Metabolite Fingerprinting *Eleutherine palmifolia* (L.) Merr. Using UPLC-QTOF-MS/MS

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ABSTRACT

Eleutherine palmifolia (L.) Merr. *(E. palmifolia)* is an Indonesian native plant that has the potential to be developed into phytopharmaca. The differences in growth locations are thought to cause variation in the content of metabolite compounds which affect differences in pharmacological activity. This study aims to determine the profile of metabolites *E. palmifolia bulb* from several regions in Indonesia. The samples were collected from six different locations, namely East Java, Central Java, West Java, East Borneo, Central Borneo, and South Borneo. Sample extraction was carried out using *Ultrasonic Assisted Extraction* (UAE) method with 96% ethanol. The analysis of the content of metabolites was carried out using UPLC-QTOF-MS/MS with a stationary phase column C_{18} (Okta Decyl Silica), mobile phase mixture of formic acid /water 0.1/99.9 (v/v), and formic acid/acetonitrile 0,1/99,9 (v/v). The results of the analysis were interpreted using software *Masslynx* and continued with chemometric analysis using the method *Principle Component Analysis* (PCA). The results showed that there were differences in the content of the metabolite compounds in *E. palmifolia* bulb originating from six different regions.

Keywords: Eleutherine palmifolia, Metabolite fingerprinting, UPLC-QTOF-MS/MS

INTRODUCTION

Metabolite fingerprinting is a fast and accurate method to determine the content of contained in a plant extract compounds (metabolome) using chromatographic techniques such *HighPerformance* Thin as Laver Chromatography (HPTLC), *High-Performance* Chromatography Liauid (HPLC), Gas chromatography (GC), and Mass spectrometry (MS) (Srivastava et al., 2010). The metabolite profiling technique can be combined with *profiling bioassays* such as cytotoxic tests, antimicrobial tests, then linked to the results of multivariate statistical analysis. Using these techniques, efforts to find new compounds can be done quickly and efficiently (Wolfendera et al., 2015).

Eleutherine palmifolia (L.) Merr., is one of the native plants of Indonesia, especially the island of Borneo, which has the potential to be developed into phytopharmaca. Epalmifolia bulb has long been used empirically for the treatment of several diseases. Similar to the onion group, *E. palmifolia* bulb is the central part of the bulb (Firdaus, 2006). *E. palmifolia* bulb contains secondary metabolites in the form of *naphthoquinone* and derivatives such elecanacine, eleutherol, as eleutherine, eleuthernone. Naphthoquinones are known as antimicrobial, antifungal, antiviral, antiparasitic, anticancer, and antioxidants (Hara et al., 1997).

*Corresponding author : Roihatul Mutiah Email : roiha@farmasi.uin-malang.ac.id Other studies have also conducted *in Vitro* with *MTT assay.* From this study, it was found that isolates of *eleutherinoside* A in *E. palmifolia* bulb which can inhibit α -glucosidase in diabetes mellitus (Ieyama, 2011).

This study aims to determine the profile of the metabolites *E. palmifolia* bulb and determine whether there are differences in the composition of the metabolite compounds *E. palmifolia* bulb based on different growing conditions. The sampling of *E*. palmifolia bulbs originated from 6 different locations, namely West Java, Central Java, East Java, South Borneo, Central Borneo, and East Borneo. In this study, the metabolite profile of the extract was Ε. palmifolia bulb analyzed using Ultra Performance Liquid Chromatography - Quadrupole Time of Flight - Mass Spectrum/Mass Spectrometry (UPLC-QToF-MS/MS) which is the best instrument currently for analyzing various compounds. UPLC-OToF-MS/MS is an instrument that combines the physical separation capabilities of liquid chromatography with mass analysis capabilities of mass spectrometry. UPLC was chosen because it has the advantage of being well used for many applications that have very high sensitivity and selectivity (Taleuzzaman, 2015). The results of UPLC-QToF-MS/MS will be analyzed using principal component analysis (PCA). PCA is a technique to reduce a lot of data (data grouping) when there are correlations between variables (Rohman, 2009). From the results of PCA visualization, it can be seen that there is a closeness

Table I. Characteristics of sampling locations of <i>E. palmifolia bulb</i>

NO.	Location	Altitude (MDPL)	Average Temperature (ºC)	Rainfall (mm)	Climate	Soil type
1.	Srengat Village, Srengat District, Blitar District, East Java	127	25.0	1819	Aw	Regosol, Litosol
2.	Kalisoro Village, Tawangmangu District, Karanganyar, Central Java	1221	19.1	3299	Am	Gray Alluvial, Dark Gray grumosol
3.	Sukaharja Village, District Cijeruk, Bogor, West Java	668	24.0	3454	Af	Regosol, latosol, Alluvial, Podsolic, and Andosol
4.	Karang Rejo, Balikpapan Tengah Subdistrict, Balikpapan City, East Borneo	29	26.4	2376	Af	Red Yellow Podsolic, and Sandland
5.	District, Baru Village, Arut Selatan District, Kotawaringin Barat District, Central Borneo	10	26.8	2765	Af	Alluvial, Latosol, Red Yellow Podzolic
6.	District of Banjarbaru, Banjarmasin, South Borneo	31	27.2	2627	Af	Alluvial

Source: www.elevationmap.net and https://id.climate-data.org (January 10th, 2018)

between objects (samples) and relationships between variables (Taufik, 2007).

METHODOLOGY

Materials

The plants used in this study were *E. palmifolia* bulb (*Eleutherine palmifolia* L.) originating from Srengat Village, Srengat District, Blitar Regency, East Java; Kalisoro Village, Tawangmangu District, Karanganyar Regency, Central Java; Sukaharja Village, Cijeruk District, Bogor Regency, West Java; Karang Rejo Village, Balikpapan Tengah District, Balikpapan City, East Borneo; Baru Village, Arut Selatan District, Kotawaringin Barat District, Central Borneo; and Banjarbaru District, Banjarmasin City, South Borneo. The characteristics of the sampling location of *E. palmifolia bulb* presented in table I.

Determination plants

Plant determination was carried out at the Materia Medika Office (UPTD) Technical Implementation Unit in Batu, East Java, Indonesia. The number of results of determination of plants is 074/348/102.7/2017. The specimens resulting stored in the Pharmacognosy Laboratory of the Pharmacy Department, Maulana Malik Ibrahim, State Islamic University of Malang.

Instrumentation

The instruments used were rotary evaporator (IKA, Ohio, USA), ultrasonic cleaner (Sonica Soltec, Milano, Italy), and Ultra Performance Liquid Chromatography-Quadrupole Time of Flight-Mass Spectrometry (UPLC-QToF-MS) (Waters, Massachusetts, USA).

Methods

Extraction

The simplicia of *E. palmifolia* bulb from each region is 200 grams. Extraction of samples *E. palmifolia* bulb using the maceration method with a combination of *Ultrasound-Assisted Extraction* (UAE). A sample of 25 grams was dissolved in ethanol 96% 500mL (comparison 1:20) and replicated three times. The collected filtrate is then separated by its solvent using a *rotary evaporator*. Next, the extract put in the oven at 40 °C.

Time (minutes)	% Eluent A	% Eluent B
0.00	95.0	5.0
2.00	75.0	25.0
3.00	75.0	25.0
14.00	0.0	100.0
15.00	0.0	100.0
19.00	95.0	5.0
23.00	95.0	5,0

Table II. Gradient Elution System

Table III. Results Extraction Powder E. palmifolia bulbous

Sample Location	Extract Weight (g)	Yield (%)
East Java	8.1034	4.0512
Central Java	10.0483	5,0215
West Java	9.3039	4.6517
East Borneo	12.3269	6.1538
Central Borneo	16.0893	8.0430
South Borneo	10.0772	5,0386

Active Compounds Identification using UPLC-QTOF-MS/MS

The UPLC-QToF-MS/MS analysis used the UPLC-MS system with QToF as an analyzer and positive ESI as an ionization source with columns in the form of Acquity C18, 1.8 μ m; 2.1 × 150 mm. The eluent used is a mixture of (A) Water (HPLC *grade*) / formic acid 99.9 / 0.1 [v / v]; (B) Acetonitrile/ formic acid 99.9 / 0.1 [v / v] with a gradient elution system with the following ratio.

The temperature for *Source temperature is* 100°C, and desolvation temperature is 350°C. A total of 10 mg of extract sample was dissolved in a 10 ml volumetric flask with absolute methanol which then injected into the UPLC-MS system, carried out three times repetition. Chromatogram data obtained, and the area expressed in percent form. Chromatograms were processed using software *Masslynx* version 4.1. Component identification based on measured m/z comparisons at Masslynx and m / z calculated on ChemDraw.

PCA Multivariate Data Analysis

Profile data of ethanol extract metabolites 96% *E. palmifolia* bulb from UPLC-QToF-MS/MS instruments were then analyzed using PCA. *The software* used in PCA analysis is Minitab version 17. Data obtained from UPLC-QToF-MS/MS entered into Minitab will then be obtained *score plot* and *loading plot*. In the *score plot*, *a* graph will appear in the form of small circles. The small circles form groups (groups) specific, and each group indicates the existence of closeness between objects (samples). In the *loading plot, a* graph will appear in the form of lines of different lengths, some pointing to the left (negative) and the direction to the right (positive). The result of *loading the plot* used is the line that leads to the right only. The longer the path to the right, the more significant the contribution to differences between groups.

RESULT AND DISCUSSION

Results of plant extraction

Extraction of *E. palmifolia* bulb was carried out to obtain a concentrated extract of tuber bulb *E. palmifolia*. The method used in this research is *ultrasound-assisted extraction*. This extraction type was chosen because it has several advantages, including faster than cold extraction methods such as maceration and percolation, safer, and can increase the crude yield of extracts (Handayani, 2016).

From Table III, it can be seen that there are differences in the percentage of yield from each region. The difference in returns, other than due to the weight of the extract, can also be influenced by several other factors such as the age of the plant, the time and process of harvesting, the variety of plants, the environment where it grows, and the way of processing plants. These factors can make one of the same plant species finally have a different yield value, the content of the compound is different, and will affect its metabolic activity (Ayunda, 2014; Distantita *et al.*, 2009).

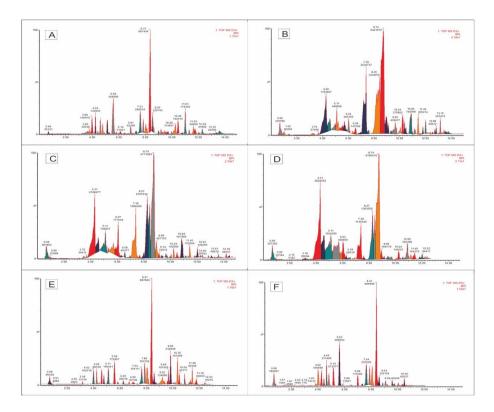


Figure 1. Results of UPLC-QTOF-MS / MS *E. palmifolia chromatogram* bulb (A: East Java; B: Central Java; C: West Java; D: East Borneo; E: Central Borneo; F: South Borneo).

Active Compounds Identification using UPLC-QTOF-MS/MS

The content analysis of compounds was E. palmifolia bulb carried out using the UPLC-QToF-MS/MS type ACOUITY UPLC (instrument Waters). The stationary phase used is C18 or ODS (Octa Desil Silica) which is capable of separating compounds with high, medium, and low polarity levels (Aulia et al, 2016). The mobile phase used is a mixture of formic acid with water 0.1/99.9 (v/v) and formic acid with acetonitrile 0.1/99.9 (v/v) with a gradient elution system, i.e. the ratio of the two solvents changes change every time. The elution results in the next stage will go to the MS detector. The sample that enters the MS system will turn into a grain of water that drips through the *needle* and will be added by a positive charge because the ion source used is ESI positive. The results of the separation will appear in the form of a chromatogram which can then be processed using the application *Masslynk* version 4.1so that the m/z spectra can be obtained from each chromatogram peak (Annisa, 2012). The results of the chromatogram of the extract are E. palmifolia bulb shown in Figure 1.

Each peak of the chromatogram indicates the presence of one compound. Chromatograms were processed using the application *Masslynx*

version 4.1 so that the m/z spectra could be identified, thus predicting the molecular formula of the result of the interpretation. The prediction of the molecular formula then searched for the name of the compound with the help of the website ChemSpider. When writing the molecular formula to the website of the ChemSpider, the number of molecules H minus 1. This is because the source of the ESI ion *positive* will add the charge H to the compound in question, so the number of m/z must also be reduced by the actual mass of H at 1, 0078. After obtaining the name of the compound and its structure through the website, compared the measured (measured) m/z with calculated (calculated) m/z by drawing the structure of the compound in question in the application ChemDraw Ultra 12.0 (Skoog, 2004). If the difference between the two $\leq 0,0005$ then it can be stated that the peak belongs to the predicted compound (Brenton, 2010). The results of the analysis prediction of the active compound on *E*. palmifolia bulb can be seen in Table IV.

The metabolite profile obtained from the results of interpretation (table IV) is that there are differences in the number and part of the compounds. With details of 40 compounds from the East Java sample, 32 compounds from the Central Java sample, 39 compounds from the West

				IA BULB OF EA	IST JAVA
Rt	%Area	Measured Mass	Calculated Mass	Molecular Formula	IUPAC Name
0.689	0,7001%	103,0996	103,0997	C ₅ H ₁₃ NO	(2S)-2-Amino-3-methyl-1-butanol
0.952	0,2750%	269,1490	269,1488	$C_{11}H_{19}N_5O_3$	2-{[4,6-Bis(ethylamino)-1,3,5-triazin-2- yl]oxy}ethyl acetate
1.352	0,3901%	120,0323	120,0324	$C_6H_4N_2O$	2-hydroxynicotinonitrile
2.415	0,1434%	120,0324	120,0324	$C_6H_4N_2O$	2-hydroxynicotinonitrile
2.781	0,0141%	120,0324	120,0324	$C_6H_4N_2O$	2-hydroxynicotinonitrile
3.284	0,1293%	149,1203	149,1205	C10H15N	4-(2-Methyl-2-propanyl)aniline 2-({4-Benzyl-5-[1-
3.650	1,6126%	431,2724	431,2819	C23H37N5OS	(dimethylamino)ethyl]-4H-1,2,4-triazol 3-yl}sulfanyl)-N,N-diisobutylacetamide
3.798	0,9256%	475,3003	475,2998	C16H42N9O5 Cl	UNKNOWN
				Gi	2-Methyl-N-(4-{[2-(3-
3.981	3,9026%	418,1279	418,1277	$C_{22}H_{18}N_4O_5$	nitrobenzoyl)hydrazino]carbonyl}phen l)benzamide
4.244	3,5627%	585,2064	585,2060	C32H27N9OS	UNKNOWN
4.427	0,4200%	578,3432	578,3428	C ₂₈ H ₄₆ N ₆ O ₇	L-Phenylalanyl-L-leucyl-L-seryl-N-[(2S) 6-amino-1-oxo-2-hexanyl]-L-
4.610	2,9136%	636,2038	636,2043	C ₃₅ H ₃₂ N ₄ O ₆ S	threoninamide 4-[Bis(5-hydroxy-3-methyl-1-phenyl- 1H-pyrazol-4-yl)methyl]-2- methoxyphenyl 4- methylbenzenesulfonate
4.793	1,7474%	615,2515	615,2516	C33H37N5O5S	N-{[5-{[2-(2,3-Dihydro-1H-indol-1-y]) 2-oxoethyl]sulfanyl}-4-(3- methylphenyl)-4H-1,2,4-triazol-3- yl]methyl}-3,4,5-triethoxybenzamide
4.896	0,2605%	244,0735	244,0736	$C_{14}H_{12}O_4$	1,4-Naphthalenediyl diacetate
5.159	3,7501%	615,2534	615,2532	C13H37N13O1 5	UNKNOWN
5.262	0,3457%	383,1345	383,1342	C17H17N7O4	7-[(2E)-2-Buten-1-yl]-3-methyl-8-[(2E) 2-(3-nitrobenzylidene)hydrazino]-3,7- dihydro-1H-purine-2,6-dione 4-(N'-
5.594	11,4730 %	256,0742	256,0743	$C_8H_{12}N_6O_2S$	Carbamimidoylcarbamimidamido)benz nesulfonamide
5.845	0,4623%	272,1057	272,1062	$C_{17}H_{12}N_4$	2,6-Diphenylimidazo[1,2- b][1,2,4]triazine
6.142	0,8314%	425,1441	425,1443	C19H27N3 04S2	Methyl 4-ethyl-5-methyl-2-({[4- (tetrahydro-2-furanylcarbonyl)-1- piperazinyl]carbonothioyl}amino)-3- thiophenecarboxylate
6.394	0,2388%	244,1311	244,1311	C12H20O5	4-(3-Methoxy-3-oxopropyl)-2,2- dimethyltetrahydro-2H-pyran-4- carboxylic acid
6.725	0,1201%	541,1953	541,1948	C28H31NO 10	(2E)-2,3-Dihydroxy-2-butenedioic acid ethyl 5-hydroxy-4-[(3-methyl-1- piperidinyl)methyl]-2-phenyl-1- benzofuran-3-carboxylate

Table IVa. Prediction of compounds from the interpretation of the data of the chromatogram in each region *sampling.*

Table IVb. Prediction of compounds from the interpretation of the data of the chromatogram in each region *sampling.*

				IA BULB OF EA	AST JAVA
Rt	%Area	Measured Mass	Calculated Mass	Molecular Formula	IUPAC Name
6.908	1,6721%	244,0740	244,0736	C15H12O4	(2E)-1-(2,4-Dihydroxyphenyl)-3-(4- hydroxyphenyl)-2-propen-1-one
7.057	0,1548%	329,1261	329,1263	C18H19NO5	(Isoliquiritigenin) Ethyl 2-[(3,5- dimethoxybenzoyl)amino]benzoate
7.126	0,1022%	385,1525	385,1526	$C_{21}H_{23}NO_6$	N-[(7S)-10-Hydroxy-1,2,3-trimethoxy-9- oxo-5,6,7,9- tetrahydrobenzo[a]heptalen-7- yl]acetamid
7.308	0,9280%	258,0900	258,0899	$C_8H_{14}N_6O_2S$	N'-Acetyl-2-[(1-propyl-1H-tetrazol-5- yl)sulfanyl]acetohydrazide
7.606	6,6528%	258,1474	258,1474	$C_6H_{22}N_6O_3S$	UNKNOWN
7.857	3,1288%	272,1053	272,1049	C16H16O4	1,1'-Disulfanediylbis(1- methylcyclohexane)
8.040	3,0623%	228,0793	228,0794	C7H12N6OS	2-Methyl-N-[(2-methyl-2H-tetrazol-5- yl)carbamothioyl]propanamide
8.372	25,9277 %	244,0740	244,0743	$C_7H_{12}N_6O_2S$	N-(Ethylcarbamoyl)-2-[(1-methyl-1H- tetrazol-5-yl)sulfanyl]acetamide
8.555	3,2360%	312,1002	312,0998	$C_{18}H_{16}O_5$	Isopropyl [(6-oxo-6H-benzo[c]chromen- 3-yl)oxy]acetate
9.001	0,7733%	270,0898	270,0899	$C_9H_{14}N_6O_2S$	6-(Dimethylamino)-N,N-dimethyl-7H- purine-2-sulfonamide
9.184	1,4197%	230,0950	230,0950	C7H14N6OS	2,2-Dimethyl-3-[(1-methyl-1H-tetrazol- 5-yl)sulfanyl]propanehydrazide
9.549	0,4033%	809,3698	809,3695	$C_{37}H_{55}N_5O_{15}$	UNKNOWN
9.870	0,0600%	541,2122	541,2119	C20H35N3O14	N'-[(1E,2S,3R)-2-{[6-O-(2-Acetamido-2- deoxy-β-D-glucopyranosyl)-β-D- galactopyranosyl]oxy}-3,4- dihydroxybutylidene]acetohydrazide
9.984	0,4157%	895,4075	895,4078	C50H61N3 010S	N-{6a-(Allyloxy)-1,2-bis(4- hydroxybutyl)-10-[2- (phenylsulfanyl)ethoxy]-4-[(tetrahydro- 2H-pyran-2-yloxy)imino]- 1,2,4,5,6,6a,11b,11c- octahydrobenzo[kl]xanthen-6-yl}-N- methyl-3-(4-nitrophenyl)acrylamide
10.28 1	3,0447%	529,4448	529,4447	C11H47N25	UNKNOWN
10.46 4	3,8059%	523,1994	523,1995	C32H29NO 6	5-(4-Ethoxyphenyl)-1-(2-furylmethyl)- 3-hydroxy-4-{4-[(3- methylbenzyl)oxy]benzoyl}-1,5- dihydro-2H-pyrrol-2-one N-(2-{[4-(1-Azepanyl)-2-
11.01 3	4,6727%	513,4523	513,4519	C30H55N7	pyrimidinyl]amino}ethyl)-N'- cyclohexyl-N-{2- [(cyclohexylmethyl)amino]ethyl}-N'- methyl-1,2-ethanediamine

			E. PALMIFOL	IA BULB OF EA	ST JAVA
Rt	%Area	Measured Mass	Calculated Mass	Molecular Formula	IUPAC Name
11.29 9	0,8062%	542,1967	542,1968	C34H22N8	2,2',2'',2'''-Benzene-1,2,4,5- tetrayltetrakis(1H-pyrrolo[2,3- b]pyridine)
11.74 5	1,5604%	300,1342	300,1340	C ₁₂ H ₂₅ O ₆ Cl	17-Chloro-3,6,9,12,15- pentaoxaheptadecan-1-ol
12.03 0	0,2794%	541,4801	541,4799	$C_{20}H_{59}N_{15}S$	UNKNOWN
12.29 3	1,1227%	481,4609	481,4608	C29H59N3O2	N1,N5-Didodecylglutamamide
12.47 6	0,0961%	283,3244	283,3239	$C_{19}H_{41}N$	N-Methyl-1-octadecanamine
12.72 8	0,0145%	426,3824	426,3822	C25H50N2O3	{Dimethyl[3- (stearoylamino)propyl]ammonio}acetat e
12.91 1	0,2142%	555,4345	555,4340	C ₁₈ H ₅₃ N ₁₇ OS	UNKNOWN
13.34 5	0,6568%	254,0945	254,0943	$C_{16}H_{14}O_3$	(2E)-1-(2-Hydroxyphenyl)-3-(4- methoxyphenyl)prop-2-en-1-one
13.64 2	1,5715%	254,0950	254,0950	C9H14N6OS	N-(1H-Tetrazol-5- ylcarbamothioyl)cyclohexanecarboxami de
11.29 9	0,8062%	542,1967	542,1968	C34H22N8	2,2',2'',2'''-Benzene-1,2,4,5- tetrayltetrakis(1H-pyrrolo[2,3- b]pyridine)
11.74 5	1,5604%	300,1342	300,1340	$C_{12}H_{25}O_6Cl$	17-Chloro-3,6,9,12,15- pentaoxaheptadecan-1-ol
12.03 0	0,2794%	541,4801	541,4799	C20H59N15S	UNKNOWN
12.29 3	1,1227%	481,4609	481,4608	C29H59N3O2	N1,N5-Didodecylglutamamide
12.47 6	0,0961%	283,3244	283,3239	$C_{19}H_{41}N$	N-Methyl-1-octadecanamine
12.72 8	0,0145%	426,3824	426,3822	$C_{25}H_{50}N_2O_3$	{Dimethyl[3- (stearoylamino)propyl]ammonio}acetat e
12.91 1	0,2142%	555,4345	555,4340	C ₁₈ H ₅₃ N ₁₇ OS	UNKNOWN
13.34 5	0,6568%	254,0945	254,0943	$C_{16}H_{14}O_3$	(2E)-1-(2-Hydroxyphenyl)-3-(4- methoxyphenyl)prop-2-en-1-one

Table IVc. Prediction of compounds from the interpretation of the data of the chromatogram in each region *sampling*.

E. PALMIFOLIA BULB OF WEST JAVA							
Rt	%Area	Measured Mass	Calculated Mass	Molecular Formula	Nama IUPAC		
0.689	2,2557%	380,0729	380,0727	$C_{22}H_{20}S_3$	4,5-Bis(phenylsulfanyl)-2,3,4,5- tetrahydro-1-benzothiepine		
0.952	0,2817%	267,0976	267,0973	$C_{11}H_9N_9$	N-(1H-Benzotriazol-1- ylmethyl)tetrazolo[1,5-b]pyridazin-6- amine		

		M		IA BULB OF WE	
Rt	%Area	Measured Mass	Calculated Mass	Molecular Formula	Nama IUPAC
1.386	0,0129%	327,1309	327,1305	C12H13N11 0	UNKNOWN
1.935	0,0071%	181,9778	181,9778	CH3N6OSCI	UNKNOWN
2.118	0,0057%	181,9779	181,9778	CH3N6OSCl	UNKNOWN
2.815	0,0026%	292,0584	292,0583	C14H12O7	[7-(Carboxymethoxy)-4-methyl-2-oxo- 2H-chromen-3-yl]acetic acid Methyl 2,3-di-0-acetyl-4,6-0-(4-
3.147	0,0325%	396,142	396,1421	C19H24O9	methoxybenzylidene)-α-D- glucopyranoside
3.513	0,2311%	438,1159	438,1162	C20H22O11	4-(β-D-Glucopyranosyloxy)-3- hydroxybenzyl 3,4-dihydroxybenzoate
3.696	0,2150%	292,0591	292,0592	C15H16O2S 2	1,1'-(2,2-Propanediyldi-5,2- thienediyl)diethanone
4.313	12,9240 %	418,1268	418,1264	C21H22O9	8,8'-(2-Furylmethylene)bis(6,10- dioxaspiro[4.5]decane-7,9-dione)
4.565	2,9606%	244,0739	244,0743	C7H12N6O 2S	N-(Ethylcarbamoyl)-2-[(1-methyl-1H- tetrazol-5-yl)sulfanyl]acetamide
5.113	4,7188%	596,2099	596,2101	C41H28N2 03	1,3-Bis(2'-hydroxy-1,1'-binaphthalen-2 yl)urea
5.342	0,2960%	214,0998	214,0994	C14H14O2	4,4'-Dimethoxybiphenyl
5.708	2,4517%	256,1104	256,1100	C16H16O3	4-(Benzyloxy)-3-ethoxybenzaldehyde (2E)-1-(2,4-Dihydroxyphenyl)-3-(4-
6.074	4,5887%	256,0739	256,0736	C15H12O4	hydroxyphenyl)-2-propen-1-one (Isoliquiritigenin)
6.291	0,2831%	272,1051	272,1049	C16H16O4	2-Hydroxy-1,2-bis(4- methoxyphenyl)ethanone
6.577	0,5048%	287,1155	287,1158	C16H17NO 4	Methyl 5-(1-acetamido-2-phenylethyl) 2-furoate
6.943	0,3376%	244,0737	244,0736	C14H12O4	4-[(E)-2-(3,5-Dihydroxyphenyl)vinyl]- 1,3-benzenediol (Oxyresveratrol)
7.389	9,3857%	244,0736	244,0740	CH6N10OCl 2	UNKNOWN
7.674	0,3336%	258,0899	258,0899	C9H20N2O 2Cl2	N'-Acetyl-2-[(1-propyl-1H-tetrazol-5- yl)sulfanyl]acetohydrazide
8.269	12,4431 %	228,0788	228,0787	C14H12O3	5-[(E)-2-(4-Hydroxyphenyl)vinyl]-1,3- benzenediol (Trans-resveratrol)
8.452	8,3760%	228,0786	228,0787	C14H12O3	5-[(E)-2-(4-Hydroxyphenyl)vinyl]-1,3- benzenediol (Trans-resveratrol)
8.738	24,6376 %	244,0733	244,0729	C6H16N2O 6S	Lysine sulfate (1:1)
8.955	[%] 2,6997%	312,1006	312,1011	C19H12N4	8,9-Diphenylfuro[3,2-
9.138	0,4306%	363,147	363,1471	0 C22H21NO 4	e][1,2,4]triazolo[1,5-c]pyrimidine Propyl 4-{[(2- naphthyloxy)acetyl]amino}benzoate
9.321	0,3363%	270,0896	270,0892	4 C16H14O4	Diphenyl succinate
9.652	0,5991%	371,1734	371,1729	CH22N170 4Cl	UNKNOWN

			E. PALMIFOL	IA BULB OF WE	EST JAVA
Rt	%Area	Measured Mass	Calculated Mass	Molecular Formula	Nama IUPAC
10.05 3	0,3888%	315,2774	315,2774	C18H37NO 3	N,N-Bis(2- hydroxyethyl)tetradecanamide
10.23 5	0,7104%	529,4463	529,4464	C30H63N3S 2	UNKNOWN
10.83 0	3,3542%	356,0903	356,0903	C12H16N6 05S	5'-Deoxy-5'- [(vinylsulfonyl)amino]adenosine
11.11 6	0,2261%	258,1261	258,1256	C16H18O3	Ethyl 2-(6-methoxy-2- naphthyl)propanoate
11.29 9	0,1186%	578,286	578,2855	C38H42O3S	UNKNOWN
11.44 7	0,8992%	497,457	497,4575	C15H51N19	UNKNOWN
11.81 3	0,1298%	621,3731	621,3729	C13H39N27 03	UNKNOWN
12.03 1	0,8637%	481,4612	481,4608	C29H59N3 O2	N1,N5-Didodecylglutamamide
12.24 8	0,1416%	533,3566	533,3564	C27H51NO 9	Hexadecyl 5-acetamido-3,5-dideoxy-6- [(1S,2R)-1,2,3-trihydroxypropyl]hex-2- ulopyranosidonicacid
12.39 6	0,2349%	416,2319	416,2314	C16H40N4 02S3	UNKNOWN
12.54 5	0,0265%	408,3751	408,3756	C30H48	(6E,10E,12Z,14E,18E)-2,6,10,15,19,23- Hexamethyl-2,6,10,12,14,18,22- tetracosaheptaene
12.87 7	0,1571%	323,282	323,2816	C15H38N5C l	4-Amino-N,N-bis(4-aminobutyl)-N-(3- aminopropyl)-1-butanaminium chloride
13.05 9	0,2498%	392,2324	392,2325	C22H28N6 0	N,N-Diethyl-1-[1-(3-methylphenyl)-1H- pyrazolo[3,4-d]pyrimidin-4-yl]-3- piperidinecarboxamide
13.31 1	0,0830%	523,3649	523,3645	C23H51N9C l2	UNKNOWN
13.60 8	0,3474%	278,2249	278,2246	C18H30O2	(9E,12E,15E)-9,12,15- Octadecatrienoic acid
13.79 1	0,1049%	412,3701	412,3705	C29H48O	(3α,5α,9ξ,14ξ,22E,24R)-Stigmasta-7,22- dien-3-ol
14.19 1	0,2837%	281,2716	281,2719	C18H35NO	1-Dodecyl-2-azepanone
14.44 3	0,2739%	470,3401	470,3396	C30H46O4	(3β,5α,14ξ,25R)-Spirost-9(11)-en-3-yl propionate
14.62 6	0,0545%	281,2145	281,2144	C20H27N	(1E)-2,2,5,9-Tetramethyl-N-phenyl- 3,4,8-decatrien-1-imine

E. PALMIFOLIA BULB EAST BORNEO							
Rt	%Area	Measured Mass	Calculated Mass	Molecular Formula	IUPAC Name		
0.689	2,1443%	380,0722	380,0719	C ₂₁ H ₁₆ O ₅ S	4-Methyl-6-oxo-6H-benzo[c]chromen-3- yl 4-methylbenzenesulfonate		
0.952	0,1879%	267,0968	267,0968	C10H13N5 04	Adenosine		
1.237	0,0270%	489,1831	489,1830	C3H24N25 Br	UNKNOWN		

Rt	%Area	Measured	Calculated	Molecular	IUPAC Name
ĸı	%Area	Mass	Mass	Formula	
1.386	0,0623%	165,0783	165,0781	C4H12N5Cl	N,N-Dimethylimidodicarbonimidic diamide hydrochloride (1:1)
2.152	0,0303%	187,0633	187,0634	C11H11NO 2	3-(1H-Indol-3-yl)propanoic acid
2.598	0,0186%	246,0521	246,0520	C8H11N4O 3Cl	N-(6-Amino-1,3-dimethyl-2,4-dioxo- 1,2,3,4-tetrahydro-5-pyrimidinyl)-2- chloroacetamide (1ξ)-1,5-Anhydro-1-(1,3,6,7-
3.181	0,2924%	422,0848	422,0849	C19H18O11	tetrahydroxy-9-oxo-9H-xanthen-2-yl)- D-allitol
3.284	0,0932%	438,1159	438,1162	C20H22O11	4-(β-D-Glucopyranosyloxy)-3- hydroxybenzyl 3,4-dihydroxybenzoate
3.433	0,0496%	396,1415	396,1413	C19H29N2 02Br	1'-(2-Bromo-4,5-dimethoxybenzyl)-1,4 bipiperidine 2-[(1H-Benzimidazol-2-
3.581	0,0654%	418,1256	418,1252	C26H18N4S	ylmethyl)sulfanyl]-4,6- diphenylnicotinonitrile
4.313	16,5162 %	418,1261	418,1264	C21H22O9	8,8'-(2-Furylmethylene)bis(6,10- dioxaspiro[4.5]decane-7,9-dione)
4.610	6,0092%	244,0741	244,0736	C14H12O4	1,4-Naphthalenediyl diacetate
5.113	8,7689%	596,2105	596,2100	C41H28N2 03	1,3-Bis(2'-hydroxy-1,1'-binaphthalen-2 yl)urea
5.708	8,1186%	256,1101	256,1100	C16H16O3	4-(Benzyloxy)-3-ethoxybenzaldehyde
5.891	1,1041%	256,0736	256,0736	C15H12O4	(2E)-1-(2,4-Dihydroxyphenyl)-3-(4- hydroxyphenyl)-2-propen-1-one (Isoliquiritigenin)
6.028	2,3862%	256,0744	256,0749	C16H8N4	Quinoxalino[2',3':3,4]cyclobuta[1,2- b]quinoxaline
6.291	0,4052%	272,1051	272,1049	C16H16O4	2-Hydroxy-1,2-bis(4- methoxyphenyl)ethanone
6.577	0,3111%	403,1617	403,1618	C18H17N11 0	[3-(1H-Tetrazol-1-yl)phenyl]{4-[6-(1H 1,2,4-triazol-1-yl)-3-pyridazinyl]-1- piperazinyl}methanone
6.760	0,0546%	342,0733	342,0732	C18H19N2 BrN2	Diphenyl[(3S)-3- pyrrolidinyl]acetonitrile hydrobromid (1:1)
6.908	0,1698%	242,0582	242,0579	C14H10O4	9,10-Dihydroxy-2,3-dihydro-1,4- anthracenedione
7.389	6,6912%	244,0738	244,0743	C7H12N60 2S	N-(Ethylcarbamoyl)-2-[(1-methyl-1H- tetrazol-5-yl)sulfanyl]acetamide
7.606	0,3505%	370,2834	370,2832	C20H38N2 04	Ethyl 4-{2-hydroxy-3-[(2-isopropyl-5- methylcyclohexyl)oxy]propyl}-1- piperazinecarboxylate
8.269	8,0759%	228,0788	228,0787	C14H12O3	5-[(E)-2-(4-Hydroxyphenyl)vinyl]-1,3 benzenediol (Trans-resveratrol)
8.452	5,4174%	228,0785	228,0787	C14H12O3	5-[(E)-2-(4-Hydroxyphenyl)vinyl]-1,3 benzenediol
8.738	24,4573 %	244,0739	244,0743	C7H12N60 2S	(Trans-resveratrol) N-(Ethylcarbamoyl)-2-[(1-methyl-1H- tetrazol-5-yl)sulfanyl]acetamide

Metabolite Fingerprinting Eleutherine palmifolia (L.) Merr.

			E. PALMIFOL	A BULB EAST	BORNEO
Rt	%Area	Measured Mass	Calculated Mass	Molecular Formula	IUPAC Name
8.955	0,9910%	312,0999	312,0998	C18H1805	(2E)-3-(3,4-Dimethoxyphenyl)-1-(2- hydroxy-4-methoxyphenyl)-2-propen-1- one
9.435	0,2784%	363,1466	363,1471	C22H21NO 4	Propyl 4-{[(2- naphthyloxy)acetyl]amino}benzoate
10.09 8	0,1821%	543,4246	543,4241	C32H62NOS Cl	UNKNOWN
10.23 5	0,8418%	529,445	529,4447	C11H47N25	UNKNOWN
10.38 4	0,0421%	523,1999	523,1999	C18H45N5S 6	UNKNOWN
10.56 7	0,2405%	523,2002	523,2002	C25H29N7 04S	Nα-{[(2S,4S)-1-[(1-Methyl-1H-pyrrol-2- yl)carbonyl]-4-{[(4-methyl-1,2,3- thiadiazol-5-yl)carbonyl]amino}-2- piperidinyl]carbonyl}-D- phenylalaninamide
10.89 9 11.15	2,2910%	513,4522	513,4519	C30H55N7	N-(2-{[4-(1-Azepanyl)-2- pyrimidinyl]amino}ethyl)-N'- cyclohexyl-N-{2- [(cyclohexylmethyl)amino]ethyl}-N'- methyl-1,2-ethanediamine Ethyl 2-(6-methoxy-2-
0	0,4624%	258,1257	258,1256	C16H18O3	naphthyl)propanoate
11.44 7	0,9595%	497,4556	497,4557	C29H59N3 03	UNKNOWN
11.81 3	0,2532%	621,3727	621,3723	C5H43N29 05S	UNKNOWN
12.03 1	0,5511%	495,3326	495,3322	C27H41N7 02	UNKNOWN
12.24 8	0,2398%	533,3563	533,3567	C28H50N2 08	(1R,2S)-2-({[(2-Methyl-2- propanyl)oxy]carbonyl}amino)cycloocta necarboxylic acid - (1S,2R)-2-({[(2- methyl-2- propanyl)oxy]carbonyl}amino)cycloocta necarboxylic acid (1:1)
12.39 6	0,2269%	416,2328	416,2325	C24H28N6 0	2-Amino-1-[4-(diethylamino)phenyl]-N- propyl-1H-pyrrolo[2,3-b]quinoxaline-3- carboxamide

		E	PALMIFOLIA	BULB CENTRA	L BORNEO
Rt	%Area	Measured Mass	Calculated Mass	Molecular Formula	Nama IUPAC
0.689	2,9195%	364,0986	364,0984	C ₆ H ₁₃ N ₁₄ O ₃ Cl	UNKNOWN
2.552	0,1490%	374,1181	374,1186	$C_{12}H_{18}N_6O_8$	4-[(1E)-3,3-Dimethyl-1-triazen-1-yl]- 1H-imidazole-5-carboxamide 2- hydroxy-1,2,3-propanetricarboxylate (1:1)
2.781	0,0229%	124,9784	124,9781	C4N3Cl	Chloromethanetricarbonitrile
3.181	0,3976%	289,1308	289,1306	C11H20N5 O2Cl	6-Chloro-N,N'-bis(3-methoxypropyl)- 1,3,5-triazine-2,4-diamine

Rt	%Area	Measured Mass	Calculated Mass	Molecular Formula	Nama IUPAC
		Mass	Mass	C11H20N5	6-Chloro-N,N'-bis(3-methoxypropyl)-
3.284	0,0266%	289,1308	289,1306	02Cl	1,3,5-triazine-2,4-diamine
3.433	0,0279%	124,9781	124,9781	C4N3Cl	Nitromethanesulfinic acid
3.650	0,0279%	124,9781	124,9781	CH3NO4S	Nitromethanesulfinic acid
5.050	0,0382%	124,970	124,9783	C19H47N7S	Niu omethanesumme actu
3.799	0,0742%	475,2994	475,2991	C19H47N75 Cl2	UNKNOWN
4.016	3,1581%	418,1263	418,1264	C21H22O9	8,8'-(2-Furylmethylene)bis(6,10- dioxaspiro[4.5]decane-7,9-dione)
4.244	2,7176%	590,1608	590,1609	C24H26N6 012	Diisopropyl 4,4'-(1,4- piperazinediyl)bis(3,5-dinitrobenzoate
				C19H32O3S	Methyl (2R)-2-{(1S,2E,3S,4R)-2- (methoxymethylene)-4-methyl-3-[2-(2
4.462	0,3536%	372,1792	372,1793	2	methyl-1,3-dithiolan-2- yl)ethyl]cyclohexyl}propanoate
					(2R)-4-{4,6-Dimethyl-9-oxo-3-[(2,3,5-
				C26H32N6	tri-O-acetyl-β-D-ribofuranosyl)oxy]-4,9
4.610	3,0357%	3,0357% 636,2026	636,2028	013	dihydro-3H-imidazo[1,2-a]purin-7-yl} 2-[(methoxycarbonyl)amino]butanoid
					acid
					4-[(E)-({[1-(4-Amino-1,2,5-oxadiazol-3
4 700	1 45 410/	4541% 620,2087	620,2092	C27H28N10 08	yl)-4-(4-morpholinylmethyl)-1H-1,2,3
4.793	1,4541%				triazol-5-
					yl]carbonyl}hydrazono)methyl]-2-
					ethoxyphenyl 3-methyl-4-nitrobenzoat
				C2(1122N/	Methyl 2-azido-4,6-0-benzylidene-2-
5.113	4,8572%	620,2078	620,2078	C26H32N6	deoxy-3-0-(3,4,6-tri-0-acetyl-2-azido-2
				012	deoxy-α-D-galactopyranosyl)-β-D-
				C71124NI70	galactopyranoside
5.296	0,0673%	321,1352	321,1350	C7H24N7O 3SCl	UNKNOWN
5.445	0,0150%	124,9782	124,9781	C4N3Cl	Chloromethanetricarbonitrile
5.445	0,013070	124,9702	124,9701	CHNSCI	(2E)-1-(2,4-Dihydroxyphenyl)-3-(4-
5.594	5,2209%	256,0735	256,0736	C15H12O4	hydroxyphenyl)-2-propen-1-one
5.574	5,220770	230,0733	230,0730	015111204	(Isoliquiritigenin)
					2-Hydroxy-1,2-bis(4-
5.845	0,4397%	272,1044	272,1049	C16H16O4	methoxyphenyl)ethanone
				C17H13N0	2-(1-Oxo-1-phenyl-2-propanyl)-1H-
6.257	0,8482%	279,0894	279,0896	3	isoindole-1,3(2H)-dione
				C6H14N2O	6-Deoxy-N-sulfamoyl-β-D-
6.474	0,1076%	242,0573	242,0573	6S	mannopyranosylamine
				C9H14N5O	2-{[4-Chloro-6-(4-morpholinyl)-1,3,5
6.691	0,5581%	259,0835	259,0836	2Cl	triazin-2-yl]amino}ethanol
				201	4-[(E)-2-(3,5-Dihydroxyphenyl)vinyl]
6.988	0,9297%	244,0734	244,0736	C14H12O4	1,3-benzenediol
0.700	0,747770	477,0734	277,0730	617111204	(Oxyresveratrol)
					(2E)-1-(2,4-Dihydroxyphenyl)-3-(4-
7.308	0,0982%	258,089	258,0892	C15H14O4	hydroxyphenyl)-2-propen-1-one
.500	0,070270	230,009	230,0092	615111404	(Isoliquiritigenin)
					tert-Butyl 3,5-dideoxy-2,4-0-
7.640	4,9683%	258,1467	258,1467	C13H22O5	isopropylidene-L-erythro-hexuronate
					2-Hydroxy-1,2-bis(4-
7.857	5,8641%	272,1049	272,1049	C16H16O4	2-11yu10xy-1,2-015(4-

				BULB CENTRA	L BORNEO
Rt	%Area	Measured Mass	Calculated Mass	Molecular Formula	Nama IUPAC
8.086	5,6093%	228,0784	228,0787	C14H12O3	5-[(E)-2-(4-Hydroxyphenyl)vinyl]-1,3- benzenediol
8.406	27,1804 %	244,0733	244,0729	C6H16N2O 6S	Lysine sulfate (1:1)
8.589	3,1634%	312,0986	312,0989	C13H17N4 03Cl	2-(4-Chloro-3-nitro-1H-pyrazol-1-yl)-N [2-(1-cyclohexen-1-yl)ethyl]acetamide
8.738	0,4153%	357,1571	357,1576	C20H23NO 5	Methyl 4-{[(3,4- diethoxyphenyl)acetyl]amino}benzoate
9.218	4,0775%	230,0939	230,0943	C14H14O3	3-Hydroxy-8,9,10,11-tetrahydro-7H- cyclohepta[c]chromen-6-one
9.687	3,2395%	529,4457	529,4455	C29H59N3 05	UNKNOWN
9.835	7,0523%	523,199	523,1995	C32H29N0 6	5-(4-Ethoxyphenyl)-1-(2-furylmethyl)- 3-hydroxy-4-{4-[(3- methylbenzyl)oxy]benzoyl}-1,5- dihydro-2H-pyrrol-2-one
10.05 2	0,2028%	895,4073	895,4072	C27H69N13 012S4	UNKNOWN
10.16 7	0,4837%	270,0893	270,0892	C16H14O4	Diphenyl succinate
10.49 8	1,9713%	356,09	356,0896	C19H1607	Methyl {[3-(4-methoxyphenoxy)-4-oxo- 4H-chromen-7-yl]oxy}acetate
10.64 7	0,1652%	288,0994	288,0998	C16H1605	1-(2,4-Dihydroxyphenyl)-2-(3,4- dimethoxyphenyl)ethanone
10.89 9	1,9083%	497,4555	497,4557	C29H59N3 O3	UNKNOWN
11.33 3	1,3305%	542,1941	542,1941	C32H3008	2-[6-(Benzyloxy)-1,3-benzodioxol-5-yl] 1-[4-(benzyloxy)-2-hydroxyphenyl]-3,3- dimethoxy-1-propanone
11.48 2	2,7945%	481,4609	481,4608	C29H59N3 02	N1,N5-Didodecylglutamamide
	1,8164%	300,1332	300,1335	C14H16N6 02	1-(4,6-dimethylpyrimidin-2-yl)-3-(4- methyl-3-nitrophenyl)guanidine
12.17 9	0,0549%	542,1925	542,1927	C29H22N10 02	3,3'-(2-Methyl-1,3-phenylene)bis(7- methyl-8-phenylpyrazolo[5,1- d][1,2,3,5]tetrazin-4(3H)-one)

			E. PALMIFOLIA	4 <i>BULB</i> SOUTH	I BORNEO
Rt	%Area	Measured Mass	Calculated Mass	Molecular Formula	Nama IUPAC
0.254	0,0160%	124,9779	124,9781	C ₄ N ₃ Cl	Chloromethanetricarbonitrile
					{[3-(2-0xo-2H-chromen-3-yl)-1-phenyl-
0.689	4,7948%	364,0964	364,0961	$C_{22}H_{12}N_4O_2$	1H-pyrazol-4-
					yl]methylene}malononitrile
2.781	0,0207%	124,9781	124,9783	CH ₃ NO ₄ S	Nitromethanesulfinic acid
3.433	0,0342%	124,9778	124,9783	CH ₃ NO ₄ S	Nitromethanesulfinic acid
3.650	0,0277%	431,2717	431,2717	C23H45NS3	1-[2-(Diethylamino)ethyl]-3-[3- (dimethylamino)propyl]-1-[(8-methyl- 2-oxo-1,2-dihydro-3- quinolinyl)methyl]thiourea

D .	0/ 4	Measured	Calculated	<u>4 BULB SOUTH</u> Molecular	
Rt	%Area	Mass	Mass	Formula	Nama IUPAC
3.833	0,3806%	475,3002	475,3006	C21H41N5 07	(1S,2S,3R,4R,6S)-4-Amino-3-{[(2R,3S)- 3-amino-6-(aminomethyl)-3,4-dihydro- 2H-pyran-2-yl]oxy}-6-(ethylamino)-2- hydroxycyclohexyl (2ξ)-3-deoxy-4-C- methyl-3-(methylamino)-L-erythro- pentopyranoside
4.016	4,5107%	418,1269	418,1264	C21H22O9	8,8'-(2-Furylmethylene)bis(6,10- dioxaspiro[4.5]decane-7,9-dione) 1-[3-(3,4-Dimethoxyphenyl)-1-phenyl-
4.279	4,1290%	590,1624	590,1624	C33H26N4 05S	1H-pyrazol-4-yl]-2-(4,5-dimethyl-1,3- thiazol-2-yl)-1,2-dihydrochromeno[2,3- c]pyrrole-3,9-dione
4.462	1,2960%	578,3432	578,3428	C28H46N6 07	L-Phenylalanyl-L-leucyl-L-seryl-N-[(2S) 6-amino-1-oxo-2-hexanyl]-L- threoninamide
4.645	5,6249%	636,2045	636,2043	C35H32N4 06S	4-[Bis(5-hydroxy-3-methyl-1-phenyl- 1H-pyrazol-4-yl)methyl]-2- methoxyphenyl 4- methylbenzenesulfonate
4.827	2,9975%	620,2097	620,2092	C27H28N10 08	4-[(E)-({[1-(4-Amino-1,2,5-oxadiazol-3- yl)-4-(4-morpholinylmethyl)-1H-1,2,3- triazol-5- yl]carbonyl}hydrazono)methyl]-2- ethoxyphenyl 3-methyl-4-nitrobenzoate
4.976	0,0933%	244,0731	244,0736	C14H12O4	1,4-Naphthalenediyl diacetate
5.159	0,2942%	520,2071	520,2069	H29N2905S 3	UNKNOWN
5.628	5,6724%	256,0726	256,0727	C7H16N2O 6S	2-Amino-N-isopropylethanesulfonamid ethanedioate (1:1)
5.891	13,2610 %	272,1042	272,1042	C8H20N2O 6S	Morpholine sulfate (2:1)
6.508	0,3749%	242,0575	242,0573	C6H14N2O 6S	6-Deoxy-N-sulfamoyl-β-D- mannopyranosylamine
6.805	0,0461%	242,0569	242,0571	C9H11N4O 2Cl	7-(2-Chloroethyl)-1,3-dimethyl-3,7- dihydro-1H-purine-2,6-dione
6.988	2,9684%	244,0741	244,0736	C14H12O4	4-[(E)-2-(3,5-Dihydroxyphenyl)vinyl]- 1,3-benzenediol (Oxyresveratrol)
7.354	0,5432%	258,0879	258,0884	C10H15N4 02Cl	3-Nitro-N-(4-piperidinyl)-2- pyridinamine hydrochloride (1:1)
7.640	8,0227%	258,1471	258,1467	C13H22O5	tert-Butyl 3,5-dideoxy-2,4-0- isopropylidene-L-erythro-hexuronate
7.903	4,9125%	272,1047	272,1049	C16H16O4	2-Hydroxy-1,2-bis(4- methoxyphenyl)ethanone
8.086	5,8744%	228,0782	228,0787	C14H12O3	5-[(E)-2-(4-Hydroxyphenyl)vinyl]-1,3- benzenediol (Trans-resveratrol)
8.406	26,4818 %	244,073	244,0729	C6H16N2O 6S	Lysine sulfate (1:1)

			E. PALMIFOLL	A BULB SOUTH	BORNEO
Rt	%Area	Measured Mass	Calculated Mass	Molecular Formula	Nama IUPAC
9.104	1,2478%	723,3323	723,3327	C33H49N5 013	(2R,3R,4R,5S)-5-[(1R)-2-Amino-1- {[(2S,3S,4S)-3,4-dihydroxy-6-{[(3S)-2- oxo-3-azepanyl]carbamoyl}-3,4- dihydro-2H-pyran-2-yl]oxy}-2- oxoethyl]-2-(2,4-dioxo-3,4-dihydro- 1(2H)-pyrimidinyl)-4-methoxytetrahyd ro-3-furanyl decanoate
9.218	0,3255%	526,1629	526,1628	C31H2608	4-({[3-(2,3-Dihydro-1,4-benzodioxin-6- yl)-4-oxo-6-propyl-4H-chromen-7- yl]oxy}methyl)-7-methoxy-2H- chromen-2-one
9.366	0,0481%	468,139	468,1389	C21H28N2 06S2	1,4-Bis[(4-methoxy-3- methylphenyl)sulfonyl]-1,4-diazepane
9.584	1,0705%	809,3669	809,3668	C33H51N11 013	Glycyl-L-seryl-N5-(diaminomethylene)- L-ornithyl-L-glutaminyl-L-tyrosyl-L- alanyl-L-glutamic acid
9.732	0,8358%	169,0883	169,0885	C4H15N3O 2S	UNKNOWN
10.05 2	0,7553%	895,4057	895,4056	C31H53N21 09S	UNKNOWN
10.16 7	0,1860%	270,0892	270,0892	C16H14O4	Diphenyl succinate
10.35 0	0,5501%	513,4505	513,4506	C29H59N3 04	UNKNOWN
10.49 8	1,6500%	356,0891	356,0896	C19H1607	Methyl {[3-(4-methoxyphenoxy)-4-oxo- 4H-chromen-7-yl]oxy}acetate
10.68 1	0,2252%	298,0834	298,0835	C9H18N2O 7S	2-[(2-{[(3- Aminopropyl)carbamoyl]oxy}ethyl)sulf onyl]ethyl hydrogen carbonate
10.78 4	0,0886%	1067,476 1	1067,4761	C73H65NO 7	UNKNOWN

			E. PALMIFOLI	A BULB CENT	RAL JAVA
Rt	%Area	Measured Mass	Calculated Mass	Rumus Molekul	Nama IUPAC
0,689	0,9161%	380,0728	380,0726	$C_{14}H_{16}N_6O_3S_2$	2-({[2-(Methoxymethyl)-5-oxo-5H- [1,3,4]thiadiazolo[3,2-a]pyrimidin-7- yl]methyl}sulfanyl)-N-(1-methyl-1H- pyrazol-4-yl)acetamide
1,020	0,1982%	293,1478	293,1475	C12H23NO7	1,2-di-O-methyl-4-[(2R)-2,4- dihydrobutyramido]-4,6-dideoxy-α-D- mannopyranoside
1,386	0,0434%	327,1317	327,1318	$C_{15}H_{21}NO_{7}$	Methyl (3,4,5-triethoxy-2- nitrophenyl)acetate
2,598	0,0034%	181,9777	181,9778	CH ₃ N ₆ OSCl	UNKNOWN
2,735	0,0046%	181,9778	181,9778	CH ₃ N ₆ OSCl	UNKNOWN
3,250	0,1746%	230,1055	230,1056	$C_{13}H_{14}N_2O_2$	Ethyl 5-methyl-1-phenyl-1H-pyrazole-4- carboxylate
3,696	0,0679%	292,0589	292,0588	C10H9N8OCl	4-Chloro-N-(4-imino-1,4-dihydro-5H- pyrazolo[3,4-d]pyrimidin-5-yl)-1- methyl-1H-pyrazole-5-carboxamide

				A BULB CENTI	RAL JAVA
Rt	%Area	Measured Mass	Calculated Mass	Rumus Molekul	Nama IUPAC
4,565	1,1948%	244,0736	244,0736	C14H12O4	1,4-Naphthalenediyl diacetate
4,976	0,5442%	596,2099	596,2094	C18H36N12 05S3	UNKNOWN
5,113	1,1116%	596,2119	596,2119	C29H32N4 010	(4aR,7aS,7bS,8R,9R)-9a-Acetoxy-4a,7b- dihydroxy-3-(hydroxymethyl)-1,1,6,8- tetramethyl-5-oxo- 1a,1b,4,4a,5,7a,7b,8,9,9a-decahydro-1H- cyclopropa[3,4]benzo[1,2-e]azulen-9-yl 5-azido-2-nitrobenzoate
5,708	1,1017%	256,1095	256,1100	C16H16O3	4-(Benzyloxy)-3-ethoxybenzaldehyde (2E)-1-(2,4-Dihydroxyphenyl)-3-(4-
5,891	0,0978%	256,0738	256,0736	C15H12O4	hydroxyphenyl)-2-propen-1-one
6,028	0,4945%	256,0744	256,0749	C16H8N4 C16H10N4	(Isoliquiritigenin) Quinoxalino[2',3':3,4]cyclobuta[1,2- b]quinoxaline 4,7-Diphenyl(1,2,5)oxadiazolo(3,4-
6,325	0,1239%	274,085	274,0855	0	d)pyridazine
6,577	0,3646%	287,116	287,1158	C16H17NO 4	Methyl 5-(1-acetamido-2-phenylethyl)- 2-furoate
7,389	6,2525%	244,0737	244,0736	C14H12O4	4-[(E)-2-(3,5-Dihydroxyphenyl)vinyl]- 1,3-benzenediol (Oxyresveratrol)
7,606	0,2237%	370,2833	370,2832	C20H38N2 04	UNKNOWN
8,452	8,4865%	228,0789	228,0787	C14H12O3	5-[(E)-2-(4-Hydroxyphenyl)vinyl]-1,3- benzenediol (Trans-resveratrol)
8,738	13,3947 %	244,0738	244,0736	C14H12O4	1,4-Naphthalenediyl diacetate
8,955	1,8921%	312,1003	312,0998	C18H1605	Isopropyl [(6-oxo-6H-benzo[c]chromen- 3-yl)oxy]acetate
9,321	0,1779%	270,0897	270,0892	C16H14O4	Diphenyl succinate
9,652	1,0856%	371,1734	371,1733	C21H25NO 5	1,2,3,10-Tetramethoxy-7- (methylamino)-6,7- dihydrobenzo[a]heptalen-9(5H)-one
10.05 3	3,3308%	315,2772	315,2774	C18H37NO 3	N,N-Bis(2- hydroxyethyl)tetradecanamide
10.23 5	9,9337%	529,4459	529,4455	C29H59N3 05	UNKNOWN
10.38 4	6,9546%	523,2003	523,2000	C17H25N13 07	UNKNOWN
11.15 0	2,9977%	258,1255	258,1256	C16H19O3	5-(Adamantan-1-yl)-2-methyl-3-furoate
11.44 7	10,4767 %	497,4562	497,4557	C29H60N3 03	UNKNOWN
11.81 3	3,9633%	541,4823	541,4819	C31H63N3 04	UNKNOWN
12.17 9	0,5320%	466,3089	466,3086	C23H50N2 OS3	UNKNOWN
12.39 6	1,0792%	341,3295	341,3294	C21H43NO 2	Ethyl N-hexadecyl-β-alaninate

	E. PALMIFOLIA BULB CENTRAL JAVA								
Rt	%Area	Measured Mass	Calculated Mass	Rumus Molekul	Nama IUPAC				
12.54 5	1,1901%	468,3241	468,3240	C30H44O4	(5ξ,9ξ)-3,11-Dioxoolean-12-en-30-oic acid				
12.76 2	2,8968%	399,2408	399,2410	C24H33NO 4	N-(3,4-Dimethoxybenzyl)-N-[3-(4- methoxyphenyl)-4- methylpentyl]acetamide				
12.87 7	3,7969%	323,2824	323,2825	C20H37NO 2	1-[(2R)-1,4-Dioxaspiro[4.11]hexadec-2- ylmethyl]piperidine				
13.16 2	8,7991%	441,2519	441,2515	C26H35NO 5	N-[3-(4-Isopropyl-2,2- dimethyltetrahydro-2H-pyran-4- yl)propanoyl]-N-(4-methoxybenzyl)-2- furamide				
13.31 1	0,8928%	523,3642	523,3640	C14H41N19 03	UNKNOWN				
13.46 0	2,2781%	299,2828	299,2825	C18H37NO 2	N-(2-Hydroxyethyl)hexadecanamide				
13.60 8	1,5235%	278,225	278,2246	C18H30O2	(9E,12E,15E)-9,12,15- Octadecatrienoic acid				
13.79 1	1,4004%	759,4714	759,4710	C46H65N0 8	3-[{6-[3,5- Bis(decyloxy)phenoxy]hexanoyl}(4- carboxyphenyl)amino]benzoic acid				

Java sample, 34 compounds from the East Borneo sample, 38 compounds from the Central Borneo sample, and 31 compounds from the South Borneo sample. The composition of the compounds in plants influenced by two factors, namely internal factors and external factors (Heuberger et al., 2013). Internal factors that affect the composition of the compound include genetic and physiological variations, while external factors such as (altitude), geographical conditions climate, humidity, light intensity, temperature, nutrient intake, and radiation (Verma and Shukla, 2015). These factors cause the number of compounds from each sample to be different.

Table IV shows the different types of compounds contained in *E. palmifolia bulbs* from six different growing locations. Each sample E. *palmifolia bulb* showed the presence of a dominant or major compound, including *N*- (*ethylcarbamoyl*) [(1-methyl-1H-tetrazole-5-yl) -2 sulfanyl] acetamide with a percent area of 25.928% at samples from Blitar, East Java; 1,4-Naphthalenediyl diacetate with a percent area of 13,395% in samples originating from Karanganyar, Central Java; Lysine sulfate (1: 1) with a percent area of 24.638% in samples originating from Bogor, West Java; N- (ethylcarbamoyl) -2 - [(1-methyl-1H*tetrazole-5-yl) sulfanyl] acetamide* with a percentage area of 24.457% in samples from Balikpapan, East Borneo; Lysine sulfate (1: 1) with a percentage area of 27,180% in samples originating from West Kotawaringin, Central Borneo; and *Lysine sulfate (1: 1)* with an area of 26.48% in samples from Banjarmasin, South Borneo.

Based on these data it is also known that E. palmifolia bulb contains the compound isoliquiritigenin and resveratrol. Previous studies reported that isoliquiritigenin could inhibit the growth of breast cancer cells and inhibit neoangiogenesis and suppress the growth of colon cancer cells in induced mice *azoxymethane* (AOM) (Takahashi et al., 2004; Wang et al., 2013). The results of the analysis showed that *isoliquiritigenin* contained in the sample *E. palmifolia* bulb originating from East Java. With a retention time of 9,608; Central Java with a retention time of 5.89; West Java with a retention time of 6.074; East Borneo with a retention time of 5.89; and Central Borneo with a retention time of 5.594; in samples from South Borneo no compounds were isoliquiritigenin found. MS spectra of compounds Isoliquiritigenin presented in Figure 2.

As a result of the spectral analysis have also been found two types *of resveratrol* in samples *E.palmifolia* studied were bulb *Trans-resveratrol* and Oxyresveratrol. *Trans-resveratrol* found in *E. palmifolia* bulb samples as samples originating from West Java with a retention time of 8,452; Central Java with a retention time of 8,452; East Borneo with a retention time of 8,8086; and South Borneo with a retention time of 5,452. In samples

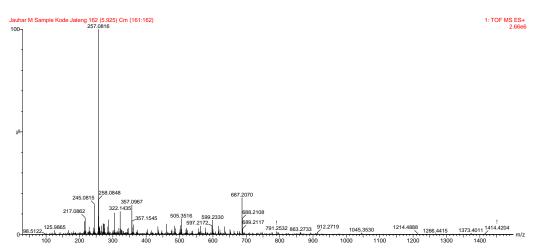


Figure 2. The spectra and structure Compound Isoliquiritigenin (C15H12O4).

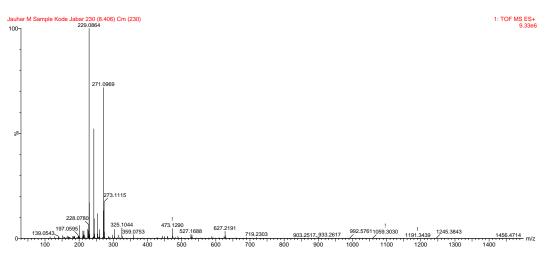


Figure 3. Spectra and structure of compounds *Trans-resveratrol* (C₁₄H₁₂O₃).

from East Java and Central Borneo, no compounds were found *trans-resveratrol. Oxyresveratrol was* found in samples *E. palmifolia* bulb from Central Java with a retention time of 7.39; West Java with a retention time of 6,943; Central Borneo with a retention time of 6,988; and South Borneo with a retention time of 6,988. In samples originating from East Java and Central Borneo, no compounds were *oxyresveratrol found*.

Resveratrol is phytoalexin in the skin of grapes, nuts, and some fruits. It is known for its effects as an antioxidant and anti-inflammatory and inhibits the proliferation of cancer cells (Smoliga *et al.*, 2011). *Resveratrol has* successfully inhibited the growth of tumors tested *in vivo* using several types of cancer, besides that in preclinical studies *resveratrol* has been shown to improve heart health, reduce blood pressure, and lower blood glucose levels (Singh *et al.*, 2014).

The spectra of trans-resveratrol and oxyresveratrol MS presented in Figures 3 and 4.

Analysis PCA Multivariate Data Using Minitab

In simple terms, PCA is a linear transformation to determine the new coordinate system of a dataset. This PCA technique reduces or reduces information on extensive data to be simpler without removing existing information (Syakhala *et al.*, 2015). PCA visualization results can be displayed through *score plots* and *loading plots*. *Score plots* describe the closeness between objects (samples), while *loading plots* describe relationships between variables, namely original variables and new variables (Taufik, 2017). The results of multivariate PCA data analysis *E. palmifolia bulb* from several locations are presented in Figures 5 and six as follows.



Figure 4. Spectra and structure of compounds *Oxyresveratrol* (C₁₄H₁₂O₄).

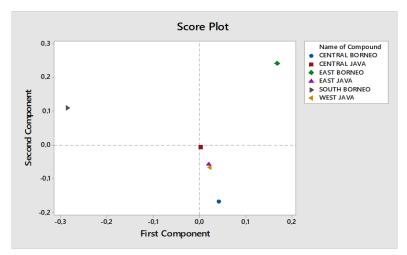


Figure 5. *Score Plot* That Shows The Similarity Of Types Of Compounds From Blitar, East Java With Bogor, West Java.

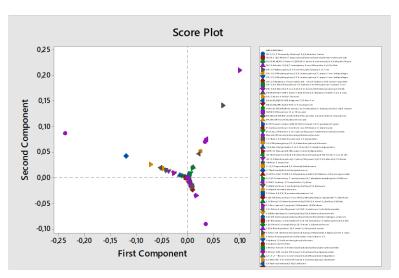


Figure 6. *Score plot* that shows the existence of compounding groups from the six regions.

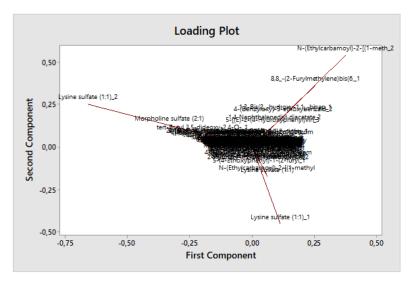


Figure 7. Loading plot that shows several major compounds.

The loading plot in Figure 7 shows the representative compounds of *E. palmifolia bulbs* originating from several regions in Indonesia. There are at least three compounds, namely *Lysine* sulfate (1: 1) yang derived from of sample West Java, Central Borneo and South Borneo; N-(ethylcarbamoyl) -2 - [(1-methyl-1H-tetrazole-5-yl) sulfanyl] acetamide obtained from samples from East Java, West Java, and East Borneo; and 8.8'- (2-Furyl methylene) bis (6,10-dioxaspiro [4.5] decane-7,9-dione) originating from samples from West Java, East Borneo, Central Borneo and South Borneo. One of the three compounds, Lysine sulfate, has excellent benefits for the body. Lysine which is a group of amino acids has an essential role in the immune system because it functions to develop antibodies. The scientists found that Lysine has antiviral activity, increases the effectiveness of L-arginine in promoting the release of human growth hormone (HGH), and can help reduce excessive anxiety (Smriga et al, 2007; Suminski et al., 1997).

Several factors cause the compound content in plants to be similar. These factors can be classified into two, namely internal factors such as genetic and physiological variations, as well as external factors such as geographical conditions (altitude), climate, humidity, light intensity, temperature, nutrient intake, and radiation (Verma N, Shukla S, 2015). The area Blitar, East Java with Karanganyar, Central Java, has a similar content of compounds thought to be caused by external factors. Previously in table I, it was explained about the environmental conditions consisting of altitude, average temperature, rainfall, climate, and soil type of all samples, it is known that the Blitar area, East Java with Bogor, West Java has an average temperature equation (^oC) and kind of soil, namely regosol.

CONCLUSION

The results showed that there were differences in the content of the metabolite compounds in bulb E. palmifolia originating from six different regions. There were 40 compounds from the East Java sample, 32 compounds from the Central Java sample, 39 compounds from the West Java sample, 34 compounds from the East Borneo sample, 38 compounds from the Central Borneo sample, and 31 compounds from the South Borneo sample, some major compounds found were isoliquiritigenin, trans-resveratrol, and oxyresveratrol.

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