Metabolite Profiling of 96 % Ethanol Extract *Marsilea Crenata* Presl. Leaves Using Uplc-Qtof-Ms/Ms

Agnis Pondinekaria Aditama*, Mangestuti Agil

Keywords *Marsilea crenata* Presl., metabolite profiling, UPLC-QTOF-MS/MS, 96% ethanol.

Abstract *Marsilea crenata* Presl. plants grow in east java area, usually consumed by local people, and was known having medical purposes. Some researches were conducted toward to the plant and showed that the plant having potential treatment to some diseases. The aim of research is to know the contain of *Marsilea crenata* Presl. compound by using UPLC MS/MS methode. *Marsilea crenata* Presl. M crenata was ekstracted using etanol 96% by using Ustrasonic Assisted Extraction methode. The first step was prepare 100 extrac ppm, and then were injected 5 µL to UPLC MS/MS. The next step, the data obtained was total ion chromatogram (TIC), and the last step, data was analyzed by using soffware Masslynx 4.1. Which shown in each equipment dichloromethane (DCM) blank 47 compound and methanol blank 50 compound. This is the first report of the application of non-targeted metabolomics in *Marsilea crenata* Presl.

1 INTRODUCTION

Marsilea crenata Presl. Contains of different phytochemical which having medical purposes. Tthe benefit explained above is the effect of metabolit secunder that was obtained in *Marsilea crenata* Presl. Secondary metabolism is chemical material that was resulted from the plant metabolism process that is useful to the plant. Secondary metabolism is classified according to chemical structured functional characteristic such as alkaloid, flavonoid, saponin, tannin, poliphenole, antraquinone and volatile oil (Manitto, 1992; Jacoeb *et al*., 2010). Some research had been done to know the activity of *Marsilea crenata* Presl. Some of them are, *Marsilea crenata* Presl. Leaves had been observed by using *Radio Immuno Assay* (RIA) and activity observation in vivo in female mice. The result showed that 96 % ethanol extract *Marsilea crenata* Presl. Leaves enable to inhibit osteoporosis to pascamenopouse woman by increasing bone remodelling process mechanism especially in the bone forming (Putra and Laswati, 2011).

The research that had been done was Gas Chromatography-Mass Spectrometry (GC-MS) analysis where the result showed that some compound such as monoterpenoid, diterpenoid, fatty acid, and other compound have not been known in n-hexane extract of *Marsilea crenata* Presl. Leaves. and Palmitat contain was assumed enable to increase the bone forming process with induction mechanism in osteoblast cell so that it can be used as phytoestrogen (Ma'arif *et al*., 2016).

According to the previous research GC-MS instrument was used in order to know *Marsilea crenata* Presl. Metabolit secondary contain, but not all secondary metabolit chemical compound can be analysed because lack of instrument, so only volatile compound can be analysed. Periodic and update library is needed because there are some compound having similar m/z model, so it is known as similarityindex (SI). Therefore metabolit profiling must be done by using Ultra Performance Liquid Chromatography-Mass Spectrometer (UPLC-MS) Instrument. UPLC-MS instrument is liquid chromatography technique with mass spectrometer detector. Bio analysis research use UPLC-MS. The instrument is specific and having wide application as well as practical method. The application of this instrument is not restricted only for volatile molecule, high flecsibility and limited time (K Naresh *et al*., 2014; Chawla and Ranjan, 2016). The using of UPLC-MS can give scientific data that is benefical for the user of the plant drug.

Metabolite Profiling of 96 .
DOI: 10.5220/0009841600002406
In *Proceedings of BROMO Conference (BROMO 2018) - Symposium on Natural Product and Biodiversity*, page 1
ISBN: 978-989-758-347-6

2 MATERIAL AND METHOD

2.1 Material

We performed UPLC-QTOF-MS/MS (*Waters*), Oasis C18 Cartridge (*Waters*), Sonicator (*Sonica)*, Moisture Analyzer (*Mettler Toledo*), Vacuum Rotary Evaporator (*Heidolph*), TLC (*Camag*), TLC Visualizer (*Camag*), analytical scales (XX), flasks, beaker glass, measuring cups, petri dishes, stirrer bars, spatulas, dropper pipes, funnel, filter paper, eppendorf, and computers.

Marsilea crenata Presl. Leaves were obtained from Benowo village at Surabaya, ethanol 96% (*Merck*), aquadest, dichloromethane (*Merck*), acetonitrile (Merck) and formic acid (Merck).

2.2 Methods

2.2.1 Sample Preparation

The extract preparation was done by simplicia of *Marsilea crenata* Presl. Leaves powder weighed 30 g and put into the Erlenmeyer flask, then dissolved with 500 ml ethanol solvent with replication 3 times (200 ml, 150 ml, 150 ml). Further extraction is done with the help of ultrasonic waves (> 20 kHz) for 6 minutes with 3 pauses every 2 minutes. Ethanol 96% extraction was performed by single extraction. The extract was evaporated using a Rotary evaporator, then stored in an oven with a temperature of 40^0C.

2.2.2 Extract Preparation to UPLC-QTOF-MS/MS Analysis

Sample was injected to instrument UPLC MS/MS 5µl, and than chromathogram was obtained and the data was processed by using software Masslynx so that peak area, retention time, spectra m/z dan elemental composition was obtained from each peak area was detected. The next step, data interpretation was done by using website Chemspider to get the level of data similarity from chromagram and spectra, so that the similarity explained above, we can get the suitable IUPAC name and it can be concluded that metabolit contain was in M.crenata extract.

3 RESULT AND DISCUSSION

The extraction method used by ultrasonic assisted extraction (UAE) which has advantages, among others, accelerating the extraction process (compared with conventional extraction eg maceration), more time efficient, and can increase the crude rendement rate of the extract. In addition, ultrasonic extraction may also be used in the extraction of heat resistant materials (Handayani *et al*., 2016).

Fourty seven compounds in DCM blank and Fifty compounds in methanol blank were obtained from UPLC MS/MS analysis. Data obtained was total ion of kromatogram (TIC) and 96 % ethanol extract from *Marsilea crenata* Presl. leaves that was processed by using software Masslynx so that peak area, retention time, spectra m/z dan elemental composition was obtained from each peak area was detected. The next step, data interpretation was done by using website Chemspider to get the level of data similarity from chromagram and spectra, so that the similarity explained above, we can get the suitable IUPAC name.

Fifty major contain were tentatively assigned based on their accurate masses, MS/MS fragmentation patterns in methanol blank and Forty-seven major contain in dichloromethane (DCM) blank, in comparison to standard compounds and references (Table 1 and 2).

The largest compound in 96% ethanol extract leaves *Marsilea crenata* Presl. on methanol blank with % area 23,3199 %; 11.9297% and 10.3549% are unknown compounds where the chemspider application does not recognize it or has never been published. Whereas in the DCM blank on % area 37.6384 % is $C_{36}H_{36}N_5O_6SCl$ after data interpretation was done by using website Chemspider and software Chemdraw so that compound similarity 4-[(N-{2-[(6-Chloro-2-methyl-4-quinolinyl)amino] ethyl}-N-[(4-methoxyphenyl) sulfonyl] -β-alanyl) amino] -3-methoxy-N-phenylbenzamide was obtained ; peak area 26.3455 % is $C_{38}H_{38}N_5O_{11}Cl$ and suitable with compound (1R, 13S, 16S, 17R, 28R) -28-Amino-20-chloro-17,25-dihydroxy-5,8,10,24-tetramethoxy-N-methyl-15,29, 31-trioxo-22-oxa-14,30,32-triazahexacyclo 14.14.2.218,21.12,6.123,27,07,12] hexatriaconta-2 (36), 3,5, 7,9,11,18,20,23 (33), 24,26,34-dodecaene-13-carboxamide and we did not obtaine the compound name that was not suitable with the compound name reference. So that we catagorized as unknown compound.

The activity of the major compound explained above had non been obtained yet before. According

to the research was done, it need to analyzed deeply in order to get the data about unknown compound.

4 CONCLUSIONS

From the analysis data, we can conclude that there are some phytochemical compound in *Marsilea crenata* Presl. leaves that was known having major unknown compound.

REFERENCES

Afriastini, J. J. 2003. *Marsilea Crenata Presl. De Winter WP, Amoroso VB*. Edited by Cryptograms. Bogor : LIPI: Ferns and fern allies.

Chawla, Gita, and Chanda Ranjan. 2016. Principle, Instrumentation, and Applications of UPLC: A Novel Technique of Liquid Chromatography.*Open Chemistry Journal* 3 (1): 1–16. https://doi.org/10.2174/18748422016 03010001.

Fiehn, O, J Kopka, P Dormann, T Altmann, R N Trethewey, and L Willmitzer. 2000. Metabolite Profiling for Plant Functional Genomics. *Nature Biotechnology* 18: 1157–61.https://doi.org/10.1038/ 81137.

Handayani Hana, Feronika Heppy Sriherfyna, and Yunianta. 2016. Ekstraksi Antioksidan Daun Sirsak Metode Ultrasonic Bath (Kajian Rasio Bahan : Pelarut Dan Lama Ekstraksi. *Jurnal Pangan Dan Agroindustri* 4 (1): 262–72.

Jacoeb AM, Nurjanah, Arifin M, Sulistiono W, Kristiono SS. 2010. Deskripsi Histologi Dan Perubahan Komposisi Kimia Daun Dan Tangkai Semanggi (Marsilea Ccrenata Presl., Marsileaceae) Akibat Perubahan. *Pengolahan Hasil Perikanan Indonesia* 13 (2).

K Naresh, S Bhawani and T Maneesh Kumar. 2014. Ultra Performance Liquid. 3 (3): 84–94.

Ma'arif, Burhan, Mangestuti Agil1 and Hening Laswati. 2016. Phytochemical Assessment On N-Hexane Extract And Fractions Of Marsilea Crenata Presl . Leaves Through GC-MS" 21 (August): 77–85.

Ma'arif, Burhan. 2015. Aktivitas Ekstrak N-Heksana Dan Fraksi Hasil Pemisahan Daun Marsilea Crenata Presl. Terhadap Diferensiasi Sel Preosteoblas MC3T3-E1 Melalui Pengukuran Alkaline Phosphatase In Vitro.

Manitto, P. 1992. *Biosintesis Metabolit Sekunder*. Edited by Koensomardiyah dan B. Sudarto. semarang: IKIP Semarang Press.

Putra, Hening Laswati. 2011. Green Clover Potentiates Delaying the Increment of Imbalance Bone Remodeling Process in Postmenopausal Women. *Folia Medica Indonesiana* 47 (2): 112–17.

Singh, Sheelendra Pratap, Nistha Dwivedi, Kanumuri Siva Rama Raju, Isha Taneja, and Mohammad Wahajuddin. 2016. Validation of a Rapid and Sensitive UPLC-MS-MS Method Coupled with Protein Precipitation for the Simultaneous Determination of Seven Pyrethroids in 100 μL of Rat Plasma by Using Ammonium Adduct as Precursor Ion. *Journal of Analytical Toxicology* 40 (3): 213–21. https://doi.org/10.1093/jat/bkw002. Waters. 2008.

Table 1. Metabolite profiling *Marsilea crenata* Presl.in methanol blank by UPLC-QTOF-MS/MS.

No	RT	% Area	**formula**	**Trivial name**	**IUPAC name**	**Activity**
1	0,200694	0,0039%	-	-	-	-
2	0,478472	0,0014%	-	-	-	-
3	1.535	2,4313%	C10H21NO5	4-(3-Hydroxypropyl)-4-nitro-1,7-heptanediol	4-(3-Hydroxypropyl)-4-nitro-1,7-heptanediol	-
4	2.232	0,1510%	C11H21NO7	2-[(tert-Butoxycarbonyl)amino]-2-deoxy-D-glucopyranose	2-Deoxy-2-({[(2-methyl-2-propanyl)oxy]carbonyl}amino)-D-glucopyranose	-
5	2.518	1,5144%	C12H23NO7	Methyl 4,6-dideoxy-4-{[(2R)-2,4-dihydroxybutanoyl]amino}-2-O-methyl-α-D-mannopyranoside	Methyl 4,6-dideoxy-4-{[(2R)-2,4-dihydroxybutanoyl]amino}-2-O-methyl-α-D-mannopyranoside	-
6	3.799	1,4856%	C15H21NO7	Methyl (3,4,5-triethoxy-2-nitrophenyl)acetate	Methyl (3,4,5-triethoxy-2-nitrophenyl)acetate	-
7	4.427	1,4055%	C5H15N3Cl2	4-Hydrazinopiperidine dihydrochloride	4-Hydrazinopiperidine dihydrochloride	-
	4.610	0,3629%	C9H6O3	3 hydroxycoumarin	3-Hydroxy-2H-chromen-2-one	Penghambatan kompetitif DAAO rekombinan manusia (Molla, 2017).
8	4.896	0,1836%	C20H24N3SCl	Prochlorperazine	2-Chloro-10-[3-(4-methyl-1-piperazinyl)propyl]-10H-phenothiazine	Analgesik (callan, 2008), amtiemetik (roberge, 2006)
9	5.228	0,9215%	C13H18N5O5Cl	Ethyl 4-[3-(4-chloro-3-nitro-1H-pyrazol-1-yl)propanoyl]-1-piperazinecarboxylate	Ethyl 4-[3-(4-chloro-3-nitro-1H-pyrazol-1-yl)propanoyl]-1-piperazinecarboxylate	-
10	5.445	0,0257%	C33H37N3	4-{Bis[4-(1-pyrrolidinyl)phenyl]methyl}-N,N-dimethyl-1-naphthalenamine	4-{Bis[4-(1-pyrrolidinyl)phenyl]methyl}-N,N-dimethyl-1-naphthalenamine	-
11	5.628	0,9906%	C10H21N3O8S	1-Azido-1-deoxy-2,3-bis-O-(methoxymethyl)-5-O-(methylsulfonyl)-D-ribitol	1-Azido-1-deoxy-2,3-bis-O-(methoxymethyl)-5-O-(methylsulfonyl)-D-ribitol	-
12	5.845	0,6908%	C29H18N4O6S	2-(2-{(E)-2-Cyano-2-[4-(2-oxo-2H-chromen-3-yl)-1,3-thiazol-2-yl]vinyl}-4-nitrophenoxy)-N-phenylacetamide	2-(2-{(E)-2-Cyano-2-[4-(2-oxo-2H-chromen-3-yl)-1,3-thiazol-2-yl]vinyl}-4-nitrophenoxy)-N-phenylacetamide	-
13	6.177	1,0895%	C25H22O11	4-(1,3-Benzodioxol-5-yl)-6-hydroxy-1-oxo-1,3-	4-(1,3-Benzodioxol-5-yl)-6-hydroxy-1-oxo-1,3-dihydronaphtho[2,3-	-

				dihydronaphtho[2,3-c]furan-5-yl hexopyranoside	c]furan-5-yl hexopyranoside	
14	6.577	0,3205%	C24H22O14	2-(3,4-Dihydroxyphenyl)-5-hydroxy-4-oxo-4H-chromen-7-yl 6-O-(carboxyacetyl)-β-D-glucopyranoside	2-(3,4-Dihydroxyphenyl)-5-hydroxy-4-oxo-4H-chromen-7-yl 6-O-(carboxyacetyl)-β-D-glucopyranoside	-
15	6.908	0,2713%	C14H21NO	1-[1-(4-Methoxyphenyl)cyclohexyl]methanamine	1-[1-(4-Methoxyphenyl)cyclohexyl]methanamine	-
16	7.206	2,0878%	C11H16O3	1-carboxy-3-hydroxyadamantane	3-Hydroxy-1-adamantanecarboxylic acid	-
17	7.423	0,6567%	C16H23NO2	UNII:891H89GFT4	1-(7-Ethyl-1-benzofuran-2-yl)-2-[(2-methyl-2-propanyl)amino]ethanol	-
18	7.640	0,2325%	C11H24N5Cl	1-Hexyl-6,6-dimethyl-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (1:1)	1-Hexyl-6,6-dimethyl-1,6-dihydro-1,3,5-triazine-2,4-diamine hydrochloride (1:1)	-
19	7.903	0,3096%	C14H22N5Cl	1-methyl-2-[(4-methylpiperazin-1-yl)methyl]benzimidazol-5-amine hydrochloride	1-Methyl-2-[(4-methyl-1-piperazinyl)methyl]-1H-benzimidazol-5-amine hydrochloride (1:1)	-
20	8.406	1,4141%	C36H46N4O	Manzamine J	(1R,2R,12R,13S,16Z)-25-(9H-β-Carbolin-1-yl)-11,22-diazatetracyclo[11.11.2.12,22.02,12]heptacosa-5,16,25-trien-13-ol	-
21	8.886	0,0560%	C17H31NO9	6-O-(N-{[(2-Methyl-2-propanyl)oxy]carbonyl}-D-leucyl)-α-D-allopyranose	6-O-(N-{[(2-Methyl-2-propanyl)oxy]carbonyl}-D-leucyl)-α-D-allopyranose	-
22	9.321	0,1071%	C18H27NO2	dyclonine	1-(4-Butoxyphenyl)-3-(1-piperidinyl)-1-propanone	Inhibitor Aldehyde Dehydrogenase 1 (ALDH1A1) (Collard, 2007). Antimicroba (Florestano,1956)
23	9.584	0,1649%	C13H29N3O4S	(3R,4R)-3-{[(2-Hydroxyethyl)(methyl)amino]methyl}-4-(hydroxymethyl)-N-isopropyl-N-methyl-1-pyrrolidinesulfonamide	(3R,4R)-3-{[(2-Hydroxyethyl)(methyl)amino]methyl}-4-(hydroxymethyl)-N-isopropyl-N-methyl-1-pyrrolidinesulfonamide	-
24	10.601	0,6568%	C12H18NO	N,N,N-Trimethyl-3-oxo-3-phenyl-1-propanaminium	N,N,N-Trimethyl-3-oxo-3-phenyl-1-propanaminium	-
25	10.830	0,3341%	C47H61N3O8S	2-({(3β,7β,8ξ,9ξ,10α,1	2-({(3β,7β,8ξ,9ξ,10α,12β,13	-

				2β,13α,14ξ,17α,20S)-3-[(2-{[(3-Acetyl-2-methyl-4-quinolinyl)amino]methyl}phenyl)ethynyl]-3,7,12-trihydroxy-24-oxocholan-24-yl}amino)ethanesulfonic acid	α,14ξ,17α,20S)-3-[(2-{[(3-Acetyl-2-methyl-4-quinolinyl)amino]methyl}phenyl)ethynyl]-3,7,12-trihydroxy-24-oxocholan-24-yl}amino)ethanesulfonic acid	
26	11.082	0,4582%	-	-	-	-
27	11.379	0,8714%	C37H47N9OS	-	-	-
28	11.562	1,7782%	C14H19N4O2Cl	Lintopride	4-Amino-5-chloro-N-[(1-ethyl-4,5-dihydro-1H-imidazol-2-yl)methyl]-2-methoxybenzamide	-
29	11.928	0,4325%	C28H49NO12	2-Methyl-2-propanyl 2-cyano-3-[(4S,5R)-5-{(5S,6R)-6-[(4R)-2,2-dimethyl-1,3-dioxolan-4-yl]-2,4,7,9-tetraoxadecan-5-yl}-2,2-dimethyl-1,3-dioxolan-4-yl]-2-(1-ethoxyethoxy)propanoate	2-Methyl-2-propanyl 2-cyano-3-[(4S,5R)-5-{(5S,6R)-6-[(4R)-2,2-dimethyl-1,3-dioxolan-4-yl]-2,4,7,9-tetraoxadecan-5-yl}-2,2-dimethyl-1,3-dioxolan-4-yl]-2-(1-ethoxyethoxy)propanoate	-
30	12.179	0,3815%	C27H49NOS2	2-[(Bis{2-[(2-methyl-2-propanyl)sulfanyl]ethyl}amino)methyl]-4,6-bis(2-methyl-2-propanyl)phenol	2-[(Bis{2-[(2-methyl-2-propanyl)sulfanyl]ethyl}amino)methyl]-4,6-bis(2-methyl-2-propanyl)phenol	-
31	12.397	1,5741%	C25H45NO9	Pederin	(2S)-N-[(S)-{(2S,4R,6R)-6-[(2S)-2,3-Dimethoxypropyl]-4-hydroxy-5,5-dimethyltetrahydro-2H-pyran-2-yl}(methoxy)methyl]-2-hydroxy-2-[(2R,5R,6R)-2-methoxy-5,6-dimethyl-4-methylenetetrahydro-2H-pyran-2-yl] acetamide	Anticancer (ghoneim, 2013)
32	12.614	1,9858%	C33H59NO14	2-(aziridin-1-yl)ethanol; decanedioic acid; 2,2-dimethylpropane-1,3-diol; 2-ethyl-2-(hydroxymethyl)propane-1,3-diol; isophthalic acid	-	-
33	12.797	2,5108%	C29H39N7O2	1-(2-Methylalanyl-5-phenyl-D-norvalyl)-4-{2-[2-(2H-tetrazol-5-yl)ethyl]phenyl}piperidine	1-(2-Methylalanyl-5-phenyl-D-norvalyl)-4-{2-[2-(2H-tetrazol-5-yl)ethyl]phenyl}piperidine	-
34	13.208	0,9465%	C30H53NO12	(3S)-16-{[(1S)-1-Carboxyethyl]amino}-2-methyl-16-oxo-3-hexadecanyl 6-O-(3-carboxypropanoyl)-β-D-glucopyranoside	(3S)-16-{[(1S)-1-Carboxyethyl]amino}-2-methyl-16-oxo-3-hexadecanyl 6-O-(3-carboxypropanoyl)-β-D-glucopyranoside	-

35	13.460	2,6423%	C29H45N5O2	8-(Benzylamino)-7-hexadecyl-3-methyl-3,7-dihydro-1H-purine-2,6-dione	8-(Benzylamino)-7-hexadecyl-3-methyl-3,7-dihydro-1H-purine-2,6-dione	-
36	13.677	2,4722%	C28H46N5O2Cl	N4-(5-Chloro-2,4-dimethoxyphenyl)-N6-hexadecyl-4,5,6-pyrimidinetriamine	N4-(5-Chloro-2,4-dimethoxyphenyl)-N6-hexadecyl-4,5,6-pyrimidinetriamine	-
37	14.409	10,3549 %	C25H50NO6Cl	-	-	-
38	14.740	2,0423%	C22H48N9Cl	N2-[3-({12-[(3-Aminopropyl)amino]dodecyl}amino)propyl]-N4-methyl-1,3,5-triazine-2,4,6-triamine hydrochloride (1:1)	N2-[3-({12-[(3-Aminopropyl)amino]dodecyl}amino)propyl]-N4-methyl-1,3,5-triazine-2,4,6-triamine hydrochloride (1:1)	-
39	15.106	23,3199 %	C8H39N23O	-	-	-
40	15.404	4,7166%	C24H50N9Cl	-	-	-
41	15.769	1,1138%	C8NO15S6Br2	-	-	-
42	15.952	0,6060%	C8NO15S6Br2	-	-	-
43	16.718	5,9510%	C36H36N5O6SCl	4-[(N-{2-[(6-Chloro-2-methyl-4-quinolinyl)amino]ethyl}-N-[(4-methoxyphenyl)sulfonyl]-β-alanyl)amino]-3-methoxy-N-phenylbenzamide	4-[(N-{2-[(6-Chloro-2-methyl-4-quinolinyl)amino]ethyl}-N-[(4-methoxyphenyl)sulfonyl]-β-alanyl)amino]-3-methoxy-N-phenylbenzamide	-
44	17.004	1,3681%	C7H24N19O9Cl	-	-	-
45	17.999	4,6577%	C46H48N5OS4Cl	-	-	-
46	18.330	11,9297 %	C8NO15S6Br2	-	-	-
47	21.509	0,0036%	-	-	-	-
48	21.726	0,0049%	-	-	-	-
49	22.389	0,0047%	-	-	-	-
50	22.755	0,0043%	-	-	-	-

Table 2. Metabolite profiling *Marsilea crenata* Presl.in DCM blank by UPLC-QTOF-MS/MS.

No	Rt	%Area	Formula	Trivial name	IUPAC name	Activity
1	0.289	0,0032%	C11H23N4O2Cl	Tert-Butyl 4-carbamimidamidopiperidine-1-carboxylate hydrochloride (1:1)	2-Methyl-2-propanyl 4-carbamimidamido-1-piperidinecarboxylate hydrochloride (1:1)	-
2	0.540	0,0278%	C16H22O4	Dibutyl phthalate	Dibutyl phthalate	Antibacteri (Khatiwora 2012), glikosidase inhibitor (Lee 2000), estrogenik (Harris 1997)
3	0.906	0,0049%	C9H22N6O2S	-	-	-
4	1.420	0,2361%				-

5	1.786	0,0096%	C11H23NO2	11-Aminoundecanoic acid	11-Aminoundecanoic acid	-
6	1.969	0,0041%	C10H23N4O3P	Propanedioic acid, 2-[[bis(1-methylethyl)phosphinyl]methyl]-, dihydrazide	2-[(Diisopropylphosphoryl)methyl]malonohydrazide	-
7	2.084	0,0670%	C11H23NO2	11-Aminoundecanoic acid	11-Aminoundecanoic acid	-
8	2.186	0,0306%				-
9	2.632	2,8001%				-
10	4.427	0,0282%	C15H27NO5	Megalanthonine	[(1S,7R,7aR)-7-Hydroxyhexahydro-1H-pyrrolizin-1-yl]methyl (2S,3S)-2,3-dihydroxy-2-isopropylbutanoate	antifeedant and antifungal (Reina 1998)
11	4.930	0,0127%	C9H21N11O	-	-	-
12	5.342	0,2477%				-
13	5.479	0,0731%				-
14	5.662	0,0912%				-
15	5.925	0,0405%	C35H41N3O	Cycloheptaneacetamide, N-(phenylmethyl)-α-[4-[(5,6,7,8-tetrahydro-4-methyl-9H-pyrido[2,3-b]indol-9-yl)methyl]phenyl]-	N-Benzyl-2-cycloheptyl-2-{4-[(4-methyl-5,6,7,8-tetrahydro-9H-pyrido[2,3-b]indol-9-yl)methyl]phenyl}acetamide	-
16	6.211	0,0164%				-
17	6.474	0,0109%				-
18	6.840	0,0031%	-	-	-	-
19	7.206	0,2253%	C11H16O3	1-Carboxy-3-hydroxyadamantane	3-Hydroxy-1-adamantanecarboxylic acid	-
20	7.457	0,0010%	-	-	-	-
21	7.640	0,0242%	C12H25NO2	Dodecanoic acid, 12-amino-	12-Aminododecanoic acid	-
22	8.006	0,1302%	C18H25NO	Dextromethorphan	(9α,13α,14α)-3-Methoxy-17-methylmorphinan	Antitussive (Manap 1999), anticonvulsant (Mohseni 2016), neuroprotective (Zhang 2004)
23	9.504	0,0908%	C20H31NO	Trihexyphenidyl	1-Cyclohexyl-1-phenyl-3-(1-piperidinyl)-1-propanol	antiparkinson antikolinergic (Takahashi 1999), anti oksidan (Ji 2008)
24	9.950	0,0080%				-
25	10.967	0,5387%				
26	11.448	2,3323%	C16H35N	Hexadecylamine	1-Hexadecanamine	antibacteri, adjuvant for diphtheria, tetanus toxoid, and influenza (Attwood 2012)
27	11.630	0,3879%	C17H37NO2	2-Amino-2-tetradecylpropane-1,3-diol	2-Amino-2-tetradecyl-1,3-propanediol	-
28	11.88	0,0775%	C19H18O	Benzylbutylphthalate	3-(1-Phenyl-2-	Estrogenik

	2		4		pentanyl)phthalate	(Harris 1997)
29	12.111	0,0640%	C17H26O5	Portentol	(1S,2S,3S,3'R,4R,4'R,5'S,6'R,8R)-4'-Hydroxy-1,3,3',5',6',8-hexamethyltetrahydro-6H,7H-spiro[5-oxabicyclo[2.2.2]octane-2,2'-pyran]-6,7-dione	Anticancer (Schröckeneder 2012)
30	12.248	0,0123%	C15H33N	Pentadecylamine	1-Pentadecanamine	-
31	12.396	0,0027%	C19H41NO2	1,2-Propanediol, 3-(hexadecylamino)-	3-(Hexadecylamino)-1,2-propanediol	-
32	12.694	0,6293%	C19H18O4	Benzylbutylphthalate	3-(1-Phenyl-2-pentanyl)phthalate	Estrogenik (Harris 1997)
33	12.842	0,9778%	C21H37N	4-Pentadecylaniline	4-Pentadecylaniline	-
34	13.894	0,9962%	C23H41N	Benzylamine, N,N-dioctyl-	N-Benzyl-N-octyl-1-octanamine	-
35	15.072	16,7611%	C12H21N25O5S	-	-	-
36	15.323	6,5543%	C12H21N25O5S	-	-	-
37	15.987	26,3455%	C38H38N5O11Cl	(1R,13S,16S,17R,28R)-28-Amino-20-chloro-17,25-dihydroxy-5,8,10,24-tetramethoxy-N-methyl-15,29,31-trioxo-22-oxa-14,30,32-triazahexacyclo[14.14.2.2^{18,21}.1^{2,6}.1^{23,27}.0^{7,12}]hexatriaconta-2(36),3,5,7,9,11,18,20,23(33),24,26,34-dodecaene-13-carboxamide	(1R,13S,16S,17R,28R)-28-Amino-20-chloro-17,25-dihydroxy-5,8,10,24-tetramethoxy-N-methyl-15,29,31-trioxo-22-oxa-14,30,32-triazahexacyclo[14.14.2.2^{18,21}.1^{2,6}.1^{23,27}.0^{7,12}]hexatriaconta-2(36),3,5,7,9,11,18,20,23(33),24,26,34-dodecaene-13-carboxamide	-
38	17.050	0,4132%				-
39	17.599	1,9907%	C35H36N4O5	Pheophorbide A	3-[(3S,4S,21R)-14-Ethyl-21-(methoxycarbonyl)-4,8,13,18-tetramethyl-20-oxo-9-vinyl-3-phorbinyl]propanoic acid	Anticancer (Cho, 2014)
40	18.433	37,6384%	C36H36N5O6SCl	Benzamide, 4-[[3-[[2-[(6-chloro-2-methyl-4-quinolinyl)amino]ethyl][(4-methoxyphenyl)sulfonyl]amino]-1-oxopropyl]amino]-3-methoxy-N-phenyl-	4-[(N-{2-[(6-Chloro-2-methyl-4-quinolinyl)amino]ethyl}-N-[(4-methoxyphenyl)sulfonyl]-β-alanyl)amino]-3-methoxy-N-phenylbenzamide	-
41	19.645	0,0049%				-
42	20.960	0,0047%				-
43	21.109	0,0065%	C12N	-	-	-
44	21.326	0,0063%				-
45	21.509	0,0074%				-

46	21.658	0,0150%	C12N	-	-	-
47	22.572	0,0466%	C7H10N2	2-Pyridylethylamine	2-(2-Pyridinyl)ethanamine	-