The Secondary Metabolite Diversity Analysis of Three 
Mangifera Foetida L. Varieties Based on Liquid Chromatography-Mass Spectrometry (LC-MS)

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Abstract. Mangifera foetida L. (Macang) is the type of mangoes that contains the highest levels of mangiferin which has activity of antioxidant, analgesic, anti-drip, anti-inflammatory, antitumor, immunomodulatory and anti-HIV. The potential of Macang as a drug needs to be assessed through phytochemical studies to obtain information on the diversity of Macang secondary metabolite content that is potential to be developed. The purpose of this study was to reveal the diversity of total secondary metabolite compounds contained in Macang with three varieties (Limus, Batu and Manis) using LC-MS analysis. Sampling was carried out use survey method at Remban and Lesung Batu, Kecamatan Rawas Ulu, Kabupaten Musi Rawas Utara, South Sumatra Province. The chromatogram was analyzed using the MassLynx program to obtain the compounds contained in the sample. Names determination of compounds based on Chemspider database and compound classes based on PubChem. The number of metabolite compounds that become characteristic of three Macang varieties in this study was 667 compounds. Limus has a specific compound of 191 compounds, Batu of 162 compounds, and Manis has a specific compound of 202 compounds. The metabolites found in this study are expected to be useful in phytopharmaca and support Macang conservation efforts that rarely found.

1. Introduction

Macang (Mangifera foetida L.) belongs to the family members of Anacardiaceae [1]. Macang grows and spreads in Malaysia, Philippines, Brunei, Vietnam, Cambodia, Laos, India, Pakistan, China, New Zealand and Indonesia [2]. The distribution of Macang in Indonesia covers the western part of Indonesia (Sumatra, Kalimantan and Java). Specifically on the island of Sumatra, M. foetida is widespread in almost all of mainland Sumatra which is also known as a cosmopolitan species [3]. This species has a high ability to grow (vigor), and the ability to adapt widely, so that it can be found growing well from an altitude of 0-2000 m above sea level.

Adaptability in Sumatra's wet tropical climate with varying heights makes this species one of the most abundant species in Sumatra, which is distributed from forests to smallholder estates. Although this species is found abundant in nature and has a Lower Risk/Least Concern status [4], it is currently under threat of extinction. The old trees of this species have the potential to be used as boards as building raw materials [5]. Society interest in this potential is higher than the potential development of
fruit cultivation, and this condition can certainly be a serious threat to the sustainability of the existence of this species in nature. In addition, the threat of loss of Mangifera foetida L. germplasm continues to increase along with the rapid deforestation occurring in Indonesia, especially in Sumatra, which is around 268,000 ha / year or 22.8% of the total deforestation in Indonesia [6].

In contrast to the cultivation of mango varieties are highly diverse, and maintained continuity. Macang is considered not so important to be preserved, even though it has superior qualities because not all Macang have a sour taste and some are sweet. In addition, this type is more resistant to pests and diseases [7]. Even among the types of mangoes, Macang is the type that contains the highest levels of mangiferin, about 2.56% higher than other types [5]. Mangiferin has antioxidant, analgesic, anti-drip, anti-inflammatory, antitumor, immunomodulatory and anti-HIV activities [8].

The potential of Mangifera foetida L. as a drug needs to be assessed through phytochemical studies to obtain information on the diversity of Mangifera foetida L. secondary metabolite content that is potential to be developed. Therefore, this research is important to be carried out in order to reveal the diversity of total secondary metabolite compounds contained in Macang. Thus, the existence of this plant can be maintained because humans know the benefits and how to use them. Study of diversity of metabolite content in plants can be done through metabolomic approaches. This approach is used to study the correlation between bioactivity and chemical profiles to identify bioactive components contained in plants [9]. According to [10], metabolomics can analyze and detect as many metabolites as possible contained in a sample at one time.

One analysis technique is Liquid Chromatography-Mass Spectrometry (LC-MS) [11]. This technique is used for the dereplication of natural substances, bioaffinity screening, in vivo screening, metabolite stability, metabolite identification, identification of purity of a drug, identification of degradants, quality control and quantitative bioanalysis of a drug [12]. This technique is very supportive in metabolomic studies because its use is not limited by volatility or stability of the sample, so that it can analyze all the compounds present in the sample as a whole [13].

2. Methodology

2.1 Materials and extract preparation
The materials used in this study were 50 g of leaf powder per Macang variety (Macang Limus, Macang batu and Macang manis), aquades, acetonitrile, methanol and dichloromethane. The samples of Macang leaves was made into powder using blander. The powder was used for extraction. The process of making Macang leaf extract was done by preparing 50 g of tiger leaf powder, then macerated with methanol and added until submerged. All macerates were collected and evaporated using vacuum rotary evaporator at 50°C until thick extracts were obtained. Then the thick extract is dissolved in water until a dilute solution is formed. Next, the solution was eluted using the OASIS SPE (Solid-Phase Extraction) method to separate the pure extract from the impurities. The solvents used in this process are methanol, acetonitrile and dichlorometan. The extracted obtained will be used for metabolite analysis using LC-MS.

2.2 Identification of Metabolic Compounds Macang Leaf Extract with LC-MS
Identification of metabolites in the sample was carried out with two injections to LC-MS. Extract of the injected sample is 5 μL per injection. The stages of analysis begin by filtering the sample extract using a 0.2 μm syringe filter, then put in a vial bottle. The samples are then injected into the LC-MS system. The resulting data is in the form of chromatograms (LC) and Spectra (MS).

2.3 LC-MS Chromatogram Data Analysis
The result of chromatogram data was processed using the MassLynx program. The stages of analysis begin by confirming the chromatogram in the form of a BPI so that it is easy to read and analyze. Each peak is analyzed one by one to get the spectra that will be used to obtain the molecular formula contained in it. Molecular formulas obtained are then matched with molecular formulas in the database.
Chemotaxonomy is the process of classifying plants based on their chemical content (secondary metabolites). Secondary metabolites can be used as characters in taxonomy because the chemical structures formed and their biosynthetic pathways are generally specific and limited to related organisms [14]. Chemotaxonomy also has an important role in addition to taxonomy. According to [15], chemotaxonomy will be the most useful guide for humans, especially in finding new drugs, development in the industrial field and becoming an important characteristic in the search for superior qualities to produce quality plants mainly because of the compounds contained there in. This chemotaxonomic data strongly supports efforts to conserve plants, whose existence is considered not so important to be preserved, as is the case with Macang.

The results of the survey field in several villages in the North Musi Rawas District showed a decrease in the number of these types, such as in Remban Village, Lesung Batu, Rupit and Rupit Beringin. The average number of individuals is less than five trees in each village. This decline in numbers is increasing rapidly due to the regional expansion process which requires the conversion of land to become regional infrastructure such as road expansion, office construction and public facilities. This makes the Macang easier to destroy because of its unknown benefits. Therefore, disclosure of chemotaxonomy of these varieties is very useful to preserve it, especially because of the belief in the present, namely "Back to Nature" by utilizing phytochemicals in the health field.

3.2 Linkages between the Use of Solvent Types and Detected Compounds
The three samples in this study were analyzed using LC-MS technology with two solvents namely methanol (polar) and dichloromethane (semi-polar). The use of these solvents is based on the nature of the polarity of the compounds to be detected. Polar compounds will dissolve easily in polar solvents, while nonpolar compounds will dissolve easily in nonpolar solvents [16]. According to [17], methanol is a universal solvent that can dissolve polar and nonpolar compounds because it has polar groups (-OH) and nonpolar groups (-CH3). The dichloromethane compounds that are semi-polar can dissolve semi-polar to nonpolar compounds [16]. The results of the analysis show that the methanol solvent provides more information on the content of chemical compounds than dichloromethane solvents. Methanol solvents extract 642 chemical compounds, while dichloromethane solvents extract 25 chemical compounds. This shows that the chemical compounds in the sample are more polar. According to [18], polar solvents will extract more and varied secondary metabolites compared to semi-polar and nonpolar solvents. The polar solvent can extract quaternary alkaloid compounds, phenolic components, carotenoid, tannin, sugar, amino acids, and glycosides.

The results of the chromatogram and spectra analysis of the three varieties using the program MassLynx produced ± 2000 chemical compound molecular formulas, but were listed on the ChemSpider database only 667 (polar to nonpolar compounds). In addition to the limitations in
completing the data on the names of chemical compounds, another difficulty is the grouping of compounds that have been identified into known classes of compounds, this is due to the lack of or even the absence of scientific publications on certain compounds. **Database-a database** that generally only provide information in the form of compounds and does not mention derivatives of compounds, so that only a few compounds which can be grouped and generally are compounds that have been identified and have been many publications on these compounds [21]. The groups of compounds most found in this study are flavonoids and amino acids (Table 1). Flavonoids are a group of natural phenolic compounds that have activities as antioxidants, antibacterial, antiviral and antimutagenic [22]. Furthermore, namely amino acids, this group is the main component making up proteins that have an important role in the metabolic process [23].

### Table 1. Groups of Compounds Detected in the Extraction of Three Macang Varieties

| Compound Group  | Chemical compounds                                                                 |
|-----------------|-------------------------------------------------------------------------------------|
| Phenylpropanoid | 6,7-Dihydroxycoumarin, Hydroquinone, Cis-Anethole, Scoparone                        |
| Flavonoids      | Chromen, Xanthen, Luteolin dan Liquiritigenin (flavon), Nobiletin (polimetoksiflavan), Quercitrin dan morin (flavon), Daidzein dan Sophoricoside (isoflavan), naringenin (Flavanon), Sophorflavone A, Diosmetin, Davidigenin, Pregnancy |
| Alkaloids       | Piperidin, Isoquinolin, Quolin, Indol, Sparteinsulfat                                 |
| Amino Acids     | Histidin, Threonine, Arginin, Methionine, Valine, Phenylalanine, Isoleusin, Lysyn, Aspartic Acid, Glutamic Acid, Glysine, Alanine, Tyrosine, Argynylglutamate |
| Aromatic        | P-Cymene, 2-Phenoxyanilin, Ethynyl imidazolyl porphyrin, 1,1-Di(2-Pyridinyl)-N,N'-Di(8-Quinolinyl)Methanediamine, 2-(2,4-Dimethoxyphenyl)-N-(4-Phenoxyphenyl)-4-Quinolinecarboxamide |
| Carboxylic Acids| Acetic acid, Phenylhexanoic Acid, Piperidinepropionic Acid                            |
| Essential Oils  | Nootkatone, Damascenone, (Z)-Ligustilide                                             |
| Seskuiterpenoid | Abscisic Acid, Curcumene                                                            |
| Medium-Chain Fatty Acid | 11-Aminoundecanoic Acid                      |
| Nucleic Acids   | 3-Benzyl hypoxanthine                                                               |

#### 3.3 Abundance of Compounds Based on Main Chromatogram

Chemical compounds in plants are generally in the form of components of mixed compounds in large and complex sizes, so that they have a low taxonomic value. Therefore, the components of these compounds must be separated from the mixture to obtain small single compounds which have high variations and are specific in nature. Data from the analysis using LC-MS instruments are in the form of chromatograms and spectra chromatograms with **peaks** Sharp and narrow sharp describe good separation of compounds [24]. Information obtained at this stage is qualitative because it is detected based on **peak** and retention time (Rt) [25]. Therefore, to get the compound quantity accurately, the sample must be analyzed by comparing the chromatogram data obtained with pure standard compound chromatogram data with the same injection volume [26]. Retention time (Rt) is the time span needed by the compound to exit the column until it is detected by the detector [27]. A good chromatographic system is that it can separate components of mixed compounds in a short time [28]. Based on the results of the chromatogram analysis in this study, the retention time passed by all the analytes to be detected by the detector was 23 minutes and the average Rt of the three samples showing good separation of compounds was up to Rt 14.0. The chromatogram data produced showed different chromatogram fragment patterns in the three macarons. It has been able to clearly describe the variation of metabolites of the three varieties.

V1 (Limus) has six peaks main (abundance above 50%) (Figure 1), namely at retention time (Rt) 6.30, 6.79, 7.10, 7.28, 7.52 and 10.21. The highest peaks are at Rt 6.79 with the highest spectra detected as luteolin based on ChemSpider database. Then in V2 (Batu), there are seven peaksmain,
namely at Rt 6.14, 6.30, 6.79, 7.10, 7.26, 7.40 and 7.52. The highest peaks are at Rt 7.10 with Methyl (1S, 4aS, 5R, 7aS)-1-(β-D-glucopyranosyloxy)-7-(hydroxymethyl)-5-\{[(2Z)-3-(4-hydroxyphenyl)-2-propenoyl]oxy\}-1,4a, 5,7-tetrahydrocyclopenta [c] pyran-4-carboxylate as the highest spectra compound (Figure 2). Furthermore V3 (Manis), has six peaks main, namely at Rt 6.12, 6.25, 6.77, 7.08, 7.38 and 10.03. The highest peaks are at Rt 6.25, but the name of the compound with the highest spectra in this Rt is not identified because it is not available in ChemSpider (Figure 3).

3.4 Abundance of Similar Compounds in All Three Macang Varieties Based on Spectra

The same compounds in the three varieties were 27 compounds of a total of 667 compounds (methanol and dichloromethane solvents). The same amount of compound is not enough to describe the similarities of the three varieties, because the abundance of these compounds may be different. This can be seen in Table 2 below. Based on compound abundance data (Table 2), the similarity of compounds with the same abundance in V1 (Limus) and V2 (Batu) are 22 compounds, V1 (Limus) and V3 (Manis) as much as 19 compounds, while V2 (Batu) and V3 (Sweet) are 22 compounds.

In addition there are also differences in abundance among these compounds. Possible causes of differences in the abundance of compounds of each individual plant have been explained by [20], that there are several factors that influence the composition, quality, and quantity of secondary metabolites, including depending on species or varieties, plant origin and climate. Different species or varieties of course will be different processes or metabolic pathways that are passed through due to internal factors (genes). The external factors (environment) will affect the secondary metabolites contained because environmental conditions can trigger stress plant which later can give rise to a response in the form of an increase in the number or production of certain compounds which under normal conditions are little or not produced as an adaptation effort [29].

| Table 2. Percentage of Compound Abundance |
|------------------------------------------|
| **Compound**                     | V1 | V2 | V3 |
|------------------------------------------|
| P-Cymene                                |    |    |    |
| Cis-Anethole                             |    |    |    |
| Ibuprofen                               |    |    |    |
| Loxoprofen                              |    |    |    |
| [(8-Methoxy-4-Methyl)-6-Oxo-6H-Benzoo[C]Chromen-3-Y]Oxy]Acetic Acid |    |    |    |
| Morin                                    |    |    |    |
| Quercitrin                               |    |    |    |
| Isoxepac                                 |    |    |    |
| 2,2-Dimethylchromene                     |    |    |    |
| 4- Allyl-2-Methoxy-3-Methylphenol        |    |    |    |
| 4,7,7-Trimethyl-3-Oxobicycle[2.2.1]Heptane-1-Carboxylic Acid |    |    |    |
| Luteolin                                 |    |    |    |
| Sophoricoside                            |    |    |    |
| Liquiritigenin                           |    |    |    |
| 1,4-Dioxane-2,3-Diy Bis(Phenoxyacetate)  |    |    |    |
| Methyl (1S,4aS,5R,7aS)-1-(β-D-glucopyranosyloxy)-7-(hydroxymethyl)-5-[[2Z]-3-(4-hydroxyphenyl)-2-propenoyl]oxy]-1,4a,5,7a-Tetrahydrocyclopenta[c]Pyran-4-carboxylate |    |    |    |

Description:
- **≥90%**
- **>50%**
- **<50%**
Daidzein
Sophoraflavone A
Nobiletin
3'-Sinapoylsweroside
Methyl 3-[3-Hydroxy-6-(Methoxymethyl)-4-Oxo-4H-Pyran-2-Yl]-3-(6-Methoxy-4-Oxo-4H-Chromen-3-Yl)Propanoate
2-Tert-Butyl-4-Methoxyphenol
4-Methoxyphenyl 4-Butylcyclohexanecarboxylate
2,4-Di-Tert-Butyl-6-(Hydroxymethyl)Phenol
Hexamethylbenzene
(+)-Abscisic Acid
Ethyl 4-[2-(4-Methylphenyl)-4-Oxo-3(4h)-Quinazolinyl]Benzoate
Figure 1. Chromatogram of Limus
Figure 2. Chromatogram of Batu
Figure 3. Chromatogram of Manis

A (m/z: 302.2) Morin
B (m/z: 539) C_{4}H_{8}NO_{3}
C (m/z: 197.2) 6-Amino-1-butyryl-3-methyl-1H-
pyrimidine-
D (m/z: 556.5) 2,4-dione
Methyl[1S,4aS,5R,7aS]-1-[β-0-
glucopyranosyl(1-)-7-
(4-hydroxymethyl)-5-
[(2Z)-3-(4-hydroxyphenyl)-2-
propenoyl]oxy]-1,4a,5,7a-
tetrahydrocyclopenta[4]pyran-4-
carboxylate
E (m/z: 418) C_{9}H_{12}N_{2}O_{3}
F (m/z: 180.2) 2,6-di-(tert-butyl)-4- methoxyphenol
Some secondary metabolites related to the response to the environment were abscisic acid (ABA) contained in the three varieties, pregnane in the Limus variety and (3α, 5α, 16α) -3,16,17-Trihydroxy pregnan-20-one in the Batu variety. ABA is a metabolite compound in the form of a hormone that plays a role in plant defenses from drought stress [30]. This is if it is related to the time of sampling, which is when the climate is in a long dry season, which greatly enables the three Macang varieties to produce ABA as a form of adaptation to the environment. The pregnane and (3α, 5α, 16α) -3,16,17-Trihydroxy pregnan-20-one found in V1 (Limus) and V2 (Batu) are compounds that play a role in reducing pollutants to organic acids, sugars, and some amino acid compounds [31]. The environmental conditions in which these two varieties grow are traffic activities that are thought to trigger the production of these compounds.

Evolution or phytochemical changes are faster than morphology [14]. This is thought to cause variations based on phytochemicals, especially at very high varieties. This is in accordance with the results of the study, namely V1 (Limus) having specific compounds amounting to 191 and V2 (Batu) having specific compounds of 162 compounds. The V3 (Manis) has a specific compound of 202 compounds. The results of the data analysis of chromatograms in this study indicate that there are multiple molecular formula of the three varieties that have been matched with a database showing the results of a compound name that should not be found in all three, such as ibuprofen, loxoprofen, Tert-Butylhydroquinone. These compounds are synthetic compounds commonly found in the phytopharmac and chemical fields. This is thought to occur because these compounds are the isomers of the original compound. According to [32], isomers are compounds of the same molecular weight, but the structure of the compounds changes. The structure of different compounds will differ in their properties and benefits. The limitations of the database make the original compounds very difficult to ascertain, even though they may be key character compounds that characterize the type of Mangifera foetida L.

4. Conclusions

Total metabolites of the three Macang varieties detected by LC-MS were 667 compounds consisting of 642 compounds in methanol and 25 compounds in dichloromethane solvents. These compounds are included in the phenylpropanoid group, flavonoids, alkaloids, amino acids, organic aromatics, carboxylic acids, essential oils, sesquiterpenoids, medium-chain fatty acids and nucleic acids. V1 (Limus) has a specific compound of 191 compounds and V2 (Batu) of 162 compounds. The V3 (Manis) has a specific compound of 202 compounds. The metabolites found in this study are expected to be useful in phytopharmac and can support Macang conservation efforts that rarely found.

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