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Volatile and Primary Compounds of *Marsdenia brunoniana* Leaves and Fruits via SPME-GC-MS Assay

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ABSTRACT
The genus Marsdenia is an essential genus with abundant phytochemistry. Its bioactive compounds contribute to the bioactivity of Marsdenia as drugs, biological control agents, and plant's essential oils. Marsdenia brunoniana is uncultivated spesies found in several places in Indonesia. There are inadequate reports in the literature concerning the chemical profiles of M. brunoniana. SPME-GC-MS is a fast, versatile, and feasible technique to identify volatile and primary compounds for further utilization of M. brunoniana. Leaves have 25 types of volatile compounds and 20 varieties in the fruit. The dominance of compounds in leaves and fruits include D-mannitol (sugar group), Octadecatrienoic acid, methyl ester; and Hexadecanoic acid (fatty acid group); Nonacosane; and Dodecanamine, N, N-dimethyl. All components have important functions and have been tested for bioactivity based on reference records. The information provided initial reference for further use and testing of M. brunoniana.

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1. INTRODUCTION

Plants are a significant source of life chemistry. Plant metabolism produces primary and secondary metabolites useful as food, biomass, the main composition in perfumes, dyes, vegetable control, and medicine (Baldwin, 2010; Tholl et al., 2021). The utilization of phytochemicals in traditional and modern medicine reaches 85%. Investigation of medicinal plants continues to be developed to find new drug alternatives (Willie, Uyoh and Aikpokpodion, 2021). *Apocynaceae* is a significant family that is present all over the world (El-Fiki et al., 2019). This family group is spread in tropical and subtropic regions, including Indonesia (Ugraiah, Karuppusamy and Pullaiah, 2012; Abd Karim, Ismail and Osman, 2022).

Members of *Apocyaneacea* are found in the mountains of Yogyakarta and the East Java region, namely *M. brunoniana* (Widodo, 2015; Kuswati and Adi, 2021). The genus Marsdenia comprises more than 400 species, varieties and cultivars (Mohammadhosseini et al., 2016). Among these are neglected and underutilized species (NUS) used as food and medicine by local communities in traditional health and nutrition systems. Most of Marsdenia genus have medicinal value. They contain many chemical compounds in example marstenacigenins (A and B), tenasogenin in *Marsdenia tenacissima;* Marsdenin, is a glycoside compound from *Marsdenia erecta* and *M. brunoniana* is one such rare medicinal for diabetes treatment as well as antioxidant agent (Ugraiah, Karuppusamy and Pullaiah, 2012; Sudha, Muthusamy and R, 2016). Due to various phytochemicals, the marsdenia genus has activities that have been reported, including potential antibacterial; antimicrobial; antioxidant; anti-inflammatory; and anti-tumor (Mirza and Navaei, 2009; Abd Karim, Ismail and Osman, 2022; Moh et al., 2022). Intensive findings on *M. brunoniana* phytochemicals boost its commercial use.

SPME (solid phase microextraction) is a fast, versatile and approved technique that can be used for testing in the fields of food, flavors and volatile environments such as plants (Farag, Rasheed and Kamal, 2015; Merck Millipore Sigma and Merck KGaA, 2018). SPME is also known as green extraction because it reduces the use of solvents in the extraction process while using relatively few samples. Plants are among the organisms with the highest diversity of VOCs that they use to protect themselves from biotic and abiotic stresses and to provide information (Baldwin, 2010; Tholl et al., 2021). The majority of plant volatiles come from four biosynthetic classes: terpenoids, fatty acid catabolites, aromatics, and amino acid-derived products. Plant volatiles has essential food aromas, flavours and scents or are used as pharmaceuticals and raw materials for industrial materials and biofuels (Olivia, Goodness and Obinna, 2021; Willie, Uyoh and Aikpokpodion, 2021). To obtain better utilization possibilities, exploring the volatile compounds from the fruits and leaves of *M. brunoniana* using SPME is necessary.

2. RESEARCH METHOD

10 g of grounded samples (leaves and fruits) were placed in vial. Then, samples incubated in a water-bath at 75^{O}C for 60 minutes. The volatile components were trapped by SPME fiber using Polydimethylsyloxane-divinilbenzena (PDMS-DVB) polimer (Supelco, USA). Then, the SPME fibers were transferred into the GCMS (Gas Chromatography-Mass Spectrometry) injector (QP2010 Plus Shimadzu) with split injector (260^{O}C). High-purity helium (P = 38,9 kPa) served as the carrier gas (1 mL/min), and the capillary column used was Restex Rt (0,25 mm x 30 m, film thickness 0.25 μm). All data generated were allingned with the WILEY7 Library. All functional data of metabolites obtained by literature study and PubChem database.

3. RESULT AND DISCUSSION

The metabolite profile of SPME- GC-MS results of *M. brunoniana* plants identified 33 compounds in the fruit and 40 compounds in the leaves (Tables 1 and 2), whose chromatograms are shown in Figure 1.

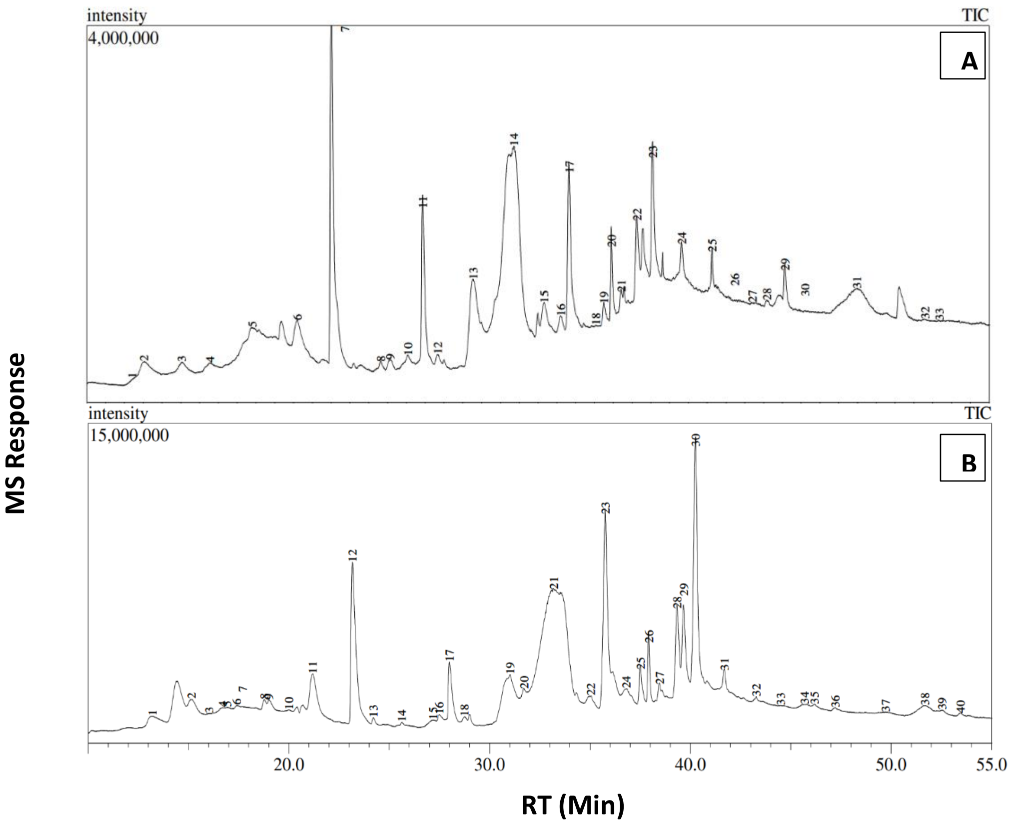

Figure 1. Representative SPME – GC/MS Chromatogram of *M. brunoniana* (A) Fruit (B) Leaves

Table 1. Relative percentage of components detected in *M. brunoniana* fruit

Peak#	RT (min)	Compounds	% Area
Amino Acid Volatile			
7	23.025	1-Dodecanamine, N, N-dimethyl- (CAS) DI-M	5,86
11	27.895	1-Tetradecanamine, N, N-dimethyl- (CAS) DI-	2,36
24	41.670	BENZYL AMPHETAMINE	7,38
Amino Acid Volatile			
5	18.812	Octanal (CAS) n-Octanal	3,81
23	40.125	9,12,15-Octadecatrienoic acid, methyl ester, (Z,	6,84

Peak#	RT (min)	Compounds	% Area
26	44.493	Ethyl sorbate	2,34
30	48.233	ETHYL ISO-ALLOCHOLATE	2,21
32	54.612	Octadecanoic acid, methyl ester (CAS) Methyl st	0,78
33	55.376	Nonahexacontanoic acid, methyl ester (CAS) M	1,18
Aromatic/Hidrokarbon Volatile			
3	15.054	Sulfone, butyl propyl (CAS) N-PROPYL N-BU	0,39
4	16.561	2-METHYLENE-CYCLOHEXANOL	0,44
8	25.653	Phenol, 2,6-dimethoxy- (CAS) 2,6-Dimethoxy	0,14
10	27.101	Phenethyl alcohol, .alpha.-butyl-.beta.-methyl-	0,23
12	28.681	2H-3,1-Benzoxazine-2,4(1H)-dione, 1-methyl-	0,36
21	38.450	Cyclohexane, 1,4-dimethoxy-2-methyl-, stereoi	2,49
28	46.205	CYCLOTRISILOXANE, 1,3,5-TRIMETHYL-	1,34
31	50.991	Nonacosane (CAS) n-Nonacosane	9,24
Terpene Volatile			
6	21.213	2,3-DIHYDRO-BENZOFURAN	2,34
19	37.531	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-	1,41
29	47.164	EICOSAMETHYLCYCLODECASILOXAN	4,23
Fat			
1	12.420	1,2,3-Propanetriol (CAS) Glycerol	0,04
17	35.673	7 Hexadecanoic acid (CAS) Palmitic acid	4,77
18	37.096	1-Hexadecanol (CAS) Cetal	0,72
22	39.284	9-Octadecenoic acid (Z)- (CAS) Oleic acid	5,62
25	43.281	EICOSAMETHYLCYCLODECASILOXANE	5,68
Carbohydrate			
13	30.566	.ALPHA.-D-LYXOFURANOSIDE, METHY	3,57
14	32.743	2-Deoxy-D-ribose	16,3
15	34.350	GLUCITOL, 1-O-NONYL-	2,67
16	35.233	alpha.-D-Glucopyranoside, methyl (CAS) M	1,17
Protein			
2	13.051	L-Arginine, N2-[(phenylmethoxy)carbonyl]- (CAS	0,59
20	37.934	1,2-Ethanediamine, N,N'-dimethyl-N,N'-bis(phe	1,7
Unidentified			
9	26.154	1H-PYRROLE-1-PROPANOIC ACID, MET	0,18
27	45.433	CYCLODODECANOL, 1-ETHENYL-	1,64

Table 2. Relative percentage of components detected in *M. brunoniana* leaves

Peak #	RT (min)	Compounds	%Area
Amino Acid Volatile			
10	20.016	Pyrrolidine, 1-nitro- (CAS) N-NITROPYRROL	0,95
12	23.176	1-Dodecanamine, N,N-dimethyl- (CAS) DI-M	5,21
16	27.501	N,N-DIMETHYL-PENTADECYLAMINE	0,16
17	28.009	1-Tetradecanamine, N,N-dimethyl- (CAS) DI-M	1,66
18	28.755	BENZOESAEUREAMID, 2-(METHYLAMI	0,26
31	41.707	BENZYL AMPHETAMINE	5,37
32	43.287	1H-Purin-6-amine, [(2-fluorophenyl)methyl]-	2,54

Peak #	RT (min)	Compounds	%Area
36	47.224	1H-Purin-6-amine, [(2-fluorophenyl)methyl]-	1,16
Fatty Acid Volatile			
4	16.719	9-Octadecenoic acid (Z)-, hexyl ester (CAS) Hex	1,09
5	16.933	4-METHYL-5H-FURAN-2-ONE	0,57
6	17.416	Methyl (E)-3-methoxy-4-nitro-3-butenoate	0,92
27	38.482	8,11,14-Eicosatrienoic acid, methyl ester (CAS)	2,28
29	39.686	Bombykol	3,38
30	40.276	9,12,15-Octadecatrienoic acid, methyl ester, (Z	9,99
39	52.548	Tetradecanoic acid, phenylmethyl ester	0,52
Aromatic / Hidrocarbon Volatile			
2	15.154	1,3-Dioxolan-2-one, 4-methyl- (CAS) Propylen	1,77
8	18.783	Cyclopropyl carbinol	0,8
11	21.191	2,3-DIHYDRO-BENZOFURAN	3,09
13	24.220	Cyclopropane, nonyl- (CAS	0,1
14	25.633	Phenol, 2,6-dimethoxy- (CAS) 2,6-Dimethoxy	0,18
38	51.695	Nonacosane (CAS) n-Nonacosane	1,3
Terpene Volatil			
7	17.707	4H-Pyran-4-one, 3-hydroxy-2-methyl- (CAS)	1,38
9	19.023	Neryl acetate	1,46
25	37.531	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[	1,5
40	53.468	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl	0,26
Fat			
20	31.728	Tetradecanoic acid (CAS) Myristic acid	1,22
23	35.783	Hexadecanoic acid (CAS) Palmitic acid	7,94
28	39.360	9-Octadecenoic acid (Z)- (CAS) Oleic acid	3,39
34	45.692	9-OCTADECENSAEURE, 12-HYDROXY-	1,2
Carbohydrate			
3	16.040	4-HEXEN-2-ONE, 3-METHYL-	0,55
15	27.192	2H-Pyran, 2,2'-[1,10-decanediylbis(oxy)]bis[tet	0,13
19	31.028	.alpha.-D-Glucopyranoside, .beta.-D-fructofur	4,09
21	33.204	D-Mannitol	22,89
22	35.047	BETA-D-LYXOFURANOSID, 5-O-(BETA-D	1,71
24	36.816	1,2,4-Cyclopentanetriol, (1.alpha.,2.beta.,4.alpha	2,43
37	49.741	Tetratriacontane (CAS) n-Tetratriacontane	1,49
Protein			
1	13.213	L-Arginine, N2-[(phenylmethoxy)carbonyl]-	0,86
26	37.949	1,2-Ethanediamine, N,N'-dimethyl-N,N'-bis(ph	1,74
Unidentified			
33	44.507	CYCLOOCTAN-1,2-DIOL	1,23
35	46.195	CYCLOTRISILOXANE, 1,3,5-TRIMETHYL	1,25

Discussion

This research aims to investigate the diversity of compounds in the fruits and leaves of *M. brunoniana*. The fruit has 61% volatile compounds with the highest percentage of aromatic/hydrocarbon volatiles (24%); fatty acid-based volatiles (18%); terpenoid and amino acid-based volatiles 9%. Meanwhile, the leaves of *M. brunoniana* have 63% volatile compounds with the dominance of amino acid-based volatile (20%); fatty acid-based volatile (18%); aromatic / hydrocarbon volatile (15%); and terpenoid volatiles (10%). Other components detected while giving flavor to the leaves and fruits of *M. brunoniana* are fats, carbohydrates, and proteins. Leaves have a high % fat content of 15%, while carbohydrates are more abundant at 18%.

Aromatic volatiles is volatile compounds with aromatic ring groups derived from L-Phenylalanine (Baldwin, 2010). A sessile lifestyle of plants significantly impacts VoC expenditure for plants. VOc mediates plant interaction with the surrounding biotic and abiotic environment (Ninkovic, Markovic and Rensing, 2021). The emission of VOc from leaves primarily functions as an allelopathic property and reduces plants' attractiveness to herbivores (Effah, Holopainen and McCormick, 2019). On the other hand, volatile also relied by fruit mainly has a role in seed dispersion. Both leaves and fruits have other metabolites that affect the flavours of both. Most abundance compounds in the leaves and fruits of *M. brunoniana* using the SPME-GC-MS technique are shown in Table 3.

Table 3. The most dominant component in the leaves and fruit of *M. brunoniana*

Leaves	Fruits
D-mannitol	2-Deoxy-D-ribose
9,12,15-Octadecatrienoic acid, methyl ester, (Z)	Nonacosane (CAS) n-Nonacosane
Hexadecanoic acid (CAS) Palmitic acid	BENZYL AMPHETAMINE
BENZYL AMPHETAMINE	9,12,15-Octadecatrienoic acid, methyl ester, (Z,
1-Dodecanamine, N, N-dimethyl- (CAS) DI-M	1-Dodecanamine, N, N-dimethyl- (CAS) DI-M

D-mannitol is a sugar component in fruits and vegetables, functioning as a sweetening agent and drug therapy for several diseases (NCBI PubChem, 2023). Both plant parts have 9,12,15-Octadecatrienoic acid, methyl ester, (Z) with a relatively high % area compared to other compounds. 9,12-Octadecadienoic acid (Z,Z) is otherwise called as omega 6 unsaturated fatty acid. This type of fatty acid has an activity as antifungal and also antibacteria (Krishnaveni, Dhanalakshmi and Nandhini, 2014). 9,12-Octadecadienoic acid is included in the FAMEs (fatty acid methyl esters) group, the composition of the fatty acids contained in vegetable oil and volatile (Kamatou and Viljoen, 2017). Furthermore, the leaves also contain Hexadecanoic acid (CAS) Palmitic acid which is also a group of unsaturated fatty acids, that one of the most abundant FAMEs of palmist oil. The Hexadecanoic acid is often used in personal and cosmetic products as skin barrier and moisturizer. Another component in both parts of the *M. brunoniana* plant is 1-Dodecanamine, N, N-dimethyl-(CAS) DI-M is an essential compound for plant and animal groups because it is involved in the process of signaling communication complex. In addition, it has a high stability value and low volatile function as food preservation, antioxidant, and antimycotoxygenic potential (Youssef et al., 2021). Nonacosane is a straight-chain alkane comprising of 29 carbon atoms. It has a role as a plant metabolite and a volatile oil component. Nonacosane has been identified within several essential oils and plays a role as a chemical communication (NCBI PubChem, 2023). Nanocosane was also found in several plants such as Moringa oleifera, Quercus salicina, Euphorbia larica, and Fumaria parviflora with nematicidal activity (Naz, Saifullah and Khan, 2013).

4. CONCLUSION

SPME-GCMS technique in profiling volatile compounds as well as primary metabolites found 33 compounds in fruits and 40 compounds in leaves. In the leaves, most compounds detected were amino acid-derived volatile groups (20%), while in the fruit, they were volatiles of the aromatic type (24%). Other dominant components in fruit and leaves are unsaturated fatty acids Octadecatrienoic acid and Hexadecanoic acid (CAS) Palmitic acid. Another compound component is D-mannitol, a group of sugars that play a role in the flavors of the leaves and fruit of *M. brunoniana.*

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