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Metabolite Profiling of *Davallia* in The Mentawai Islands, West Sumatra, Indonesia

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ABSTRACT
This study revealed the metabolite compounds of the *Davallia* species in the Mentawai Islands, West Sumatra, through metabolite profiling. This study aimed to determine the chemical compounds in the leaves of the *Davallia* species. Leaf samples of three species, *D. denticulata* (Burm. f.) Kuhn var. *denticulata*, *D. heterophylla* Sm., and *D. solida* (G. Forst.) Sw. var. *solida* were collected from the Mentawai Islands. Metabolite characterization used the Gas Chromatography-Mass Spectrometry (GC-MS). The results were obtained in 25 compounds originating from 15 accessions belonging to six metabolite compounds. The compounds found included 13.04% hydrocarbons, 26.09 % fatty acids, 8.7% benzenoids, 8.7% tocopherols, 13.4% phytosterols, 17.39% terpenoids and 13.04% fatty acids ethyl esters. The most identified compounds was fatty acids. The detected compounds are potential as drug ingredient in pharmacology.

Keywords: *Davallia, GC-MS, Metabolomics, Terpenoids*

1. INTRODUCTION

Davallia is a genus of Davalliaceae, which lives as an epiphyte on host plants [1]. Oil palm (*Elaeis guineensis* Jacq.) and Sengon (*Albizia Chinensis* (Osbeck) Merr.) are the most suitable host plants as habitats for *Davallia*. *Davallia* was able to grow in a variety of habitats, from lowlands to highlands. This genus is found growing in conservation areas on the islands of Mentawai Islands regency [2]. The Mentawai islands are part of the range of islands in the western part of Sumatra [3]. The Mentawai Islands consist of Siberut, Sipora, North Pagai, and South Pagai [4]. The island is thought to have separated from mainland Sumatra more than 500,000 years ago [5]. Flora and fauna in this area are thought to have originated from the early Sundaland communities. In addition, the plant species in this area have partially evolved into different forms of the living communities found in mainland Asia [6].

A study of the active chemical compounds of several *Davallia* species used in traditional medicine in China reported the presence of antioxidant activity and high polyphenol content from the rhizomes of six fern species, *Drynaria fortunei* (Kaze.) J. Sm., *Pseudodrynaria coronans* (Wall. Ex Mett.) (Polypodiaceae), *Davallia divaricata* Bl., *Davallia mariesii* Moore ex Bak, *Davallia solida* (Forst.) Sw., and *Humata griffithiana* (Hk.) C. Chr., which is used as traditional medicine in Taiwan [7]. Using 2,2-diphenyl-1-picrylhydrazyl (DPPH) analysis also showed that the ethanolic extract of *Davallia solida* had the highest activity. Fresh rhizome extract of *Davallia formosana* significantly inhibits osteoclast differentiation and has therapeutic potential for treating osteoclast bone disease

[8]. The results of this study indicated that ferns in the genus *Davallia* contain metabolites that are useful as antioxidants and have potential as ingredients for herbal medicines [9].

The content of active chemical compounds, which are the result of metabolism in plants, has been widely studied but is still very limited to species of ferns of the genus *Davallia*. Complete information on the content of active chemical compounds can be disclosed using the metabolomic studies. Metabolite profiling is a form of analysis that quantifies and identifies metabolites in cells, tissues, and biological fluids [10]. The metabolomic profile is important information related to pharmacology in plants.

Differences in organs can cause variation in biosynthesis so that the chemical compounds in each organ will also be different [11]. Based on the above background, it is necessary to analyze the content of active chemical compounds with metabolite profiles in *Davallia* using different organs, species, and locations from previous studies. In this study, fresh leaf organs were collected directly from four islands in the Mentawai Islands. This study aimed to reveal the diversity of the metabolite content in *Davallia* species growing in the Mentawai Islands, West Sumatra, by analyzing their metabolomic profiles.

2. METHODOLOGY

This research was carried out through exploration activities in four islands in the Mentawai Islands, consisting of Siberut Island, Sipora Island, North Pagai Island, and South Pagai Island, located in the Mentawai Islands Regency, West Sumatra Province, Indonesia (Figure 1) Furthermore, the GC-MS analysis for metabolomic profiling was carried out at the Regional Health Laboratory (LABKESDA) Jakarta. This research was conducted from August to December 2020.

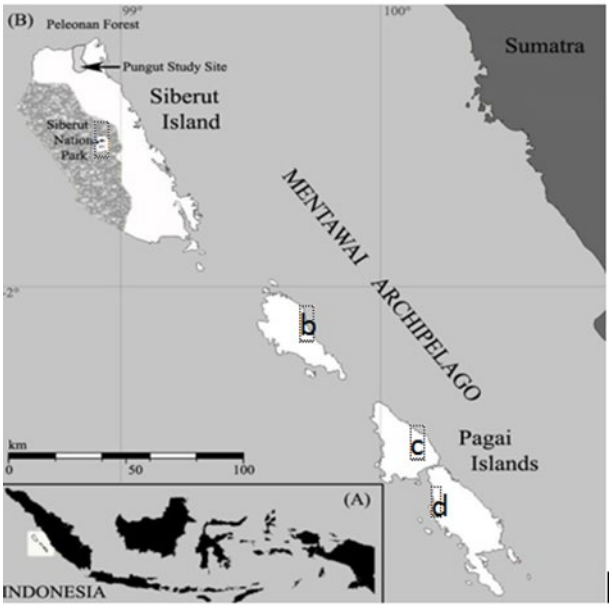

Figure 1. Location of *Davallia* collection in Mentawai Islands a. Siberut Island, b. Sipora Island, c. North Pagai Island and d. South Pagai Island

Data collection begins with sample collection directly in the field. A total of 15 accessions represented three species in the genus *Davallia* consisting of *D. denticulata* (Burm. f.) Kuhn var. *denticulata, D. heterophylla* Sm., and *D. solida* (G.Forst.) Sw. var. *solida* was collected from four islands in the Mentawai Islands (Figure 2). Sampling for metabolomic analysis was carried out by taking fresh leaves of *Davallia* species in the field, then put in plastic. The preparation of plant material was carried out by taking fresh *Davallia* leaves and then washing them thoroughly, then

Figure 2. *Davallia* spesies **e.** *D. denticulata* (Burm. f.) Kuhn var. *denticulata,* **f.** *D. heterophylla* Sm., **g.** *D. solida* (G.Forst.) Sw. var. *solida*

as much as 100-200 g were coarsely ground. Furthermore, the sample was directly distilled using the hydrodistillation method.

Davallia's metabolomic profile analysis was carried out in several stages. First, leaves of three *Davallia* species from several locations in the Mentawai Islands were extracted following [12] using absolute ethanol. Second, the extracted fraction was used for metabolite characterization by untargeted metabolomics analysis using *Gas Chromatography-Mass Spectrometry* (GC-MS). The GC-MS analysis was carried out using Agilent Technologies 7890 Gas Chromatography with Autosampler and selective MS detector Agilent 5975 (Agilent Technologies) with an Agilent HP ultra two capillary column (30 m length, 0.2 mm diameter).

Metabolite compound data from the GC-MS analysis were selected based on the Pressure Cycling Technology (PCT) area, which has a percentage of 80%, then the data were analyzed using the R version 3.4.4 program with the metabolomics package (http://www.r-project.org/). The metabolomics package was used to perform hierarchical group analysis compiling heatmaps and PCA. The Heatmaps were used to visualize large amounts of metabolite data and their differences in sample groups [13]. The metabolites were determined based on databases at Chemspider and PubChem (National Center for Biotechnology Information).

3. RESULTS AND DISCUSSION

This research was conducted using the GC-MS approach, which is one of the methods for determining the quality and quantity of chemical compounds with isolation, purification, and determination of the structure of standardized natural metabolites using plant extracts. The GC-MS instrument is better than LC-MS because it can better display metabolites with a *more comprehensive Physico-chemical* range [14]. Thus the study of the content of metabolomic compounds carried out in plants will provide information about the benefits of these plants in the pharmacological world.

The metabolomic analysis (*metabolite profiling*) carried out in three *Davallia* species, *D. denticulata* (Burm. f.) Kuhn var. *denticulata, D. heterophylla* Sm., and *D. solida* (G.Forst.) Sw. var. *solida.* This study has revealed the content of metabolites contained in plant

Table 1. Metabolites from non-targeted metabolic analysis using GC-MS on 15 accessions of *Davallia*

Code	Metabolite	Formula Molecular	Molecule Weight	PubChem CID	Group
C10	11,13-Dimethyl-12-tetradecane-1-ol acetate	C18H34O2	283	549821	Hydrocarbons
C19	2-(((2-Ethylhexyl)oxy)carbonyl) benzoic acid	C16H22O4	278	20393	Hydrocarbons
C39	9,12-Octadecadienoic acid	C18H32O2	280	3931	Fatty acids
C41	9-Octadecenoic acid, ethyl ester	C20H38O2	311	5364430	Fatty acids
C43	Benzoic acid	C7H6O2	122	243	Benzenoid
C45	beta-Tocopherol	C28H48O2	417	6857447	Tocopherols
C49	Campesterol	C28H48O	401	173183	Phytosterols
C54	Clionasterol	C29H50O	415	457801	Phytosterols
C60	delta-Iraldeine	C14H22O	206	5372195	Others
C61	Diploptene	C30H50	411	92155	Terpenoid
C64	Dodecahydro-as-indaceno[4,5-b]oxirene	C12H18O	178	549006	Others
C67	Ergot-5-en-3-ol	C28H48O	401	18660356	Phytosterol
C71	Ethyl linoleate	C20H34O2	307	5367460	Ethyl ester fatty acids
C72	Ethyl oleate	C20H38O2	311	5363269	Ethyl ester fatty acids
C73	Ethyl palmitate	C18H36O2	285	12366	Ethyl ester fatty acids
C81	Linoelaidic acid	C18H32O2	280	5282457	Fatty acids
C82	Linolenic acid	C18H30O2	278	5280934	Fatty acids
C87	Neophytadiene	C20H38	279	10446	Terpenoid
C91	Palmitic acid	C16H32O2	256	985	Fatty acids
C94	Phthalic acid	C8H6O4	166	1017	Benzenoid
C95	Phytol	C20H40O	297	5280435	Terpenoid
C98	Squalene	C30H50	411	638072	Terpenoid
C105	trans-13-Octadecenoic acid	C18H34O2	283	6161490	Fatty acids
C106	Tridecanedial	C13H24O2	212	544162	Hydrocarbons
C107	Vitamin E	C29H50O2	431	14985	Tocopherol

tissues of *Davallia*. Based on the interpretation of the chromatogram data from the GC-MS metabolomic analysis originating from several locations in the national park in the Mentawai Islands, the class of metabolite compounds of species in the genus *Davallia* is presented in Figure 3. The class of compounds consists of hydrocarbons 13.04%, fatty acids 26.09%, benzenoids 8.7%, tocopherols 8,7%, phytosterols 13.4%, terpenoids 17.39% and fatty acids ethyl esters 13.04%. The most identified number of compounds was the fatty acids class of 26.09% of the total identified metabolites. The metabolites identified in species in the genus *Davallia* were 25 compounds from 15 accessions of *Davallia* found in the Mentawai Islands (Table 1).

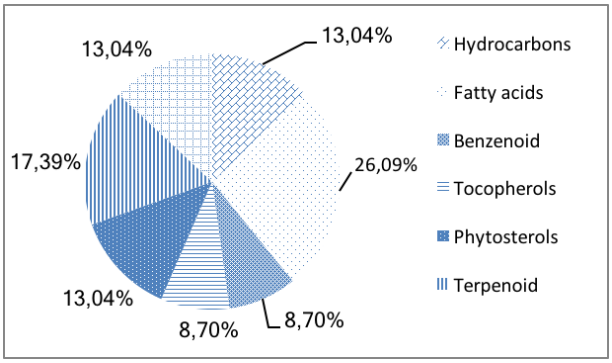

Figure 3. Class of compounds detected in the leaves of species of *Davallia* found in the Mentawai Islands.

The classes of hydrocarbons compounds which amount to about 13.04%, include 11,13-dimethyl-12-tetradecane-1-ol acetate, 2-(((2-Ethylhexyl)oxy)carbonyl) benzoic acid and tridecanedial. This compound can be used as a source of fuel or chemical raw materials, especially for the use of plant biomass [15]. The classes of fatty acid compounds, which amount to about 26.09%, include 9,12-octadecadienoic acid, linoelaidic acid, 9-octadecenoic acid ethyl ester, linolenic acid, palmitic acid, and trans-13-octadecenoic acid. These compounds are also found in ferns *Aglaomorpha quercifolia* (L.) Hovenkamp & S. Linds. which is widely used as traditional medicine [16]. The fatty acid compounds in ferns are also known as valuable sources of essential fatty acids [17]. The class of benzenoid compounds, which account for about 8.7% of their compounds, are benzoic acid and phthalic acid. This compound was also found in the fern *Cheilanthes farinosa* (Forssk) Kaulf (Pteridaceae) [18].

The classes of tochoperols compounds, which amount to about 8.7%, include beta-tocopherol and vitamin e. These compounds contain molecular substances in the form of antioxidants that can prevent various cellular target molecules from oxidative damage (Halder1 and Chakraborty 2018) [9]. The phytosterol class of compounds which amounts to about 13.04% of its compounds was campesterol, clionasterol and Ergot-5-en-3-ol. In general, herbal plants such as seed plants, ferns, algae, phytoplankton, and unicellular eukaryotes that are rich in phytosterols have anti-inflammatory activity [19]. The class of terpenoid compounds, which amount to about 17.39% of them are diploptene, neophytadiene, phytol, and squalene. Terpenoids are a class of compounds commonly found in medicinal plants [20]. The classes of fatty acid ethyl ester compounds, which amount to 13.04% of the compounds, are ethyl linoleate, ethyl oleate, and ethyl palmitate. These compound are also found in the plant *Stenochlaena palustris* in the form of ethyl linoleic compounds ranging from 2.57% [21].

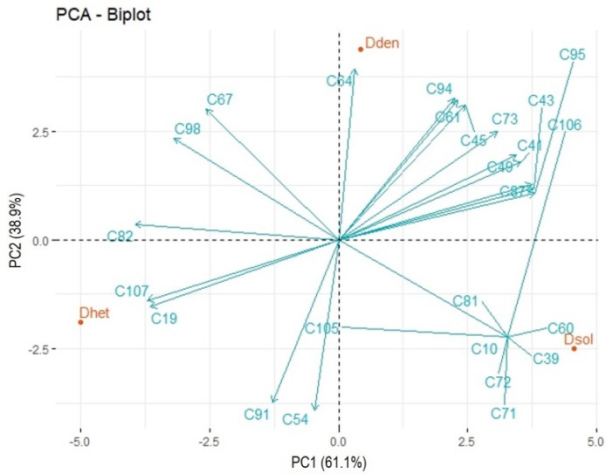

Figure 4. Results of analysis of the main components of leaf metabolites of *Davallia* based on species found in the Mentawai Islands. Dden = *D.denticulata* (Burm. f.) Kuhn var. *denticulata,* Dhet = *D. heterophylla* Sm., and Dsol = *D. solida* (G.Forst.) Sw. var.*solida*.

Principal Component Analysis on three *Davallia* in the Mentawai Islands, West Sumatra using GC-MS can reveal the metabolites' grouping in these plant species. Figure 4 shows the compounds correlated with the species found. However, some compounds are not correlated with the species found. The number of compounds used in this study was 25 compounds that comes from compounds occured twice in each of the analyzed data. This step was done to get the best grouping picture based on PCA analysis. The cumulative value of the resulting PCA analysis reached 100%, with PC1 of 61.1% and PC2 of 38.9%. This result shows that PCA is only able to explain 100% of the total variation.

Figure 4 also shows that each *Davallia* has a characteristic compound. The species identifying compounds found were 11,13-Dimethyl-12-tetradecen-1-ol acetate, 9,12-Octadecadienoic acid, delta-Iraldeine, Ethyl linolenate, Ethyl linolenate, Ethyl oleate, Linoelaidic acid, Neophytadiene, trans-13-Octadecenoic acid, and Tridecanedial. These compounds were predominantly found in *D. solida*

(G.Forst.) Sw. var. *solida*. The compounds have the highest contribution value using PCA analysis and seen in the longitudinal direction. Contrast to *D. solida* (G.Forst.) Sw. var. *solida*, *D. denticulata* (Burm. f.) Kuhn var. *denticulata* and *D. heterophylla* Sm. have PCA values in different dimensions, which is caused by differences in their metabolites. Both species having PCA values in opposite dimensions where *D. denticulata* (Burm. f.) Kuhn var. *denticulata* is in the northern part of the central axis, while *D. heterophylla* Sm. is to the west of the central axis. This data explains that different species have different content of metabolite compounds [22], so the species that have many compounds in common will combine into a separate group.

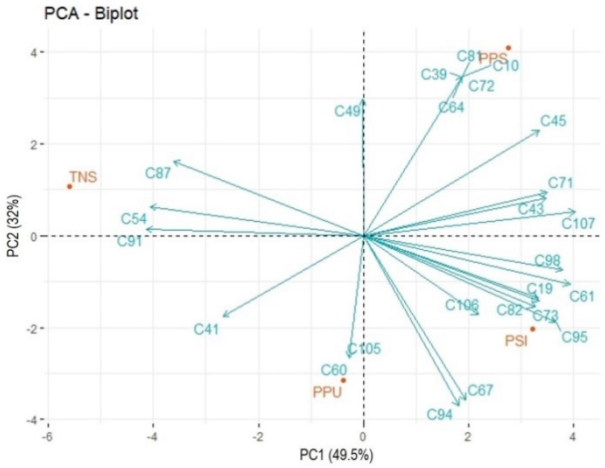

Figure 5. Results of analysis of the main components of leaf metabolites of *Davallia* based on the location of species in the Mentawai Islands. PPS = South Pagai Island, PPU = North Pagai Island, PSI = Sipora Island, TNS = Siberut National Park.

In this study, the result of PCA analysis (Figure 5) showed the presence of compounds that correlated with the location of the species found. However, there are also some compounds that are not correlated to their origin sites. The cumulative value of PCA analysis reached 81.5%, with PC1 at 49.5% and PC2 at 32.0%. PCA is able to explain 81.5% of the total variation. The metabolites that were not grouped according to the location found were 9-Octadecenoic acid, ethyl ester, Palmitic acid, Campesterol, Clionasterol, beta-Sitosterol, and Neophytadiene. The data indicate that these compounds are not specific to one area only.

Based on the sampling location, there were metabolite variations from different areas. However, several metabolites found at several sampling locations were similar. Some areas where have the same metabolite compounds include North Pagai Island and Sipora Island Island. Differences in the metabolite content of *Davallia* species between locations indicate the response of each species to different environmental conditions to adapt. Several environmental factors that affect differences in the metabolite contents are the availability of groundwater, temperature, and light intensity [23].

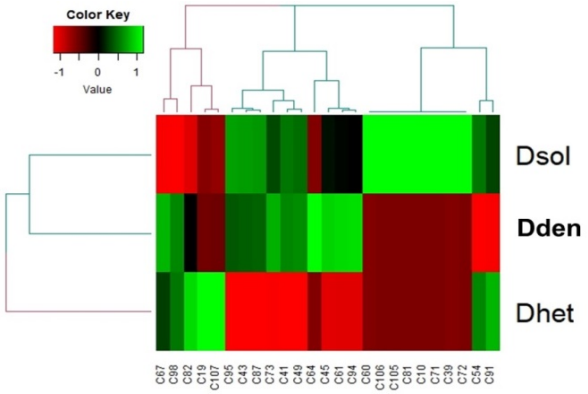

Figure 6. Heatmap of metabolites using GC-MS on 15 accessions of *Davallia,* reviewed by species. Dden = *D. denticulata* (Burm. f.) Kuhn var. *denticulata,* Dhet = *D. heterophylla* Sm. and Dsol = *D. solida* (G.Forst.) Sw. var. *solida*.

Analysis of metabolites using the R ver program. 1.1.442 with the metabolomics package in *Davallia* showed a clear division of groupings between *Davallia* species. The grouping differences result when the heatmap was combined with hierarchical cluster analysis (Figure 6). The results were presented in a heatmap picture that describes the types and concentrations of the metabolite compounds, and the appeared species. Based on the heatmap formed, the color difference determines the concentration of the metabolite compounds. Metabolite compounds having a lighter green color can be interpreted having a higher concentration than other compounds.

Based on metabolomic analysis using heatmap, it is known that species diversity factors affect the concentration of metabolites found in *Davallia.* Based on the color on the heatmap, it can be seen that the compound which has a higher concentration is found in *D. solida* (G.Forst.) Sw. var. *solida* include delta-Iraldeine, Tridecanedial, trans-13-Octadecenoic acid, Linoelaidic acid, 11,13-Dimethyl-12-tetradecane-1-ol acetate, Ethyl linolenate, 9,12-Octadecadienoic acid, and Ethyl oleate. Compounds having higher concentrations were found in *D. denticulata* (Burm. f.) Kuhn var. *denticulata* is Dodecahydro-as-indaceno[4,5-b]oxirene. Compounds having higher concentrations found in *D. heterophylla* Sm. are 2-(((2-Ethylhexyl)oxy)carbonyl)benzoic acid and Vitamin E. The metabolites found in these three species also have different concentrations.

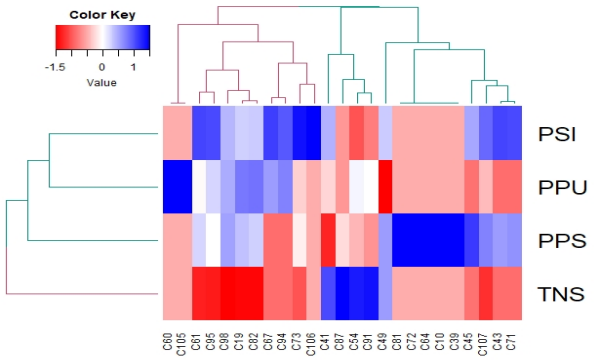

Figure 7. Heatmap of metabolomics using GC-MS on 15 accessions of *Davallia* reviewed by location. PPS = South Pagai Island, PPU = North Pagai Island, PSI = Sipora Island, TNS = Siberut National Park (Siberut Island).

The heatmap of the results of hierarchical group analysis based on location found non-targeted metabolomic compounds using GC-MS in 15 species of *Davallia* in the Mentawai Islands, West Sumatra, is presented in Figure 7. The results of the metabolomics package analysis show a clear group division between sampling locations. The formed heatmap describes the type of compound, its concentration, and the area where the compound appears. Based on the heatmap formed, metabolite compounds with a darker blue color indicate higher concentrations than other compounds. The compounds delta-Iraldeine and trans-13-Octadecenoic acid had the highest concentrations at four locations, namely South Pagai Island, North Pagai Island, Sipora Island.

Results of leaf extract analysis using GC-MS from 15 accessions representing *D. denticulata* (Burm. f.) Kuhn var. *denticulata, D. heterophylla* Sm., and *D. solida* (G.Forst.) Sw. var. *solida* found in the Mentawai Islands succeeded in identifying as many as 25 compounds. The class of compounds consists of hydrocarbons 13.04%, fatty acids 26.09%, benzenoids 8.7%, tocopherols 8.7%, phytosterols 13.4%, terpenoids 17.39% and fatty acids ethyl esters 13.04%. The characteristic compounds in *Davallia* found in the Mentawai Islands are 11,13-Dimethyl-12-tetradecen-1-ol acetate, 9,12-Octadecadienoic acid, delta-Iraldeine, Ethyl linolenate, Ethyl linolenate, Ethyl oleate, Linoelaidic acid, Neophytadiene, trans-13-Octadecenoic acid, and Tridecanedial. The compounds found have potential as medicinal ingredients such as antioxidants and anti-inflammatory based on pharmacological studies.

AUTHORS' CONTRIBUTION

The authors confirm contribution to the paper as follows: study conception and design: Mildawati (A), and Tatik Chikmawati (B); data collection: A; analysis and interpretation of results: A, B, Sulistijorini (C), and Sobir (D); draft manuscript preparation: A and B. All authors reviewed the results and approved the final version of the manuscript.

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REFERENCES

[1] H.P. Nooteboom, Flora Malesiana, Series 2. Ferns and Ferns Allies. Vol III. Rijksherbarium/Hortus Botanicus. Leiden. Neitherland. 33, 1998, pp. 44-48

[2] Mildawati, Sobir, Sulistijorini, T. Chikmawati, The Diversity of Pteridophytes in Siberut National Park, Mentawai Islands, West Sumatra, Indonesia, Biodiversitas vol 21(7), 2020, pp. 3200-3208.

[3] R.P.J. de Kok, M. Briggs, D. Pirnanda, and D. Girmansyah, Identifying targets for plant conservation in Harapan rainforest, Sumatra, Tropical Conservation Science vol. 8(1), 2015, pp. 28-32.

[4] A.J. Whitten, J. Damanik, Anwar, N. Hisyam, The Ecology of Sumatera, Gadjah Mada University Press, Yogyakarta, 2000.

[5] H.T. Verstappen, On palaeoclimates and landform development in Malaysia. In: G.J. Bartstra & W. A. Caspari (eds.) Modern Quaternary Research in Southeast Asia, Rotterdam, Balkema, 1975.

[6] C.T. Roos, J.K. Ziegler, H. Hodges, C. Zischler, Abegg, Molecular phylogeny of Mentawai macaques: taxonomic and biogeographic implications, Molecular Phylogenetics and Evolution vol. 29, 2003, pp. 139-150.

[7] H.C. Chang, G.J. Huang, D.C. Agrawal, C.L. Kuo CL, C.R. Wu and H.S. Tsay, Antioxidant activities and polyphenol contents of six folk medicinal ferns used as "Gusuibu." Botanical Studies, 48, 2007, pp. 397-406.

[8] T.H. Lin, R.S. Yang, K.C. Wang, D.H. Lu, H.C. Liou, Yunma, S.H. Chang, and W.M. Fu, Ethanol Extracts of Fresh *Davallia Formosana*. Inhibit Osteoclast Differentiation By Suppressing Rankl-Induced Nuclear Factor-κb Activation. Hindawi Publishing Corporation Evidence-Based

Complementary And Alternative Medicine. Volume 2013, 2013. DOI: http://dx.doi.org/10.1155/2013/647189H.

[9] K. Halder and S. Chakraborty, An account of Antioxidant Potential in Pteridophytes : A Biochemical Perspective Intl. J. Bioinformatics and Biological Sci., vol. 6, no.1, 2018, pp. 15-24.

[10] K.M. Lee, J.Y. Jeon, B.J. Lee, H. Lee, H.K. Choi, Application of Metabolomics to Quality Control of Natural Product Derived Medicines, Biomolecules and Therapeutic, 2017, pp. 559-568.

[11] F.N. Maslakhah, R. Mutiah, A. Hakim, R. Aprinda, & A. Suryadinata, Metabolite Profiling Bagian Akar, Batang, Daun, dan Biji *Helianthus annuus* L. Menggunakan Instrumen UPLC-MS, MPI (Media Pharmaceutica Indonesiana), vol. 2(2), 2019, pp. 64-81. DOI:https://doi.org/10.24123/mpi.v2i2.1361

[12] J. Lisec, N. Schauer, J. Kopka, L. Willmitzer, A.R. Fernie, Gas chromatography-mass spectrometry-based metabolite profiling in plants, Nat Protoc., vol. 1(1), 2006, pp.387-96.

[13] X. Huang, Y.J. Chen, K. Cho, I. Nikolskiy, P.A. Crawford, and G.J. Patti, X13CMS: global tracking of isotopic labels in untargeted metabolomics. Anal. Chem., vol. 86, 2014, pp. 1632–1639.

[14] A. Cuadros-Inostroza, S. Ruíz-Lara, E. González, A. Eckardt, L. Willmitzer, H. Peña-Cortés, GC–MS metabolic profiling of Cabernet Sauvignon and Merlot cultivars during grapevine berry development and network analysis reveals a stage- and cultivar dependent connectivity of primary metabolites, Metabolomics vol. 12, 2016, pp. 1–17.

[15] D. Kalita, Hydrocarbon Palnt-New souce of energy future, Renewable and Sustainable Energy Reviews vol. 12, 2008, pp. 455–471. DOI:10.1016/j.rser.2006.07.008

[16] H.R. Costa, I. Simão, H. Silva, P. Silveira, A.M.S. Silva, D.C.G.A. Pinto, *Aglaomorpha quercifolia* (L.) Hovenkamp & S. Linds a Wild Fern Used in Timorese Cuisine, Foods, vol. 10, 2021, pp. 87. DOI: https://doi.org/10.3390/ foods10010087

[17] A.R. Genrich, Fatty acid composition of fern spore lipids, Phytochemistry vol. 16, 1977, pp :1044-1046.

[18] P.N. Ghorpade, M.V. Kale, S.B. Thakar, Phytochemical Analysis Of Bioactive Components *Cheilanthes Farinosa* (Forssk.) Kaulf. Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences vol. 1(1) No. 33, 2015.

[19] S. Darnet, A. Blary, Q. Chevalier, and H. Schaller, Phytosterol Profiles, Genomes and Enzymes – An Overview, Front Plant Sci., vol. 12, 2021, pp. 665206. DOI: 10.3389/fpls.2021.665206

[20] N. Saeed, M.R. Khan, M. Shabbir, Antioxidant activity, totalphenolic and total flavonoid contents of whole plant extracts Torilisleptophylla L., BMC ComplementAltern Med., vol. 12(1), 2012, pp. 1–12.

[21] Erwin, D. Anggeraini, and Suryani, Chemical analysis and antibacterial activity of the ethanolic extract of *Stenochlaena palustris,* Der Pharmacia Lettre, vol. 8 (1), 2016, pp. 233-236.

[22] A. Gargallo-Garriga, J. Sardans, V. Granda, J. Llusià, G. Peguero, D. Asensio, R. Ogaya, I. Urbina, L.V. Langenhove, L.T. Verryckt, J. Chave, E.A. Courtois, C. Stahl, O. Grau, K. Klem, O. Urban, I.A. Janssens, and J. Peñuelas, Different "metabolomics niches" of the highly diverse tree species of the French Guiana rainforests. Scientific Reports vol. 10, 2020, pp.6937

[23] V. Arbona, M. Manzi, C.D. Ollas, A. Gomez-cadenas, Metabolomics as a tool to investigate abiotic stress tolerance in plants, Int. J. Mol. Sci., vol. 14(3), 2013, pp. 4885– 4911.