

Metabolite Profile and Antioxidant Activity of *Gelam* (*Melaleuca cajuputi* subsp. *cumingiana*) Leaves Growing on Peatland

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Abstract

This study aimed to determine the metabolite profile and antioxidant activity of *gelam* (*Melaleuca cajuputi* subsp. *cumingiana*) leaf extract growing in the peatland of Sriwijaya University campus. Metabolite profile analysis used the GC-MS method. Antioxidant activity was based on the IC₅₀ value of the DPPH assay. Twenty secondary metabolite compounds were identified by GC-MS analysis from *gelam* leaf extract growing in peatland and classified into 7 groups of secondary metabolite compounds, namely terpenoids, aldehydes, quinones, flavonoids, esters, phenols, and fatty acid methyl ester (FAME). Metabolite-assigned compounds were dominated by β -Caryophyllene (21.97%), 2-Hydroxy-5-methoxybenzaldehyde (17.98%), and 14,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z (9.84%), 1,4-Naphthoquinone, 5,8-dihydroxy-2-methoxy (7.10%), and β -Caryophyllene Oxide (6.10%) Based on the DPPH assay, the extract of *gelam* leaves grown on peatlands has very strong antioxidant activity with an IC₅₀ value of 21.71 ppm.

Keywords: *Melaleuca cajuputi* subsp. *cumingiana*; metabolite profile; antioxidant activity.

INTRODUCTION

Melaleuca cajuputi subsp. *cumingiana*, also known as *gelam*, is a member of the Myrtaceae family that is naturally distributed across Indonesia, Thailand, Malaysia, Vietnam, and Myanmar. In Indonesia, *gelam* is naturally distributed in western Kalimantan, western Java, and Sumatra (Craven & Barlow, 1997; Tran et al., 2013). *Gelam* has the ability to grow in various types of soil, from waterlogged peat soil to well-drained mineral soil (Mansur et al., 2022). In South Sumatra, *gelam* can be found naturally distributed in swamp ecosystems, from tidal peat swamp forests to fully submerged peat swamp forests (Wibisono et al., 2022). On the campus of Sriwijaya University, Ogan Ilir, South Sumatra, *gelam* thrives on peatlands (peat soil) and non-peat lands (mineral soil).

Phytochemical screening of *gelam* leaves from South Kalimantan's peat swamp forests revealed the presence of secondary metabolites including saponins, alkaloids, polyphenols, flavonoids, and quinones (Wardhani et al., 2018). These secondary metabolites contribute to strong antioxidant activity. Antioxidants can prevent reactive oxygen species-related diseases, which trigger carcinogenesis, cardiovascular disease, and premature aging in humans (Fitri et al., 2023). The accumulation and biosynthesis of secondary metabolites are influenced by various factors, including soil pH, soil type, soil

mineral content, climate, and geographic variations, including geographic location (Xu et al., 2022; Yang et al., 2024). Therefore, *gelam* grown in Sumatra or other regions may have a different metabolite profile than *gelam* grown in Kalimantan.

Identification of bioactive compounds can be carried out using a metabolomics approach in the form of metabolite profile analysis to identify primary and secondary metabolites in plants comprehensively. The metabolite profile analysis method involves LC-MS (Liquid Chromatography-Mass Spectrometry) and GC-MS (Gas Chromatography-Mass Spectrometry) (Heryanto et al., 2023). The GC-MS method is used in compound identification and is capable of analyzing compounds simultaneously, equipped with a standard metabolite spectrum library, and allows for rapid and accurate metabolite analysis (Hotmian et al., 2021). This study aims to determine the metabolite profile and antioxidant activity of *gelam* leaf extract (*Melaleuca cajuputi* subsp. *cumingiana*) grown in peatland on the Sriwijaya University campus, Ogan Ilir, South Sumatra.

MATERIALS AND METHODS

Study area

This research was conducted from September to December 2025 in the Genetics and Biotechnology

Laboratory, Department of Biology, and the Chemical Analysis, Instrumentation, and Testing Laboratory, Faculty of Mathematics and Natural Sciences, Sriwijaya University. The tools and materials used in this study included analytical balances, funnels, ovens, blenders, volumetric flasks, beakers, test tubes, droppers, micropipettes, water baths, cuvettes, a UV-Vis spectrophotometer, GC-MS, rotary evaporator, mortar and pestle. The materials used in this study included *gelam* leaves, sterile distilled water, Whatman No. 1 filter paper, and 96% methanol. *Gelam* leaf samples were collected from *gelam* trees growing in peatland (3°13'16.4"S, 104°39'42.3"E) on the Indralaya campus of Sriwijaya University.

Procedures

Three hundred grams of leaf powder was macerated with 96% methanol at a ratio of 1:5 for 72 hours. Filtration was performed using Whatman No. 1 filter paper, then evaporation was carried out using a rotary evaporator at 60°C with 50 rpm until a thick extract was obtained.

Metabolite profile analysis was carried out using the GC-MS (Gas chromatography–mass spectrometry) method. This was carried out by adding 10 ml of 96% methanol to the thick *gelam* leaf extract and injecting 1 µl into the GC-MS according to the method instrument based on the UI HP-5MS Column GC-MS work protocol. Antioxidant activity was determined by determining the IC₅₀ (half maximal inhibitory concentration) value using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay (Ao, 2019). Various dilutions of

gelam leaf extract (0.1 mL) were mixed with 2.5 mL of methanol and 0.1 mM DPPH solution, incubated at 25°C for 30 minutes, and absorbance (Ad) was measured at 517 nm using a UV spectrophotometer. Ascorbic acid was used as a positive control. GC-MS and IC₅₀ analyses were conducted in the Chemical Analysis Laboratory of the Faculty of Mathematics and Natural Sciences, Sriwijaya University.

Data analysis

Chromatogram data and metabolite profiles from GC-MS analysis were used to identify compounds using the PubChem, ChEBI, and Spectrabase websites. Metabolite profile identification included molecular formula, molecular weight, and compound class. IC₅₀ value data were analyzed descriptively for antioxidant activity.

RESULTS AND DISCUSSION

The chromatogram of the results of the analysis of *gelam* leaf extract samples using Mass Spectrometry (MS), *gelam* leaf extracts grown in peatlands showed several peaks with 4 main peaks. The four main peaks were identified as Caryophyllene compounds with a retention time of 11.24 minutes, 1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z compounds (11.72 minutes), 2-Hydroxy-5-methoxybenzaldehyde compounds (15.99 minutes), and 1,4-Naphthoquinone, 5,8-dihydroxy-2-methoxy (19.48 minutes).

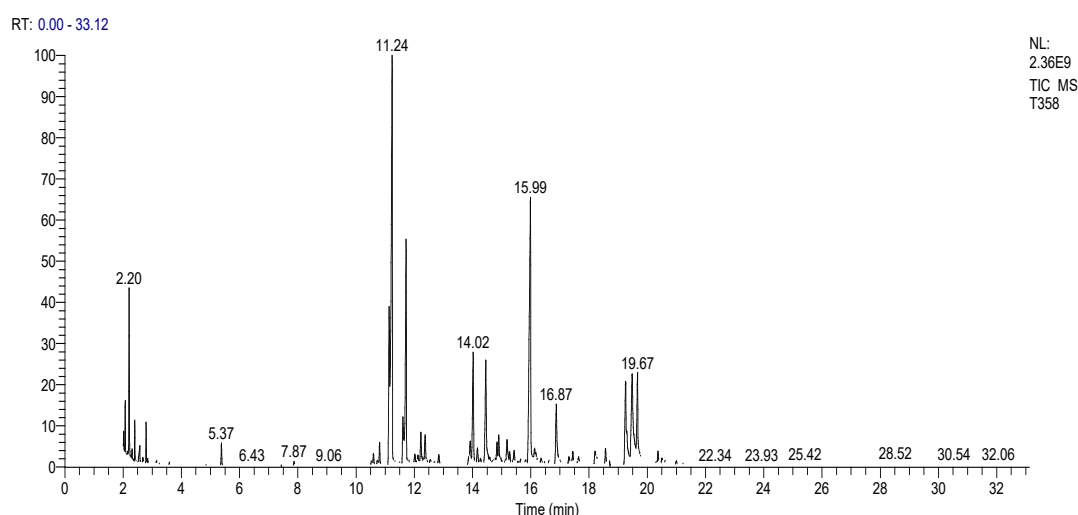


Figure 1. Chromatogram of GC-MS analysis results of peatland *gelam* leaf extract.

Based on the chromatogram of the GC-MS analysis results, the *gelam* leaf extract growing in the peatland of Sriwijaya University campus identified 20 metabolite-assigned compounds. (Table 2.) The twenty metabolite compounds identified by the GC-MS analysis of the

gelam leaf extract growing in the peatland were classified into 7 main metabolite compound classes, namely terpenoids, aldehydes, quinones, flavonoids, esters, phenols, and fatty acid methyl esters (FAME) (Table 1.).

Table 1. Class of compounds extracted from gelam leaves (*Melaleuca cajuputi* subsp. *cumingiana*) which grows on peat land.

Compound Classes	Peak Area (%)	Number of Compounds
Terpenoid	57.88	13
Aldehyde	17.98	1
Quinone	7.10	1
Flavonoid	6.69	2
Ester	5.34	1
Phenol	4.19	1
Fatty Acid Methyl Ester (FAME)	0.80	1

The results of GC-MS analysis showed that the extract of gelam leaves grown in the peatland of the Sriwijaya University campus in South Sumatra was dominated by terpenoid compounds, with an abundance (peak area) of 57.88% and a total of 13 metabolite-assigned compounds. This result differs from the phytochemical screening results of *gelam* leaf extracts from the peat swamp forests of South Kalimantan, which did not contain terpenoid compounds (Wardhani et al., 2018). This indicates that differences in distribution and geographical location can affect the accumulation and

biosynthesis of secondary metabolites, as reported by Xu et al. (2022) and Yang et al. (2024). The dominance of terpenoid compounds in *gelam* leaves growing on peatlands is closely related to the growing environment. Peatlands are marginal habitats characterized by low pH and limited nutrient availability. Plants growing in limited abiotic environments will increase the biosynthesis of secondary metabolites, including phenolics and terpenoids, to maintain and repair cell stability from oxidative damage (Gaude & Jalmi, 2025).

Table 2. Metabolite profile of GC-MS analysis results from gelam leaf extract (*Melaleuca cajuputi* subsp. *cumingiana*) growing on peat land.

RT (min)	Assigned compounds	Molecular Formula	Compound Classes	Peak Area (%)
11.13	β -caryophyllene Oxide	C ₁₅ H ₂₄	Terpenoid	6,10
11.24	β -Caryophyllene	C ₁₅ H ₂₄	Terpenoid	21,97
11.61	Humulene (α -Caryophyllene)	C ₁₅ H ₂₄	Terpenoid	2,13
11.72	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z	C ₁₅ H ₂₄	Terpenoid	9,84
12.22	β -Selinene	C ₁₅ H ₂₄	Terpenoid	1,52
12.37	α -Selinene	C ₁₅ H ₂₄	Terpenoid	1,57
13.92	(-)-Spathulenol	C ₁₅ H ₂₄ O	Terpenoid	1,41
14.02	Caryophyllene oxide	C ₁₅ H ₂₄ O	Terpenoid	4,78
14.45	2-Propenoic acid, 3-(3-hydroxy-2,6,6-trimethyl-1- cyclohexen-1-yl)-, methyl ester	C ₁₄ H ₂₂ O ₃	Ester	5,34
14.84	Calarene epoxide	C ₁₅ H ₂₄ O	Terpenoid	0,82
14.90	Tetracyclo [6.3.2.0(2,5).0(1,8)] tridecan	C ₁₅ H ₂₄ O	Terpenoid	1,31
15.18	Globulol	C ₁₅ H ₂₆ O	Terpenoid	1,30
15.99	2-Hydroxy-5-methoxybenzaldehyde	C ₈ H ₈ O ₃	Aldehyde	17,98
16.13	Isoaromadendrene epoxide	C ₁₅ H ₂₄ O	Terpenoid	1,23
16.87	2,4,6-Trimethoxyacetophenone	C ₁₁ H ₁₄ O ₄	Terpenoid	3,90
18.20	4H-1-Benzopyran-4-one, 5-hydroxy-7-methoxy-2-methyl	C ₁₂ H ₁₀ O ₄	Flavonoid	1,10
18.56	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	FAME	0,80
19.26	Benzo[b]dihydropyran, 6-hydroxy-4,4,5,7,8-pentamethyl	C ₁₄ H ₂₀ O ₂	Flavonoid	5,59
19.48	1,4-Naphthoquinone, 5,8-dihydroxy-2-methoxy	C ₁₅ H ₁₄ O ₆	Quinone	7,10
19.67	Phenol 2 6-bis(1 1-dimethylethyl)-4-ethyl	C ₁₆ H ₂₆ O	Phenol	4,19

Of the 20 metabolite-assigned compounds identified by GC-MS analysis of *gelam* leaf extracts growing in the peatland of Sriwijaya University campus, there were 5 dominant secondary metabolite compounds, namely β -Caryophyllene with an abundance (peak area) of 21.97%, 2-Hydroxy-5-methoxybenzaldehyde compound (17.98%), 1,4,7-Cycloundecatriene compound, 1,5,9,9-tetramethyl-, Z,Z,Z (9.84%), 1,4-Naphthoquinone, 5,8-dihydroxy-2-methoxy (7.10%), and β -Caryophyllene Oxide (6.10%).

The results of the analysis of antioxidant activity based on the IC₅₀ value using the DPPH assay, the

gelam leaf extract grown in the peatland of the Sriwijaya University campus has an IC₅₀ value of 21.71 ppm. Based on the IC₅₀ value of less than 50 ppm, the *gelam* leaf extract grown in the peatland of the Sriwijaya University campus has very strong antioxidant activity (Table 3.)

Table 3. Antioxidant activity of *gelam* leaf extract (*Melaleuca cajuputi* subsp. *cumingiana*) based on IC₅₀ DPPH assay.

Samples	IC ₅₀ (ppm)	Antioxidant Activity
Leaf extract	21,71	Very strong
Ascorbic acid	4,33	Very strong

The very strong antioxidant activity of the *gelam* leaf extract indicates that the metabolite compounds found in the *gelam* leaves growing on the peatland of the Sriwijaya University campus have bioactivity as antioxidants. This high antioxidant activity is closely related to the dominance of the terpenoid group, especially the abundantly detected Caryophyllene compound (Table 2), which is thought to be the main contributor to this antioxidant bioactivity. β -Caryophyllene compounds are known to have significant antioxidant potential through the mechanism of chain-breaking oxidation reactions and scavenging reactive oxygen species and increasing levels of endogenous antioxidant enzyme like glutathione peroxidase (GPx) (Wu et al. 2022).

Gelam leaf extract that grows in the peatlands of Sriwijaya University campus has great potential to be developed and applied in preventing and treating diseases related to reactive oxygen species, which trigger carcinogenesis, cardiovascular disease, and diabetes in humans. Many studies have reported that β -Caryophyllene as a natural product from plants has significant anticancer activity, influencing the growth and proliferation of various cancer cells (Ahmed, 2025; Gbadebo et al., 2025). β -Caryophyllene can play a role in repairing acute myocardial injury by reducing infarction and restoring myocyte enzymes so that it can improve heart function (Younis & Mohamed, 2019).

CONCLUSIONS

Gelam (*Melaleuca cajuputi* subsp. *Cumingiana*) leaves growing on the peatland of the Sriwijaya University campus in South Sumatra contain 20 types of metabolite compounds classified into 7 main metabolite groups: terpenoids, aldehydes, quinones, flavonoids, esters, phenols, and fatty acid methyl esters (FAME). *Gelam* leaves growing on the peatland of the Sriwijaya University campus in South Sumatra have very strong antioxidant activity.

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Competing Interests: The authors declare that there are no competing interests.

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