties engaged in this investigation, according to the authors.

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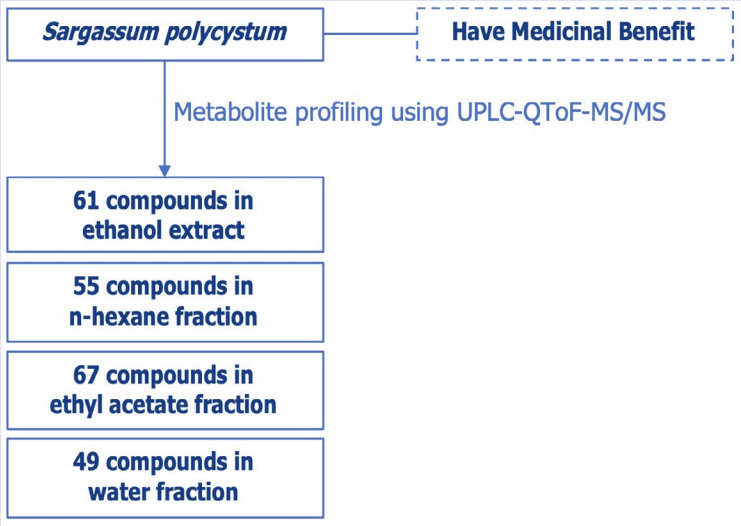
GRAPHICAL ABSTRACT



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Cite this article: Riwanti P, Prasetyanti IK, Ma'arif B. Metabolite Profiling of Compounds from *Sargassum polycystum* using UPLC-QToF-MS/MS. Pharmacogn J. 2023;15(3): 321-333.