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Metabolite Profile of Moringa Leaves (*Moringa oleifera* Lam.) from Several Regions in South Sumatra, Indonesia

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ABSTRACT: Moringa (*Moringa oleifera* Lam.) is a medicinal plant which is recognized as having many health benefits and has been widely used as a herbal medicine to treat certain diseases. To make an ingredient into herbal medicine, standardization of testing is required, because the chemical composition of a plant is not always the same. The growth of Moringa in South Sumatra is spread with varying altitudes, ranging from low, medium and high altitudes. Not much is known about the profile of metabolites of Moringa leaves based on the altitude where they grow. The altitude factor is one of the abiotic factors that can affect plant composition. The purpose of this study is to determine the metabolite profile and pharmacological activity of Moringa leaf metabolites based on different growing heights using a non-target metabolomics analysis approach using the GC-MS instrument. Sampling locations were determined using a stratified purposive sampling method based on the height where they grow. It is known that Moringa leaves from Bangun Rejo Village (795 masl) and Masam Bulau Village (570 masl) showed 29 types of metabolites, while Tebing Gerinting Village, Ogan Ilir (35 masl) showed 27 types of metabolites. Each Moringa leaf growing at an altitude of 35 masl showed dominant metabolites Palmitic acid methyl ester and Linolenic acid methyl ester, a height of 570 masl with Linolenic acid methyl ester and Oleic acid methyl ester compounds and a height of 795 masl with Palmitic acid methyl ester compounds and Oleic acid methyl ester, which has bio-activity as an antioxidant, antiandrogenic, antiproliferative, antieczemic, antihistamine, antibacterial, antifungal, hypocholesteromiic and antitumor.

KEYWORDS: Altitude, GC-MS, Metabolite profile, *Moringa oleifera* Lam., Bio-activity.

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INTRODUCTION

Herbal medicine has been widely accepted by the world community in dealing with various diseases. Knowledge, experience and skills regarding medicinal plants have been passed down from one generation to the next for generations. The World Health Organization (WHO) recommends herbal medicines for the maintenance of public health, because herbal medicines have relatively fewer side effects than modern medicines. The use of these herbal medicines must still pay attention to the accuracy of the dosage, the time of use, the method of use, the correctness of the drug, and the accuracy in choosing drugs for certain diseases (Sumayyah and Salsabilla, 2017).

Indonesia has great potential in the supply of herbal medicinal plants. *Moringa oleifera* Lam. Or Moringa with the local name Daun Kelor in Indonesia is a medicinal plant that is recognized as having many health benefits. The use of Moringa leaves can overcome several diseases, such as hyperglycemia, inflammation, bacterial or viral infections, and cancer (Tiloke *et al.*, 2018), as well as inhibit growth disorders caused by exposure to alcohol (Wardani and Suryono, 2019).

The results of the phytochemical tests of Moringa leaf extract showed compounds that act as drugs in treating certain diseases. The phytochemical content of Moringa leaf extract includes alkaloids, flavonoids, saponins, phenols, steroids/tripenoids, and tannins which have a role in maintaining health (Putra *et al.*, 2016). Oka *et al.* (2016) explained that the active compound hexane fraction of Moringa leaf powder extract with GC-MS contains 8 compounds that provide antioxidant effects and belong to the phenol group.

One of the analyzes that can be used to determine the composition of metabolites is by metabolomics analysis. Metabolomics is the total analysis of metabolite compounds in a sample and cells or tissues of an organism (Qi and Zhang, 2014). Metabolomics analysis is a powerful initial approach to determine the metabolite profile of a substance. One approach to

metabolomics analysis is untargeted metabolomics analysis which is widely used as a first step for more in-depth and detailed research. The aim of the analysis is to collect as much information as possible about the metabolites contained in biological samples (De Vos *et al*. 2007). Roessner and Beckles (2009), stated, metabolomics is the latest 'omics' technology with the aim of separating and measuring metabolite compounds resulting from plant biological activity.

Gas Chromatography-Mass Spectrometry (GC-MS) is one of the instruments used to identify metabolites in a material. The working principle of GC-MS is separation with high chromatography and being able to identify the metabolites of a material, such as essential oils, fatty acids, hydrocarbons, lipids, and others (Kaushik *et al.*, 2002). According to Sprakman *et al*. (2011) the advantage of GC-MS is that it has a high level of sensitivity, so it can be used to analyze compounds with small concentrations.

Moringa can grow in lowland areas to highlands, in range 0-1000 masl (Krisnandi, 2014). The composition of Moringa leaf metabolites is thought to be different at different heights where it grows. The growth of Moringa in South Sumatra is spread with varying altitudes, ranging from low, medium and high lands. Therefore, research is needed regarding the profile of Moringa leaf metabolites originating from that area in South Sumatra, to be used as a source of data and information regarding the type and abundance of metabolite compounds, as well as the dominant compounds in Moringa leaf samples from different heights.

MATERIAL AND METHODS

Sampling of Moringa leaves was carried out in several places in South Sumatra, in Tebing Gerinting Village, Ogan Ilir (35 masl), Masam Bulau Village, Lahat (570 masl), and Bangun Rejo Village, Pagar Alam (795 masl) South Sumatra. Moringa leaves methanol extract were analyzed for profile of metabolite

Research procedure

Sampling

Sampling locations were determined using a stratified purposive sampling method based on altitude, at lowland, medium, and high.

Preparation and Making of Simplicia

The obtained Moringa leaves were separated from each stalk. Furthermore, washed with running water, then drained and in the oven with a temperature of 50°C for 3x24 hours. After drying, it was weighed again to determine the dry weight. Then it was mashed using a blender until it became simplicia powder and sifted through a 12 mesh sieve. The refined simplicia powder is put into a jar that has been labeled according to the sampling location.

Extraction

The simplicia powder used was 100 g and 500 ml methanol solvent or a ratio of 1: was macerated for 1x24 hours with methanol solvent which was placed in a dark room at room temperature. After maceration is complete, it is filtered using filter paper, then concentrated using a rotary evaporator at 50°C until a thick extract is obtained.

Analysis of Moringa Leaves Using GC-MS

Sample preparation was carried out using methanol solvent. Moringa leaf methanol extract which had been added with 10 ml of methanol was injected as much as 1µl into the GC-MS according to the instrument method based on the work protocol of GC-MS TraceTM 1310 ISQ

Data Analysis

The results of the identification of metabolites were analyzed by descriptive quantitative analysis in the form of data tabulation. GC-MS results data is in the form of a chromatogram containing a graph complete with a list of detected chemical components, chemical structure, retention time, and area. The profile of metabolites detected in each sample was calculated by calculating the total type and abundance of metabolites. The total abundance of types of metabolites was determined by calculating all the abundances of types of metabolites in each sample of Moringa leaves. Furthermore, the dominant metabolite compound was determined based on the largest percentage of area, then a biosynthetic pathway was traced to the dominant compound using the PubChem, KEGG, ChEBI, PlantCyc, and Spectrabase websites.

RESULTS AND DISCUSSION

Research on Moringa leaf metabolite profiles from several regions in South Sumatra based on differences in altitude, is presented in Table 1 as follows.

Table 1. Moringa leaf metabolite profile, bio-activity based on altitude

Metabolite Compounds	Molecular Formulas	Bio-activities	References	Presence of Metabolites		
				TG	MB	BR
2-Myristynoyl pantetheine	C25H44N2O5S	-	-	+	+	+
N-[5-(1-Cyano-2-furan-2-yl-vinyl)-[1,3,4]thiadiazol-2-yl]-benzamide	C16H10N4O2S	Antitumor	Holmes & Twentyman (1995)	-	-	+
Deoxyspergualin	C17H37N7O3	Antioxidant and anti-inflammatory	Eryanti *et al*. (2011)	+	-	-
Propanoic acid, 3-ethoxy-, ethyl ester	C7H14O3	-	-	-	+	+
Cyclopentanone, 2-methyl	C6H10O	-	-	+	-	-
2-(2 Butynyl)cyclohexanone	C10H14O	-	-	+	-	-
4-(2,4,4-Trimethyl-cyclohexa-1,5-dienyl)-but-3-e n-2-one	C13H18O	-	-	-	+	-
Toluene, m-ethyl-	C9H12	-	-	+	-	-
Benzene, p-dichloro-	C6H14Cl2	-	-	+	-	-
Tertbutyloxyformamide, N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl]-	C14H24N2O2	-	-	-	-	+
(4-Carbomethoxy)benzyl p-toluate	C17H16O4	Antimicrobial, anesthetic, antioxidant, antiseptic, anticancer.	Mary dan Giri (2008)	-	-	+
Benzoic acid, 4-methyl, [4(methoxycarbonyl)phenyl] methyl ester	C17H16O4	-	-	-	+	-
Acetonitrile, (p-hydroxyphenyl)	C8H7NO	Drugs for disorders of the urinary system, antiasthmatics	Subramanian *et al.* (2020)	-	-	+
17-Pentatriacontene	C35H70	-		-	+	-
Methyl 2-ethylhexyl phthalate	C17H24O4	-	-	-	+	-
1-Methoxy-2-propyl acetate	C6H12O3	-	-	-	-	+
Ethanol, 2-(9-octadecenyloxy)	C20H40O2	-	-	-	-	+
Octaethylene glycol monododecyl ether	C28H58O9	-	-	+	-	-

Metabolite Compounds	Molecular Formulas	Bio-activities	References	Presence of Metabolites		
				TG	MB	BR

9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,	C27H44O3	Vitamins, drugs for autoimmune disorders and bone diseases, antidermatitis, anti-inflammatory	Brintha *et al.* (2017)	+	+	+
Methyl 9-cis,11-trans-octadecadienoate	C19H38O2	-	-	+	+	-
9,12,15-Octadecatrienoic acid	C27H52O4Si2	-	-	-	+	-
Methyl N-(N-benzyloxycarbonyl-beta-l-aspartyl)-beta-d-glucosaminide	C19H26N2O10	Alzheimer's disease drug	Rajmoahamed *et al.* (2017)	+	+	+
Linolenin, 1-mono	C21H36O4	-	-	+	-	-
Linolenic acid, methyl ester	C19H34O2	Hypocholesterolemic, antiandrogenic, antiacne, antihistamine, antieczemic, antiproliferative, antifungal	Nishanthini *et al.* (2014)	+	+	+
Oleic acid, 3-(octadecyloxy)propyl ester	C39H76O3	-	-	+	+	+
Oleic acid, methyl ester	C19H36O2	Antibacterial, antitumor	Teh *et al*. (2017), Pinto *et al*. (2017)	-	-	+
Oleic acid, eicosyl ester	C38H74O2	Antibakterial	Awa et al. (2012)	+	+	+
Methyl tetradecanoate	C15H30O2	Antibakterial	Chandrasekara *et al.* (2011)	+	+	+
i-Propyl 12-methyl-tridecoate	C17H34O2	-	-	+	-	-
Palmitic acid, methyl ester	C17H34O2	Antioxidant, antiandrogenic	Kumar *et al*. (2010)	+	+	+
Palmitic acid, 2-(hexadecyloxy)ethyl ester	C34H68O3			-	+	-
Palmitic acid, ethylene ester	C34H66O4	-	-	-	+	-
Methyl 11-hexadecenoate	C17H32O2	-	-	-	-	+
Methyl 10-methylundecanoate	C13H26O2	-	-	-	-	+
Methyl stearate	C19H38O2	Antioxidant, antidiarrheal, antiinflammatory, antimicrobial, anticancer	Abdel-Hady *et al.* (2018)	-	-	+
Methyl isostearate	C19H38O2	-	-	+	+	-
i-Propyl 14-methyl-pentadecanoate	C19H38O2	-	-	+	-	-
Gentamicin a	C18H36N4O10	Antibakterial	Scholar (2007)	+	-	-

Metabolite Compounds	Molecular Formulas	Bio-activities	References	Presence of Metabolites		
				TG	MB	BR
D-Mannosamine	C6H13NO5	Antitumor	Onoda *et al.* (1985)	-	+	-
Phenol, 2,4-bis(1,1-dimethylethyl)	C14H22O	Antimicrobial, antioxidant	Teresa *et al.* (2014)	+	-	-
2-Methoxy-4-vinylphenol	C9H10O2	-	-	-	+	+
Ethyl iso-allocholate	C26H44O5	-	-	+	+	+
7,8-Epoxylanostan-11-ol, 3-acetoxy-	C32H54O4	Antimicrobial, anti-inflammatory	Zekeya *et al.* (2014)	+	+	+
5á)Pregnane3,20á-diol	C28H43NO6	-	-	-	+	-
Dasycarpidan-1-methanol, acetate (ester)	C20H26N2O2	Anti-inflammatory	Mohammad *et al.* (2016)	+	+	-
Pyridine, 2-(1-methyl-2-pyrrolidinyl)	C10H14N2	-	-	-	+	-
Dihydromorphine, di(trimehylsilyl) ether	C23H37NO3Si2	-	-	-	+	-
Imidazolo	C19H15N3O	-	-	-	-	+
4-(4-Chlorophenyl)-N-[(2E)-1-methylpiperidinylidene]-5-isothiazolamine	C15H16ClN3S	-	-	-	-	+
Phorbol 12,13-dihexanoate	C32H48O8	-	-	+	+	-
Lycopene	C42H64O2	Antioxidant	Miller *et al.* (1996)	-	-	+
Octadecane	C26H54	-	-	-	+	-
Phytol	C20H40O	Antioxidant, antidiarrheal, antimicrobial, anticancer, antiinflammatory, antihyperalgesic, Antiarthritic	Carvalho *et al.* (2020), Abdel-Hady *et al.* (2018)	-	-	+

Description: TG = Tebing Gerinting Village (45 masl), MB = Masam Bulau Village (570 masl), BR= Bangun Rejo Village (795 masl), minus sign (-) = not detected

The same metabolites detected in the Moringa leaf samples included 2-Myristynoyl pantetheine, 9,10-Secocholesta-7,10(19)-triene3,24, 25 -triol, Oleic acid eicosyl ester, Oleic acid 3-(octadecyloxy)propyl ester, Linolenic acid methyl ester, Methyl tetradecanoate, Palmitic acid methyl ester, Methyl N-(N-benzyloxycarbonyl-beta-l-aspartyl)-beta-d-glucosaminide, Ethyl iso-allocholate. and 7,8-Epoxylanostan-11-ol 3-acetoxy.

The same metabolite compounds were detected in samples of Moringa leaves from Tebing Gerinting Village (35 masl), Masam Bulau Village (570 masl), and Bangun Rejo Village (795 masl) showing several health benefits, namely as an antioxidant, antiandrogenic, anti-acne, antifungal, antieczemic, antihistamine, antibacterial, antimicrobial, antiproliferative, anti-inflammatory, antidermatitis, hypocholesterolemic, vitamins, drugs for autoimmune disorders, bone disease, and Alzheimer's (Table 1).

Moringa leaves from Tebing Gerinting Village (35 masl), Masam Bulau Village (570 masl), and Bangun Rejo Village (795 masl) each have unique metabolite compounds or compounds that were only detected in Moringa leaves from Tebing Gerinting

Village (35 masl). N The compounds are Deoxyspergualin, Cyclopentanone, 2-methyl, 2-(2 Butynyl)cyclohexanone, Toluene m-ethyl-, Benzene p-dichloro, Octaethylene glycol monododecyl ether, Linolenin, 1-mono, i-Propyl 12-methyl-tridecoate , i-Propyl 14-methyl-pentadecanoate, Gentamicin a, Phenol, 2,4-bis(1,1-dimethylethyl),and Phthalic acid methyl tetradecyl ester. According to Teresa et al. (2014), Phenol, 2,4-bis(1,1-dimethylethyl) is a compound that can be used as an antimicrobial correlated with its antioxidant activity, and Gentamicin a according to Scholar (2007) has a function as a pharmacological agent that can slow down protein synthesis in bacteria.

Unique metabolite compounds in Moringa leaves from Masam Bulau Village (570 masl), namely 4-(2,4,4-Trimethyl-cyclohexa-1,5-dienyl)-but-3-e n-2-one, Benzoic acid, 4 -methyl, [4(methoxycarbonyl)phenyl]methyl ester, 17-Pentatriacontene, Methyl 2-ethylhexyl phthalate, 9,12,15-Octadecatrienoic acid, Palmitic acid 2-(hexadecyloxy)ethyl ester, Palmitic acid ethylene ester, Mannosamine, Octadecane , 5á) Pregnane3,20á-diol, Pyridine, 2-(1-methyl-2-pyrrolidinyl), and Dihydromorphine, di(trimehylsilyl) ether. According to Onoda et al. (1985), D-Mannosamine is a metabolite that is known to be used as an antitumor which combined with unsaturated fatty acids can provide a cytotoxic effect on T-lymphocyte cells.

The only metabolite detected in Moringa leaves from Bangun Rejo Village (795 masl) was N-[5-(1-Cyano-2-furan-2-yl-vinyl)-[1,3,4]thiadiazol-2-yl] -benzamide, Tertbutyloxyformamide, N-methyl-N- [4-(1-pyrrolidinyl)-2-butynyl]-, (4-Carbomethoxy)benzyl p-toluate, Acetonitrile, (p-hydroxyphenyl), 1-Methoxy-2- propyl acetate, 1,1-Cyclopropanedicarboxylic acid, 2-[2-cyano-1,1 bis(methoxycarbonyl)propyl dimethyl ester, Ethanol 2-(9-, octadecenyloxy), Methyl 11-hexadecenoate, Methyl 10-methylundecanoate, Methyl stearate, Methyl arachisate, Imidazolo, 4-(4-Chlorophenyl) -N-[(2E)-1 methylpiperidinylidene]5isothiazolamine, Lycopene, and Phytol. The unique metabolites identified as having bio-activity are Methyl stearate, Methyl arachisate, Lycopene, and Phytol while other unique compounds have not been identified. Phytol is a diterpene class compound which is only found in Bangun Rejo Village (795 masl). According to Mach (2015), Phytol is a degradation of chlorophyll which becomes a tocopherol or vitamin E precursor in plants with the help of the enzyme phytol kinase which phosphorylates phytol into phytyl-phosphate. Ogunlesi et al. (2009) stated, Phytol provides good prevention and a promising treatment against rheumatoid arthritis and possibly other chronic inflammatory diseases.

CONCLUSION

Moringa leaves from Tebing Gerinting Village (35 masl), Masam Bulau Village (570 masl), and Bangun Rejo Village (795 masl) show metabolite compounds which are known to have bio-activities such as antioxidant, antiandrogenic, anti-acne, antifungal, antieczemic, antihistamine, antibacterial, antimicrobial, antiproliferative, anti-inflammatory, antidermatitis, hypocholesterolemic, vitamins, medications for autoimmune disorders, bone disease, and Alzheimer's. Each sample of Moringa leaves showed metabolites which were only detected at one of the sampling locations and some of these metabolites are known to have health benefits.

SUGGESTION

Further research is needed regarding the effect of edapic factors on the metabolite profile of Moringa leaves based on altitude using GCMS analysis.

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