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NATURAL COMPOUNDS AQUATIC PLANT BIODIVERSITY OF LAKE LEDULU, ROTE DEAD SEA AREA-INDONESIA FOR RECOMMENDATIONS OF CONSERVATION

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Abstract

The Rote Dead Sea is a unique area in Rote Ndao, Indonesia. There are 24 saltwater lakes and two freshwater lakes. Lake Ledulu is one of the freshwater lakes with unique aquatic vegetation biodiversity and has potential active compound content. This study aims to explore the unique content of essential compounds from aquatic plants in Lake Ledulu. The Gas Chromatography-Mass Spectrometry (GC-MS) method was used to identify and analyze the content of essential compounds in six species of aquatic plants found in Lake Ledulu. The results obtained were Schoenolectiella mucronate (L.) J. Jung & H.K. Choi has 19 compounds; Monochoria vaginalis (Burm.f.) C. Presl ex Kunth 24 compounds; Ludwigia hyssopifolia (G. Don) Exell (24 compounds); Portulaca oleraceae L 17 compounds; Ottelia alismoides (L.) Pers. (young) 24 compound; Ottelia alismoides (L.) Pers. (old) 25 compounds, and Nymphaea nouchali Burm.f. 17 compounds. Each of these compounds has specific characteristics that are only found in certain species, such as squalene (a compound found in shark fins) as well as in Ottelia alismoides (L.) Pers. The gas chromatography-mass spectroscopy analysis showed that aquatic plant from lake ledulu had various essential compounds can be used to pharmacy, vegetable health, cosmetics, and human nutrition in the future.

Keywords*: Endemic; Lake Ledulu; Biodiversity; Aquatic plants; Natural compounds.*

Introduction

Geographically, Rote Island is located 10°25’ - 11°00’ South Latitude and 121°49’ - 123°26’ East Longitude [1]. Rote Island is composed of coral limestone from a quaternary formation, and complex Bobonaro formations spotted in the island’s center. Bobonaro Formations are clay rock formations formed in the Neogene period [2]. Field mapping and analysis of foraminifera from the synorogenic pelagic units of Rote and Savu Islands, Indonesia, revealed high surface uplift rates of the incipient Banda arc-continent collision in the

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past 1.8myr. Rote has a core of strongly folded sediments, overlain by quaternary coral reefs up to 430m above sea level [1, 3]. The rote island was formed by the subduction of the Eurasian Plate against the Indo-Australian Plate. When Rote Island was formed, it was separated from the Indo-Australian Plate, and then it moved horizontally to the northeast towards the island of Timor. Then an uplift occurred at a speed of 13-15cm/year [4]. The Rote Island originally formed two new islands in the south and north. This northern island would become the Rote Dead Sea Area [4]. Later many mud volcanoes formed between the southern and northern parts. These two islands became one forming the Rote island [3].

Lake Ledulu was formed due to non-uniform vertical deformation, resulting in the formation of high and steep cliffs in the east and hills in the west. The rapid uplift movement in the east and west caused a lake to form. The land in Lake Ledulu had low vertical deformation. The basin that was formed then filled with water, and eventually, Lake Ledulu was formed. Lake Ledulu was formed in the Quaternary period with Cenozoic constituents and Neogene deposits based on geological formation. The forming formation in the Lake Ledulu region is Sediment: Chemical: Limestone [3].

During the Oe expedition carried out in 2018-2019 in the Rote Ndao Island area, 82 lakes were found. In addition, in the Dead Sea area on the island of Rote Ndao, there are 24 saltwater lakes, with salinity ranging from 35 to more than 100ppt [3]. The three freshwater lakes are Lake Ledulu, Lake Lendeoen, and Lake Oendui. Lake Ledulu is administratively located in Daiama Village, Landuleko District, the Regency of Rote Ndao. Lake Ledulu covers of about 7.6ha surrounded by secondary forest, and in the west, it is bordered by a relatively high cliff along the west side of the lake. In Lake Ledulu, many terrestrial plants are tolerant of standing water. The water has a normal pH ranging from 6-7. The biodiversity of lake water vegetation in Ledulu is unique, engaging, and the active compounds are potentially beneficial.

Lake Ledulu is the habitat of the Rote snake neck turtle (*Chelodina mccordi*), which is an endemic turtle to Rote Island [5]. Currently, this animal is included in the red list of The International Union for Conservation of Nature (IUCN). Lake Ledulu has relatively high biodiversity. The biodiversity of an ecosystem is essential in symbiotic functions and in supporting habitat functions [6].

The ecosystem function shows a positive asymptotic relationship with increased biodiversity involving many species [7]. Aquatic plants affect the habitat and growth of the flora and fauna that live in the area [8]. Allelochemistry plants can be utilized as medicinal compounds, nutritional sources for humans, effective and environmentally friendly bioherbicides for weed control, and so on [9]. Aquatic plants are also bioremediation agents in absorbing ecosystem pollutants [10]. There are no studies of the biodiversity of aquatic plants in Lake Ledulu. Therefore, this study aimed to examine the biodiversity of aquatic plants and the content of natural essential chemical compounds in each species.

Experimental part

Lake Ledulu location at Rote Island Indonesia

Lake Ledulu area is located at coordinates 100^0 33' 37 "S; 123^0 20' 59" E with an elevation of 12m from Mean Sea Level (MSL), with a shallow depth of about 0 to 3m.

Lake Ledulu location map and habitat condition are presented in figures 1 and 2.

Sampling

Aquatic plants were collected from Lake Ledulu in Rote Ndao Island in July 2019. The sample was taken by collecting aquatic plants that live on the surface of the lake. The leaves were cleaned from contaminants/dirt, dried in direct sunlight, and an oven. Then it was powdered with a blender until a fine powder was obtained (40 mesh) [11]. The identification of the aquatic plants was conducted by the Botanical Division, Research Center for Biology, Indonesian Institute of Sciences (No. 1984/IPH.1.01/If.07/X/2019).

Extraction

The dried leaves of Ladelu lake aquatic plants were powdered and extracted with the maceration method. The maceration extraction method was carried out by soaking the substance in ethanol 70% (1:10) in a closed vessel for 24h with occasional stirring. This step was repeated three times. After that, both liquid extracts were evaporated using a rotary vacuum evaporator and a water bath at 50ºC. The extraction yield (%) was calculated by dividing the weight of the thick extract obtained by the weight of the initial substance [11].

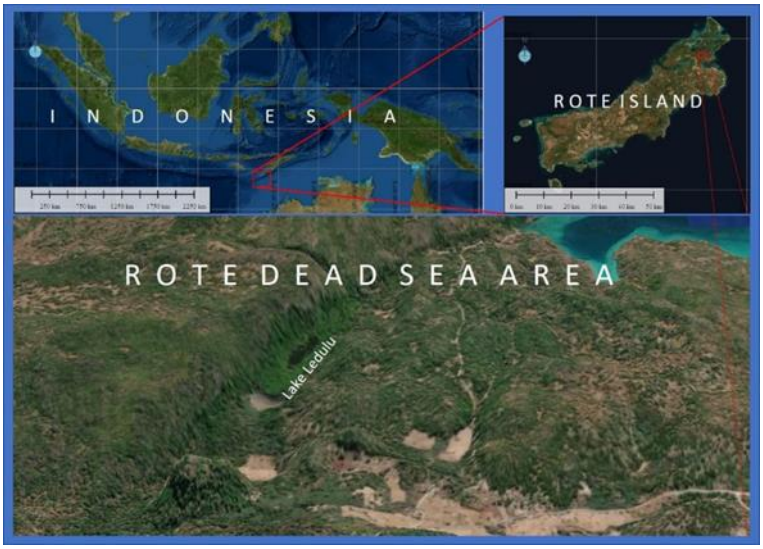

Fig. 1. Map of the location of Lake Ledulu

Fig. 2. Lake Ledulu habitat condition

GC-MS analysis

The final residue obtained from the samples was subjected to GC-MS analysis. GC-MS analysis was performed using an Agilent Technologies 7890 Gas Chromatograph with an autosampler, 5975 Mass Selective Detector, and Chemstation Data System. Compounds were

separated on H.P. Ultra 2, with a capillary column of 30m x 0.20mm I.D x 0.11m film thickness. Samples were injected with ionization mode electron impact with an electron energy of 70eV. Other treatments were 80ºC for 0min, raised 3ºC per min to 150ºC, held for 1.0min, and finally raised 20ºC per min to 280ºC and held for 26min. The ion source temperature was maintained at 230ºC, the interface temperature at 280ºC, quadrupole temperature at 140ºC; the carrier gas was helium, column mode was at a constant flow with a flow column of 1.2 mL min-1, and the injection volume was 5mL split 8:1. This method was developed by the Indonesian Spice and Medicinal Crops Research Institute (ISMCRI).

Results and Discussion

Lake Ledulu aquatic plant Species

Six species of surface water plants in Lake Ledulu (Table 1) were obtained.

Table 1. Lake Ledulu aquatic plant species

No	Species name	Family
1.	*Schoenoplectiella mucronate* (L.) J. Jung & H.K. Choi	Cyperaceae
2.	*Monochoria vaginalis* (Burm.f.) C. Presl ex Kunth	Pontederiaceae
3.	*Ludwigia hyssopifolia* (G. Don) Exell	Onagraceae
4.	*Portulaca oleraceae* L.	Portulacaceae
5.	*Ottelia alismoides* (L.) Pers. (tua)	Hydrocharitaceae
6.	*Ottelia alismoides* (L.) Pers. (Muda)	Hydrocharitaceae
7.	*Nymphaea nouchali* Burm.f.	Nymphaeaceae

Gas Chromatography-Mass Spectrometry (*GC-MS) analysis result*

The results of the GC-MS analysis in the form of compound content found in each species are shown in tables 2-8. A graph of the results of GC-MS is shown in figure 3-9.

Table 2. Results of essential compounds analysis of *Schoenoplectiella mucronate* (L.) J. Jung & H. K. Choi using GC-MS method

No	Natural compound name	Content (%)	Quality	Retention Time (RT)
1.	(+)- Isololiolide	1,12	92	26,452
2.	Neophytadiene	3,92	93	44,800
3.	2-Cyano-5-Azabicyclo [3.3.0] oct-7-En-6-One	3,40	72	29,189
4.	Hexadecanoic Acid	3,37	99	29,444
5.	Phytol	8,15	91	29,589
6.	Tetradecanamide	3,74	62	30,044
7.	2-Tridecenal, (Z)-	20,75	46	30,396
8.	Tetradecanal	4,11	55	30,513
9.	14-Beta-H-Pregna	2,44	94	30,741
10.	Octasecanal	4,49	53	30,961
11.	2-Hydroxy-1-(Hydroxymethyl) Etyl Palmitate	11,12	91	31,575
12.	9E-9-Octadecenoic Acid	1,15	86	31,871
13.	cis, cis, cis-7, 10, 13-Hexadacatrien	4,84	90	32,568
14.	9-Octadecebanide, (Z)-	1,09	95	33,044
15.	2,3-Dimethoxystrtchdin 29 One	1,54	25	35,767
16.	Ergost-5-EN-3-ol	5,12	99	37,857
17.	Stigmasterol	6,16	99	38,374
18.	Cholest-5-EN-3-ol, 23-Ethyl-(3. Beta, 23 S)-	1,58	99	39,505
19.	Stigmast – 4-en-3-one	2,20	96	42,035

Table 2 and figure 3 chromatogram results of *Schoenoplectiella mucronate* (L.) J. Jung & H. K. Choi, the most active major compound in this species is 2-Cyano-5-Azabicyclo [3.3.0] oct-7-En-6- One and 2-Tridecenal, (Z)- or also called fatty aldehyde. Fatty aldehyde functions in cell differentiation and epidermal function [12].

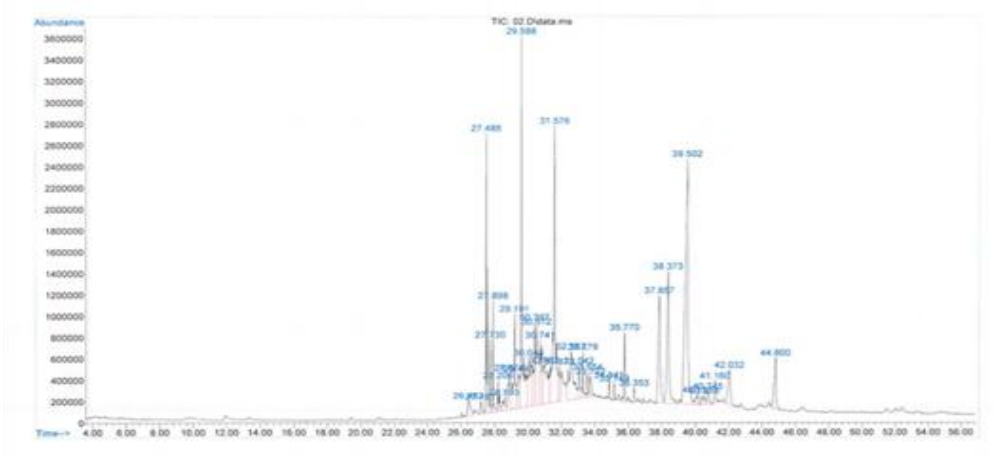

Fig. 3. Chromatogram Results of *Schoenoplectiella mucronate* (L.) J. Jung & H. K. Choi Extract with GC-MS method

Table 3. Results of essential compounds analysis of *Monochoria vaginalis* (Burm.f.) C.Presl ex Kunth using GC-MS method

No	Natural compound name	Content (%)	Quality	Retention Time (RT)
1.	Hexanal, 5-methyl-	2,34	38	6,655
2.	(+)-Isololiolide	1,10	35	26,472
3.	Neophytadiene	2,24	99	27,486
4.	Hexadecanoic Acid, Methyl Ester	1,30	98	28,286
5.	1-fluornafhalen	2,21	52	29,156
6.	(2E)-3,7,11,15-Tetramethyl-2-Hexadecen-1-ol	12,50	90	29,596
7.	Hexadecenoic Acid	16.56	99	30,106
8.	Undecal, 2-methyl-	7,07	49	30,479
9.	2-Aminoethanethiol Hydrogen Sulfate (Ester)	6,68	96	30,809
10.	2-Hexadecanoyl Glycerol	8,50	94	31,575
11.	1-Phenyl-3-Hydroxyfluoren-9-One	4,82	90	321,588
12.	Endo-7-Hydroxy-8,8-Dimethylbicyclo [4,3,0] non-1 (9)-EN-2-ONE	2,39	53	33,050
13.	3,3,6,6-Tetramethyltricyclo [3,1,0,0-2,4-] Hexane	2,87	25	33,223
14.	Pyridine-3-catboxamide, oxime, N-(2-trifluoromethylphenyl)-	1,06	91	33,795
15.	Octadec-9Z-enol trimethylsilyl ether	1,89	52	33, 947
16.	Methyl 3-(3,4-Dimethoxyphenyl)	1,54	20	34,561
17.	(3H,6H), Thieno [3,4-c] isoxazole, 6 ethyl-3a, 4-dihydro	1,85	30	34,960
18.	Octadenoic acid, 6,6-dimthoxy-, methyl ester	1,40	41	36,043
19.	Campesterol	1,39	99	37,836
20.	Stigmasterol	4,12	99	38,387
21.	Cholest-5-EN-3-ol., 23-Ethyl-(3Beta, 23S)-	3,31	99	39,373
22.	4,22-Stigmastadiene-3-one	1,28	99	40,780
23.	Vitamin E	1,91	89	42,035
24.	22-Stigmasterol-3-one	3,62	44	44,821

The most abundant and active compound in *Monochoria vaginalis* (Burm.f.) species C. Presl ex Kunt was (2E)-3,7,11,15-Tetramethyl-2-Hexadecen-1-ol (12.50%). This compound is what functions as an anti-microbial, anti-inflammatory and inhibit the activity of COX 1 receptors [13].

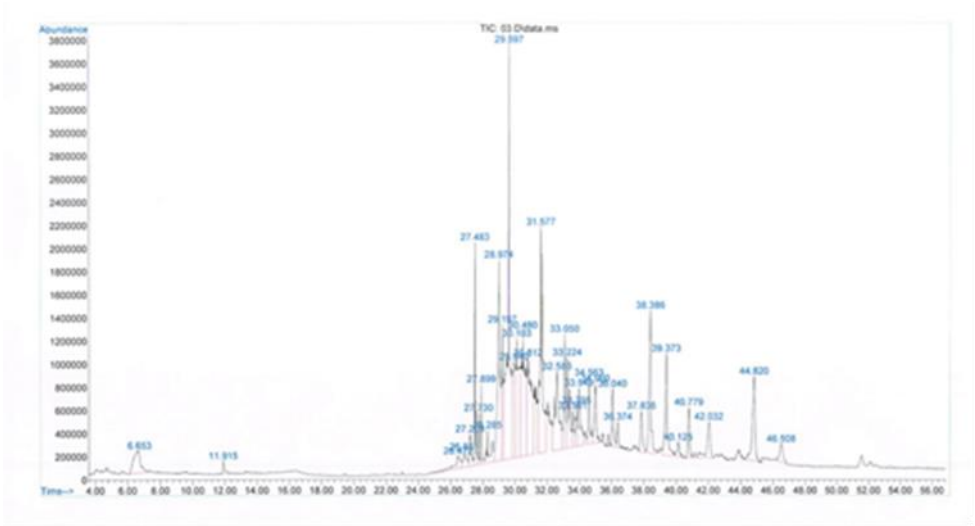

Fig. 4. Chromatogram Results of *Monochoria vaginalis* (Burm.f.) C. Presl ex Kunth Extract with GC-MS method

Table 4. Results of essential compounds analysis of *Ludwigia hyssopifolia* (G. Don)

No	Natural compound name	Content (%)	Quality	Retention Time (RT)
1.	1,2,3-Benzenetriol	7,84	97	17,446
2.	(-)-Loliolide	1,38	84	27,162
3.	Neophytadiene	3,67	94	27,507
4.	1-Hexadecene	3,40	98	27,948
5.	Hexadecanoic Acid	5,86	99	28,920
6.	n-Nonadecanol – 1	3,67	93	29,423
7.	Cyclopropanecarboxylic acid, but-e-yn-2-yl ester	1,38	50	29,596
8.	(9E,12E)-9,12-Octadecadienoic Acid	9,50	99	29,941
9.	(2E,6E)-4-Methyl-2,6-Octadiene	7,23	53	30,630
10.	Cyclooctanone	2,43	42	31,423
11.	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	2,08	91	31,713
12.	Fumaric acid, undecyl tetrahydrofurfuryl ester	1,01	72	32,016
13.	Behenic alcohol	1,99	93	32,444
14.	Pyrrolidine, N-(4-methyl-4-pentenyl)	1,09	53	32,795
15.	(+)-P,1R,3S)-5-(4,5-Dimethoxy-2-Methyl-1-Naphthyl)-6,8-Dimethoxy-2-Methyl-1-2,3-Trimethyl-1,2,3,4-Tetrahydroisoquinoline-(+)-O-Methylancistrocline	1,31	83	33,754
16.	6,8-Doixatetradecane	3,72	27	34,195
17.	Alpha-1-Mannopyranose, 6-deoxy-tetra acetate	2,58	52	34,374
18.	2-(3-Butoxy-2-Hydroxypropyl) Malonohydrazide	4,88	35	34,567
19.	Alpha-Pyrrolidone, 5-acetoxymethyl-	1,22	49	35,078
20.	2-Methoxyphenyl 2,3,4-Tri-O-Acetyl-6-Deoxyhexopyranoside	8,23	37	36,574
21.	Acetic acid, 6-acetoxymethyl-2,2- dimethyl tetra hydro [1,3] dioxolo [4,5-c] pyran-7-yl ester	5,67	32	36,967
22.	Campesterol	1,48	98	37,974
23.	Stigmasterol	1,33	95	38,470
24.	Gamma – Sitosterol	10,91	93	39,773

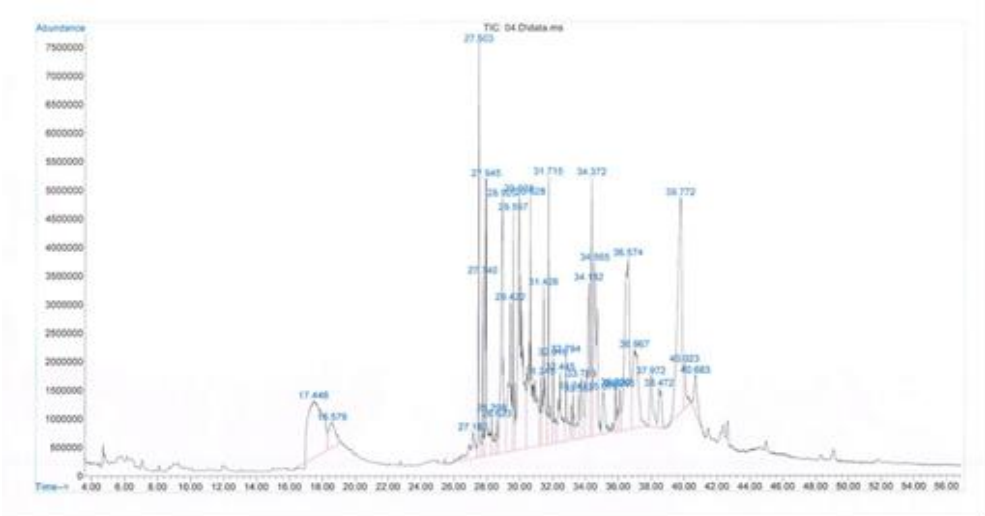

Fig. 5. Chromatogram Results of *Ludwigia hyssopifolia* (G. Don) Exell Extract with GC-MS method

The most abundant compound in *Ludwigia hyssopifolia* (G. Don) was Gamma-Sitosterol (10.91%). Gamma-Sitosterol functions as a Tocopherol synergist, PPAR gamma antagonist [14].

Table 5. Results of essential compounds analysis of *Portulaca oleraceae L*. using GC-MS method

No	Natural compound name	Content (%)	Quality	Retention Time (RT)
1.	Beta, D-Glucopyranose,1,6-anhydro-	4,42	43	25,728
2.	1,2-Oxathiolene, 3,3,4,5-Tetramethyl-5-(1-Methylethenyl)-,2-Oxide	5,71	53	27,734
3.	1-(4-Fluorophenyl)-2-[(4-hydroxy-6-methylpyrimidin-2-yl) thio] ethan-one	11,13	43	27,907
4.	Hexadecanoic acid	15,61	99	60,295
5.	Cyclotetradecane	1,24	59	29,410
6.	(2E) -3,7,11,15-Tetramethyl-2-Hexadecen-1-ol	1,84	90	29,589
7.	Linolenic acid	12,25	99	29,934
8.	Cyclohexanone, 3-vinyl-3-methyl-	3,92	60	30,644
9.	Eicosanoic acid	1,64	89	30,899
10.	Methyl (7E,10E,13E)-7,10,13-Hexadecatrienoate	5,81	93	32,395S
11.	Stigmasterol	5,10	99	38,470
12.	Gamma-Sitosterol	1,72	99	38,470
13.	N-[(8E)-2-Methoxy-4A,6A-Dimethyl-9-Methyleneoctadecahydro-8H-Naphtho [2`,1`:4,5] Indeno [1,2-BFuran-8-Ylidene] Cyclohexanamide	2,57	91	44,249
14.	Neophytadiene	6,75	87	44,993
15.	Lamost-8-en-3-one	5,71	45	46,358
16.	Lanosterol	3,14	60	48,668
17.	1(2H)-Isoquinolinone, 3-(2-Ethenyl-4,5-Dimethoxyphenyl)-5,7,8-Trimethoxy-2-methyl	3,17	64	50,496

The most abundant compound in *Portulaca oleraceae* L. Extract is Hexadecanoic acid (15.61%) and Linolenic acid (12.25%). Hexadecanoic acid functions as Antioxidant, Flavor, Anti-fibrinolytic, Hypocholesterolemic, Antiandrogenic, Lubricant, Hemolytic, 5-Alpha reductase inhibitor, Nematicide, Antialopecic. Linolenic acid is an essential acid and have important role in body cells metabolism [15].

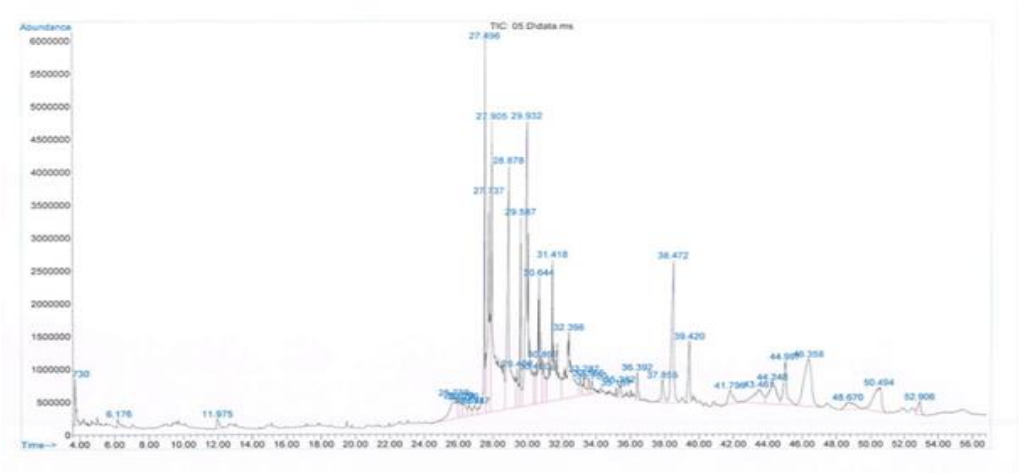

Fig. 6. Chromatogram Results of *Portulaca oleraceae* L. Extract with GC-MS method

Table 6. Results of essential compounds analysis of *Ottelia alismoides* (L.) Pers. (young leaves) using GC-MS method

No	Natural compound name	Content (%)	Quality	Retention Time (RT)
1.	Hexahydrofarnesy acetone	1,05	91	27,569
2.	Methyl palmitoleate	1,23	99	28,3114
3.	Hexadecanoic acid, Methyl ester	2,24	99	28,307
4.	Hexadecanoic acid	10,44	99	28,893
5.	Methyl (9Z,12Z,15Z)-9,12,15-Octadecatrienoate	3,72	96	29,500
6.	Phytol	4,30	91	29,603
7.	Oleic acid	3,76	99	29,899
8.	Tricosyl trifluoroscetate	5,41	38	30,113
9.	Hexadecane	3,64	95	30,492
10.	Bicyclo [4.1.0]-3-heptene, 2-isopropenyl-5-isopropyl-7,7-dimethyl-	4,79	35	30,817
11.	14-Beta-H-Pregna	2,04	97	30,934
12.	Cyclopropanecarboxamide, N-Methallyl-	7,86	41	31,099
13.	Dotriacontyl trifluoroscetate	1,72	93	31,410
14.	Docosanoic acid, methyl ester	4,63	95	31,568
15.	1-Isoproyl-6-Methyl-3-(1-Methylethylidene)-6-Vinyl-1-xcylohexene	3,20	42	32,044
16.	1-Ethenyl-1,2,3,3,7,7A-Hexahydro-3- [(3-Hydro-4-Methoxyphenyl) Methyl]-7-Methylene-4H-Inden-4-One-Isomer	1,68	53	32,216
17.	5-Amino-1-(4-Methoxy-Benzyl) 1H-(1,2,3) Triazole-4-Carboxylic acid (3-Chloro-Phenyl)-Amide	7,14	38	12,554
18.	Trans-13-Decosenamide	2,82	83	33,071
19.	Squalene	1,54	99	33,292
20.	Cholesta-4-, 6-Dien-3-ol, 6-Fluoro-, (3Beta)-	1,10	53	33,761
21.	Campesterol	2,87	99	37,891
22.	Stigmasterol	9,11	99	38,532
23.	Cholest-5-EN-3-ol, 23-Ethyl-, (3Beta,23S)-	4,16	99	39,791
24.	Alpha-Elemene	3,40	55	48,468

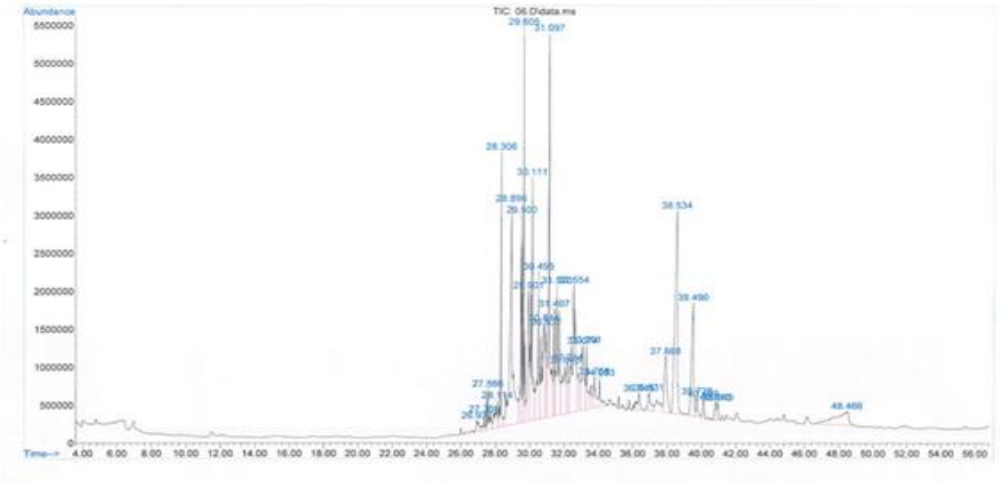

Fig. 7. Chromatogram Results of *Ottelia alismoides* (L.) Pers. (Young leaves) Extract with GC-MS method

Table 7. Results of essential compounds analysis of *Ottelia alismoides* (L.) Pers. (old leaves) using GC-MS method

No	Natural compound name	Content (%)	Quality	Retention Time (RT)
1.	Erythritol	3,04	39	4,752
2.	Hexahydrofarnesyl acetone	1,15	95	27,562
3.	Hexadecanoic acid, methyl ester	1,87	99	28,293
4.	Hexadecanoic acid	6,29	99	28,796
5.	Phytol	6,27	91	29,582
6.	Benzyl (Dideuterated) Methyl Ester	6,74	41	30,093
7.	Behenic alcohol	4,11	93	30,465
8.	1-Docosene	6,49	59	30,796
9.	Dotriacontyl trifluoroacetate	4,14	87	31,051
10.	14-Beta-H-Pregna	8,18	95	31,575
11.	Tricosanoic acid, Methyl ester	4,28	86	32,044
12.	1-Ethenyl-1,2,3,3,7,7A-Hexahydro-3-[(3-Hydro-4-Methoxyphenyl) Methyl] -7-Methylene-4H-Inden-4-One-Isomer	2,63	76	32,196
13.	5-Amino-1-(4-Methoxy-Benzyl) 1H (1,2,3) Triazole-4-Carboxylic acid (3-Chlorophenyl)-Amide	8,46	49	32,533
14.	9-Octadecenamide, (Z)-	2,88	90	33,057
15.	Squalene	3,63	98	33,278
16.	1-Hexacosene	1,82	95	33,795
17.	i-Propyl 9-octadecenoate	2,15	90	34,037
18.	2H-1-Benzopyran-6-ol,3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl) - [2R-[2R*(4R*,8R*)]}	2,79	91	34,374
19.	Cyclopent-2-eme-1-carboxylic acid, 2,3-dimethyl-1-ethyl-ethyl este	3,04	70	34,836
20.	Cholesterol	1,74	99	36,326
21.	1,4-Bis (6-Phenylfulven-6-yl) Benzen	2,83	40	37,801
22.	Stigmasterol	3,58	99	38,319
23.	Gamma - Sitosterol	2,38	99	39,312
24.	9,19-Cyclopropane carboxamide, 2-cyclopropyl-2-methyl-N-(1-cyclopropyletyl)-	1,45	95	41,966
25.	9-Tricosene, (Z)-	1,63	55	55,371

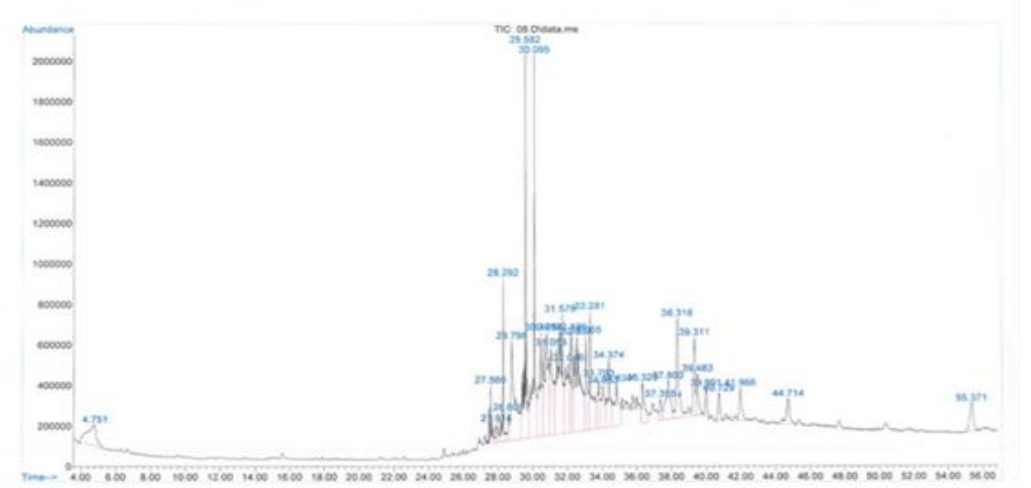

Fig. 8. Chromatogram Results of *Ottelia alismoides* (L.) Pers. (old leaves) Extract with GC-MS method

Ottelia alismoides (L.) Pers. (young leaves) have Hexadecanoic acid contain (10, 44%), and 6,29% at old leaves the same with several important compounds such as stigmasterol in young leaves (9.11%) and reduced in old leaves (3.58%). The phytol content increased in old leaves (6.27%), while in young leaves phytol was still slightly formed (4.30%). The content of squallene compounds in old leaves (3.63%) and 1,54% in young leaves. Production of chemical compounds in this spesies is different at each stage of age.

Table 8. Results of essential compounds analysis of *Nymphaea nouchali Burm*.f. using GC-MS method

No	Natural ccompound name	Content (%)	Quality	Retention Time (RT)
1.	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	1,75	94	7,214
2.	Pyrogallol	16,35	97	17,557
3.	Neophytadiene	5,26	99	45,028
4.	Hexadecanoic Acid	24,46	99	29,113
5.	(2E)-3,7,11,15-Tetramethyl -2-Hexadecen-1-ol	1,05	93	29,624
6.	Linolenic acid	19,73	97	30,079
7.	(9E)-9-Octadecenoic acid	1,60	86	30,506
8.	Cis-7, cis-11-Hexadecadien-1-yl acetate	1,09	90	30,665
9.	Eicosanoic acid	4,17	99	30,961
10.	1,2-Benzenedicarboxylic Acid	1,90	91	31,733
11.	1,4-Benzenediol 2,6-dimethyl-	1,77	72	34,167
12.	5-Heptadecatri-8(Z), 11(Z), 14(Z)-Enylresorcinol	2,34	94	35,119
13.	Ethyl Vallesiachotamate	1,04	83	35,815
14.	Stigmasterol	3,47	99	38,443
15.	Gamma-Sitosterol	3,92	99	39,642
16.	12-(O-Nitrophenylseleno)-5,7. H.11. Betta. H-Eudesm-3-EN-6, Alph-ol	1,73	60	41,883
17.	24-Methylenecycloartanol	3,58	97	42,373

Nymphaea nouchali Burm.f, has the highest content of Hexadecanoic acid (24.46%) and Pyrogallol (16.35%).

The results of GC - MS show that every aquatic plant species found in Lake Ledulu has a dominant compound content. The dominant types of compounds found in each species are listed in (Table 9). In addition to the dominant natural compounds, several other types of compounds can be found in several species of aquatic plants in Lake Ledulu (Table 10).

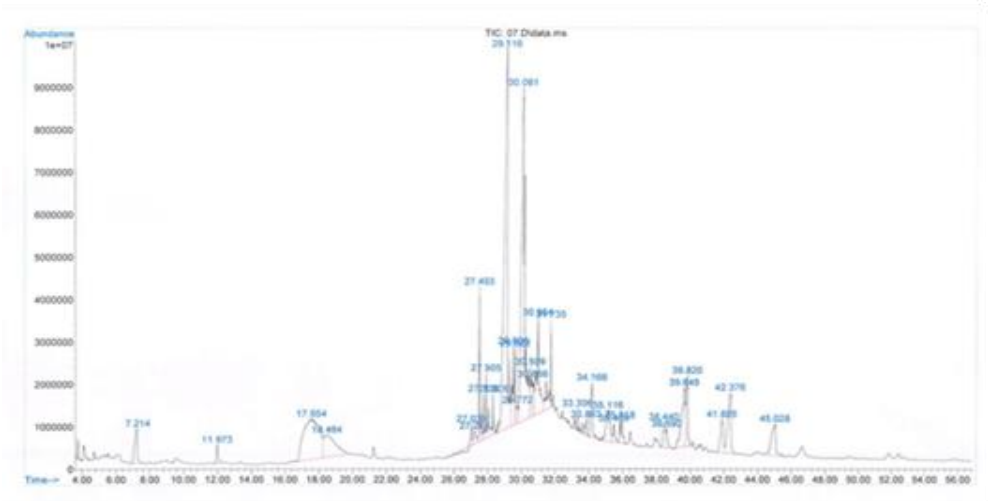

Fig. 9. Chromatogram Results of *Nymphaea nouchali Burm* f. Extract with GC-MS method

Table 9. Species with dominant chemical compounds in Lake Ledulu

No	Species Name	Natural compound name	Content (%)
1.	*Schoenoplectiella mucronate* (L.) J. Jung & H.K. Choi	2-Tridecenal, (Z)-	20,75
		2-Hydroxy-1-(Hydroxymethyl) Etyl Palmitate	11,12
2.	*Monochoria vaginalis* (Burm.f.) C. Presl ex Kunth	(2E)-3,7,11,15-Tetramethyl-2-Hexadecen-1-ol	12,50
		Hexadecanoic Acid	16.56
3.	*Ludwigia hyssopifolia* (G. Don) Exell	(9E,12E)-9,12-Octadecadienoic Acid	9,50
		Gamma – Sitosterol	10,91
4.	*Portulaca oleraceae* L.	Hexadecanoic acid	15,61
		Linolenic acid	12,25
5.	*Ottelia alismoides* (L.) Pers. (tanaman muda)	Hexadecanoic acid	10,44
		Stigmasterol	9,11
6.	*Ottelia alismoides* (L.) Pers. (tanaman Tua)	5-Amino-1-(4-Methoxy-Benzyl)1H (1,2,3) Triazole-4-Carboxylic acid (3-Chlorophenyl)-Amide	8,46
		14-Beta-H-Pregna	8,18
7.	*Nymphaea nouchali* Burm.f.	Hexadecanoic Acid	24,46
		Linolenic acid	19,73

Table 10. Natural compounds found in several species of aquatic plants in Lake Ledulu

Natural compounds	*Schoenoplectiella mucronate* (L.) J. Jung & H.K. Choi	*Monochoria vaginalis* (Burm.f.) C. Presl ex Kunth	*Ludwigia hyssopifolia* (G. Don) Exell	*Portulaca oleraceae* L.	*Ottelia alismoides* (L.) Pers. (young leaves)	*Ottelia alismoides* (L.) Pers. (old leaves)	*Nymphaea nouchali* Burm.f.
Neophytadiene	+	+	+	+			+
Hexadecanoic	+	+	+	+	+	+	+
Acid Phytol	+				+	+	
Stigmasterol	+	+	+		+	+	+
Campesterol		+	+		+		
Eicosanoic acid				+			+
Gamma-Sitosterol				+		+	+

Gas Chromatography-Mass Spectrometry (GC-MS) result analysis show that each type of aquatic plant has a different character of metabolite compounds. In general, plants only have two compounds' functions, i.e., for metabolism and reproduction [16]. Its derivatives provide

secondary metabolites that are very useful. It is estimated that there are about 20,000 types of secondary metabolites in the plant kingdom. Using the gas mass spectrometry, each of these compounds could be identified [17]. The results of our analysis showed that some specific compounds are only found in certain species, namely:

Neophytadiene is an anti-inflammatory compound found in the marine algae *Turbinaria ornate* [18]. *Neophytadieneis* is reported to be antipyretic, analgesic, anti-inflammatory, antimicrobial, and antioxidant, while squalene is reported to be anti-bacterial, antioxidant, antitumor, immunostimulant, chemo-preventive, a lipoxygenase inhibitor, diuretic. It can also be used as a pesticide, perfume, and sunscreen [17].

Hexadecanoic acid or palmitic acid is found in every aquatic plant species in the Lake Ledulu ecosystem. This compound is commonly found in plants. The results obtained show that all aquatic plants from Lake Ledulu contain Hexadecanoic acid.

Pyrogallol is only found in Nymphaea nouchali Burm.f. Squalene is a compound that is only found in *Ottelia alismoides* (L.) Pers in young and old leaves. Pyrogallol in *Nymphaea nouchali Burm*.f contains 16.35% Pyrogallol with a quality of 97 and a retention time of 17.557. Pyrogallol is a molecular adhesive compound that binds soluble DNA and RNA proteins, which form covalent bonds. Therefore, they can form hydrogels as new biomaterials for the beauty industry, biomedical devices, energy, and others [19]. Pyrogallol can also be used as a chitosan-based adhesive for bones in regenerative medicine. Pyrogallol exhibited two-fold more extraordinary adhesion ability in wet conditions than did fibrin glue, a commercially available surgical glue. The ability of pyrogallol polymer to help blood coagulation is significantly higher than other commercial polymers [20, 21], reported that Pyrogallol is more efficient in scavenging O_2 as it has O_2 solid scavenging activity. The high content of pyrogallol in *N. nouchali* gives it the potential to be used as an adhesive with hemostatic hydrogel organic biomaterials from aquatic plants.

Lanosterol is a compound found in *Portulaca oleraceae* L by 3.14%. One of the functions of lanosterol that has been reported is to prevent protein aggregation in animals which causes cataracts [22].

Vitamin E, which was discovered in 1922, is an essential compound in the pharmaceutical and cosmetic fields. This vitamin is an antioxidant, immunoregulator, anti-inflammatory neuroprotector and photoprotective, and thus, it is essential for health and cosmetics. Vitamin E is soluble and easily damaged at high temperatures, light, oxygen and alkaline conditions, and water [23]. *Monochoria vaginalis* (Burm.f.) C. Presl ex Kunth contains Vitamin E (1.91%). The amount of Vitamin E found in this species is small and it is estimated that it will dissolve and evaporate during the identification process.

Phytol (3,7,11,15-tetramethylhexadec-2-en-1-ol) belongs to phytanic acid which is a valuable essential oil used in perfumery and various applications in the pharmaceutical and biotechnology industries. These compounds have anxiolytic, metabolism-modulating, cytotoxic, antioxidant, autophagy- and apoptosis-inducing, antinociceptive, anti-inflammatory, immune-modulating, and antimicrobial effects [24]. Pharmaceutical industries need for phytol is around 0.1 to 1.0 metric tons per year [25]. Aquatic plants from Lake Ledulu *Schoenoplectiella mucronate* (L.) J. Jung & H.K. Choi and *Ottelia alismoides* (L.) Pers. (Old leaves) have potential phytol content ranging from 4.30 - 6.27-8.15%.

Stigmasterol is an essential compound in regulating metabolism to form progesterone, androgen, estrogen, and corticoid hormones [26]. It also functions as a precursor of progesterone which acts as an intermediate in the biosynthesis of androgens, estrogens, anti-osteoarthritics, anti-hypercholesterolemic, cytotoxics, antitumours, hypoglycemics, antimutagenics, antioxidants, anti-inflammatories, and analgesics [14]. Stigmasterol is found in almost all aquatic plants of Lake Ledulu except for *Portulaca oleraceae* L. The range of stigmasterol contained in each species is between 1.08 - 9.11 %

Campesterol is an unsaturated fatty acid that is beneficial for the body. This compound is found in many food plants. Aquatic plants from Lake Ledulu containing Campesterol are *Monochoria vaginalis* (Burm.f.) C. Presl ex Kunth, *Ludwigia hyssopifolia* (G. Don) Exell, *Ottelia alismoides* (L.) Pers. (young plants), with a range of 1.39 - 2.87%.

Squalene. This compound is only owned by *Ottelia alismoides* (L.) Pers. This compound is a polyunsaturated hydrocarbon with the formula $C_{30}H_{50}$. It is found in shark fat and human sebum. Squalene functions as an anti-cancer, antioxidant, drug carrier, detoxicant, skin hydrating substance. This compound is one of the essential compounds in the nutraceutical, pharmaceutical, and cosmetic industries [27]. Squalene also has anti-free radical activity, so it helps cure cancer. It can also be used as an anti-cholesterol as well as an alternative treatment and heart health supplement. Squalene is an anti-oxidant, anti-cancer agent, anti-aging, chemo-preventive agent, anti-bacterial agent, adjuvant for vaccines and drug carriers, and detoxifier [28, 29].

The content of squalene in *Ottelia alismoides* (L.) Pers (old leaves) is 3.63 and 1.54% in *Ottelia alismoides* (L.) Pers (young leaves). Other plants that contain squalene are spinach [30] and olives [31]. In 2014, the global squalene market demand was approximately 2.67 kilotons [30], with a projected value of 241.9 million USD in year 2022 [32]. This demand is so significant that it is necessary to find new and renewable sources of squalene for medical and pharmaceutical needs from aquatic plants. *Ottelia alismoides* (L.) Pres has the potential to increase the supply of squalene.

Conclusions

Lake Ledulu, which covers a lake surface area of 7.2h with a depth of 3m, is a virgin lake that has not been touched by humans and has not been exposed to pollution. Aquatic plants that live in it have originality in the content of chemical compounds which have implications for conservation. We think these results are important for obtaining pharmaceutical knowledge from aquatic plants in areas that are still conservative and virgin. This article provides important information about aquatic plant species and allelochemical compounds that are important for pharmaceutical applications. In the future, aquatic plants can be conserved and cultivated as an important and safe medicinal ingredient for treating disease and can be used as a natural antibiotic agent. This finding supports ethnobotanical knowledge derived from aquatic herbs. The results also indicate the presence of important phytocomponents in aquatic plant extracts from Lake Ledulu that provide biological benefits. Further research on the isolation and characterization of phytochemicals will further explain the effectiveness of these compounds in medicine. We believe the results of this study provide a new perspective on aquatic flora and their medicinal benefits. We provide the view that aquatic ecosystems need to be preserved along with their aquatic flora and fauna. because from our findings in a virgin lake, there are many benefits for humans. The large number of pollutants in the water will eliminate the potential of natural compounds that are important in the pharmaceutical field for humans. We hope that the frame of our research can be used as a mirror for water conservation. Therefore, we suggest preserving the environment of the river lakes and water ecosystems as habitat for aquatic flora. These secondary metabolites from lake ledulu aquatic plant have the potential to be utilized for pharmaceuticals, cosmetics, and human nutrition for people in Rote Island and global.

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Author statement

Luki Subehi, funding acquisition LIPI's COREMAP-CTI 2021 – 2022. Atriyon Julzarika, Dany Puguh Laksono, Kayat designed the survey location, conducted the field sampling. Atriyon Julzarika, Luki Subehi, Kayat, Aan Dianto, Media Fitri Isma Nugraha, Dany Puguh Laksono, Hanhan A. Sofiyuddin, organized the expedition. Luki Subehi, Atriyon Julzarika, Dany Puguh Laksono, Aan Dianto visulasation ecosystem sample area. Media Fitri Isma Nugraha conceptualization, methodology and conducted the bioactive compound and quality control. All author curated the specimens. All author contributed in formal analysis, data processing, data preparation data analysis, drafting, writing, review, revising, improved and approved the manuscript. All authors are main contributors. This research is multidisciplinary and part of the Expedition Oe 2018 - 2021 in the Rote Islands.

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