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# Phytochemical Analysis of Volatile Compounds from Two Varieties of *Leucaena Leucocephala* (Taramba and Wondergraze) Using Gas Chromatography-Mass Spectrometry (GC-MS) Method

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ABSTRACT

*Leucaena leucocephala* var. Taramba and Wondergraze, which are leguminous trees native to Central America, show great potential as fodder crops for livestock. The volatile compounds of these Malaysian varieties have yet to be reported; thereupon, it is critical to understand their pharmacological values. This study determined the volatile compounds in the methanolic extract of fresh leaves of *L. leucocephala* (var. Taramba and Wondergraze) and in those after being ensiled for 30 days. Using gas chromatography-mass spectrometry (GC-MS), we identified over 20 volatile compounds in both fresh and ensiled samples. The compounds exhibited different patterns between the two varieties; for instance, α-tocopherol and thiophene were commonly found in both varieties, while β-amyrin was exclusively present in Wondergraze and campesterol in Taramba. Notably, the primary compounds such as *n*-hexadecanoic acid, 9,12,15-octadecadienoic acid, and β-sitosterol were similar across both varieties. In the ensiled samples, some volatile compounds initially present in the fresh samples were missing, while new compounds, which include linoelaidic acid and α-tocospiro B, were then discovered. Many of the identified compounds possess antioxidant, anti-inflammatory, antimicrobial, and cholesterol-lowering activities, which could enhance livestock production by improving animal health, boosting immune function, and reducing reliance on synthetic feed additives or antibiotics.

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## INTRODUCTION

*Leucaena leucocephala,* commonly known as Petai belalang, is a fast-growing species of small tree that belongs to the Mimosoideae subfamily of the Fabaceae family. It is one of approximately 24 species within the genus Leucaena, which includes various trees and shrubs native to the tropical and subtropical regions of the Americas. This species is often referred to as the "miracle tree" due to its global success as a long-lived and highly nutritious forage source. It is utilised for various purposes, including firewood, timber, human food, green manure, shade, and erosion control. It is estimated that *L. leucocephala* covers between 2 to 5 million hectares worldwide [1]. Phytochemical analyses have identified various compounds in *L. leucocephala*, particularly in the leaf extract, which contains several secondary metabolites. These include phlobatannins, alkaloids, cardiac glycosides, tannins, flavonoids, saponins, and glycosides [2].

The analysis of the volatile compounds in the leaves of local Malaysian *L. leucocephala* using the GC-MS method revealed the presence of 30 main compounds. The major chemical compounds identified were squalene (41.02%), phytol (33.80%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (30.86%), 3,7,11-tridecatrienenitrile, and 4,8,12-trimethyl (25.64%) [3]. These are different from those of *L. leucocephala* species from Mexico, in which the main compounds are 2(H)-benzofuranone-5,6,7,7a-tetrahydro-4,4,7a-trimethyl, pentadecanoic acid-14-methyl-methyl ester, and 6,10,14-trimethyl-2-pentadecanone [4], while those from China were ficaprenol-11 (polyprenol), squalene, lupeol, sitostenone, *trans*-coumaric acid, *cis*-coumaric acid, pheophytin-A, pheophorbide-A methyl ester, methyl-132-hydroxy-(132-S)-pheophorbide-B, and aristophyll-C [5].

The chemical composition of *L. leucocephala* varieties Taramba and Wondergraze, cultivated in Malaysia, has never been reported before. Taramba is characterized by its bushier growth habit, broader leaflets, and greater tolerance to psyllid infestation [6]. In contrast, Wondergraze features a more upright growth form, finer stems, and a higher leaf-to-stem ratio, making it especially suitable for intensive grazing systems [7]. Both varieties show significant potential as livestock forage due to their nutritional value and adaptability. However, the silage process that is commonly used for forage preservation can significantly alter or degrade volatile compounds associated with plant aroma, palatability, and potential bioactivity. These changes may impact forage quality and acceptability. Therefore, it is essential to study the volatile profiles of both fresh and silage leaves to understand how fermentation influences these chemical constituents. We report herein the composition of volatile compounds found in fresh and silage leaves of *L. leucocephala*, highlighting the differences between the two samples.

## EXPERIMENTAL

### *Plants Material*

The fresh and ensiled leaves of two *L. leucocephala* varieties, Taramba and Wandergraze, were harvested from trees growing at Unit Ladang, UiTM Pahang, Jengka Campus, Pahang, Malaysia. A voucher specimen was deposited at the Herbarium of Universiti Teknologi MARA Pahang, Jengka Campus, Malaysia.

### *Sample Preparation*

To prepare the silage sample, fresh leaves were sealed in an airtight container for four weeks. This process reduces the anti-nutritional compounds in *L. leucocephala* leaves, namely mimosine and tannin content, to below 1% [8]. After ensiling, both fresh and silage samples were dried in an oven at 60°C for two days. This drying temperature preserves the integrity of most thermos labile and volatile compounds, making the samples suitable for subsequent chemical analysis and nutritional evaluation.

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The dried leaves were then ground using a grinder mill to achieve an average particle size of 0.5 mm.

### *Sample Extraction for GC-MS Analysis*

Approximately 25 g of dried leaf material was weighed and transferred into a clean extraction flask, where it was fully immersed in methanol and incubated overnight at room temperature. The resulting extract was filtered through Whatman No. 1 filter paper containing 2 g of anhydrous sodium sulfate to remove residual water and particulates. To facilitate flow and prevent clumping, the filter paper and sodium sulfate were pre-wetted with 95% ethanol prior to filtration. The filtrate was air-dried at room temperature, and the concentrated residue was reconstituted in n-hexane. A 1 µl sample was diluted in 100 µl of methanol and injected into the GC-MS. The analysis utilized a non-polar DB-5 cross-linked column (30 m long × 0.25 mm ID × 0.25 µm film thickness made of 5% phenyl methyl polysiloxane) with helium as the carrier gas at a flow rate of 1 mL/min. The initial temperature was set at 50°C and held for two minutes, then increased to 300°C at a rate of 6.5°C/min, and the final temperature was maintained for 10 minutes.

## RESULTS AND DISCUSSION

This is the first study on the phytochemical screening of volatile constituents found in the leaves of *L. leucocephala* varieties Taramba and Wondergraze, cultivated in Malaysia. The volatile compounds in the methanol extracts of the sampled leaves were identified using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. More than 20 volatile compounds were identified from the extracts by comparing the mass spectra with those in the NIST library. The findings indicate that both fresh and silage leaves of the Taramba and Wondergraze varieties of *L. leucocephala* contain a diverse range of phytochemical constituents (Tables 1-4).

**Table 1.** Phytochemicals in *L. leucocephala* leaves var. Wondergraze

| R. Time | Name of the Compound | Molecular Formula | Molecular weight | Peak Area % |
|---|---|---|---|---|
| 29.514 | *n*-α-Serylserine | $C_9H_{17}N_3O_5$ | 247.12 | 0.81 |
| 29.608 | Thiophene | $C_4H_4S$ | 84.14 | 0.90 |
| 29.737 | Hexadecanoic acid, methyl ester | $C_{17}H_{34}O_2$ | 270.45 | 1.73 |
| 30.086 | α-Methyl mannofuranoside | $C_7H_{14}O_6$ | 194.18 | 0.91 |
| 30.150 | 4-*O*-Methyl mannose | $C_7H_{14}O_6$ | 194.18 | 1.11 |
| 30.359 | Thiophene | $C_4H_4S$ | 84.14 | 1.23 |
| 31.124 | *n*-Hexadecanoid acid | $C_{16}H_{32}O_2$ | 256.00 | 11.17 |
| 31.232 | Pentadecanoid acid | $C_{15}H_{30}O_2$ | 242.40 | 1.65 |
| 31.823 | Phytol | $C_{20}H_{40}O$ | 296.00 | 0.81 |
| 35.632 | 9,12-Octadecadienoic acid | $C_{19}H_{40}O$ | 292.00 | 1.55 |
| 36.682 | 9,12,15-Octadecatrienoic acid | $C_{18}H_{30}O_2$ | 278.43 | 9.36 |
| 37.494 | Octadecanoic acid | $C_{18}H_{36}O_2$ | 284.48 | 1.63 |
| 43.334 | 2-Thiophenecarboxylic acid | $C_4H_3CO_2H$ | 128.15 | 0.83 |
| 53.835 | Squalene | $C_{30}H_{50}$ | 410.00 | 1.52 |
| 58.721 | α-Tocopherol | $C_{29}H_{50}O_2$ | 430.71 | 3.23 |
| 61.093 | Stigmasterol | $C_{29}H_{48}O$ | 412.70 | 0.80 |
| 62.601 | γ-Sitosterol | $C_{29}H_{50}O$ | 414.70 | 3.00 |
| 64.542 | Lupeol | $C_{30}H_{50}O$ | 426.72 | 2.95 |

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**Table 2.** Phytochemicals in *L. leucocephala* leaves var. Taramba

| R. Time | Name of the Compound | Molecular Formula | Molecular weight | Peak Area % |
|---|---|---|---|---|
| 29.725 | Hexadecanoic acid, methyl ester | $C_{17}H_{34}O_2$ | 270.45 | 1.12 |
| 31.125 | *n*-Hexadecenoic acid | $C_{16}H_{32}O_2$ | 256.42 | 12.82 |
| 34.422 | Phytol | $C_{20}H_{40}O$ | 296.53 | 10.34 |
| 35.932 | 9,12-Octadecadienoic acid | $C_{18}H_{32}O_2$ | 280.44 | 9.12 |
| 36.149 | 9,12,15-Octadecatrienoic acid | $C_{18}H_{30}O_2$ | 278.43 | 5.79 |
| 36.868 | Octadecanoic acid | $C_{18}H_{36}O_2$ | 284.48 | 3.29 |
| 38.204 | 3,7,11, 15-Tetramethylhexadec | $C_{20}H_{40}$ | 280.50 | 0.82 |
| 51.641 | Linoelaidic acid | $C_{18}H_{32}O_2$ | 280.45 | 0.86 |
| 53.877 | Supraene | $C_{30}H_{50}$ | 410.70 | 7.70 |
| 53.990 | Phthalic acid | $C_6H_4(CO_2H)_2$ | 166.14 | 1.05 |
| 54.207 | α-Tocospiro B | $C_{29}H_{50}O_2$ | 430.71 | 0.83 |
| 55.701 | 2,6,10-Dodecatrien 1-ol | $C_{15}H_{26}O$ | 222.37 | 0.91 |
| 55.764 | 2-Methyl-3 methylbut-2-enyl | $C_{15}H_{26}O$ | 222.37 | 1.06 |
| 58.659 | Vitamin E | $C_{29}H_{50}O_2$ | 430.71 | 3.04 |
| 60.351 | Campesterol | $C_{28}H_{48}O$ | 400.70 | 1.27 |
| 61.078 | Stigmasterol | $C_{29}H_{48}O$ | 412.70 | 1.07 |
| 62.621 | γ-Sitosterol | $C_{29}H_{50}O$ | 414.70 | 6.95 |
| 63.177 | β-Amyrin | $C_{30}H_{50}O$ | 426.70 | 1.49 |
| 65.008 | Lupeol | $C_{30}H_{50}O$ | 426.72 | 6.79 |

In the Wondergraze variety (Table 1), the predominant volatile compounds identified in the fresh leaf extract include lupeol, a pentacyclic triterpenoid known for its anti-inflammatory properties; γ-sitosterol, a plant sterol that may help lower cholesterol levels; stigmasterol, another plant sterol with potential health benefits; α-tocopherol, a form of vitamin E recognised for its antioxidant effects; and squalene, a natural organic compound that contributes to skin hydration [9]. Likewise, the Taramba variety shows its own unique profile of major chemical constituents in its fresh leaf extract (Table 2). These include lupeol and β-amyrin, which are triterpenoids studied for their anti-cancer properties; γ-sitosterol and stigmasterol, as in Wondergraze; campesterol, a phytosterol that helps improve heart health; and vitamin E, an essential nutrient that plays a critical role in protecting cell membranes from damage [10]. This study shows that each variety presents a unique blend of beneficial compounds, emphasizing its possible nutritional and therapeutic uses.

In a separate investigation on the ensiled samples from the leaves of two *L. leucocephala* varieties, Taramba and Wondergraze, we discovered a rich array of major chemical constituents in the silage made from both varieties. The Wondergraze variety contains lupeol, γ-sitosterol, α-tocopherol, campesterol, and 9,12-octadecadienoic acid (*Z,Z*), all of which contribute significantly to its nutritional value and health benefits (Table 3). In contrast, the ensiled leaf extract from the Taramba variety features a different set of important constituents, including lupeol, β-sitosterol, stigmasterol, campesterol, and vitamin E, as well as 1,2-benzenedicarboxylic acid and 9,12,15-octadecadienoic acid (*Z,Z*) (Table 4).

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**Table 3.** Phytochemicals in *L. leucocephala* var. Wondergraze silage

| R. Time | Name of the Compound | Molecular Formula | Molecular weight | Peak Area % |
|---|---|---|---|---|
| 29.514 | *n*-α-Serylserine | $C_9H_{17}N_3O_5$ | 247.12 | 0.81 |
| 29.608 | Thiophene | $C_4H_4S$ | 84.14 | 0.90 |
| 29.737 | Hexadecanoic acid, methyl ester | $C_{17}H_{34}O_2$ | 270.45 | 1.73 |
| 30.086 | α-Methyl mannofuranoside | $C_7H_{14}O_6$ | 194.18 | 0.91 |
| 30.150 | 4-*O*-Methylmannose | $C_7H_{14}O_6$ | 194.18 | 1.11 |
| 30.359 | Thiophene | $C_4H_4S$ | 84.14 | 1.23 |
| 32.91 | Tetratetracontane | $C_{44}H90$ | 618.00 | 1.59 |
| 34.05 | 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester | $C_{16}H_{22}O_4$ | 278.00 | 8.41 |
| 35.13 | Etratetracontane | $C_{44}H_{90}$ | 618.00 | 6.35 |
| 36.188 | 9,12,15-Octadecadienoic acid | $C_{19}H_{32}O_2$ | 292.71 | 7.09 |
| 37.22 | 9,12-Octadecadienoic acid (Z,Z) | $C_{19}H_{34}O_2$ | 294.01 | 8.38 |
| 40.310 | Campesterol | $C_{28}H_{48}O$ | 314.00 | 8.43 |
| 51.021 | α-Tocopherol | $C_{29}H_{50}O_2$ | 412.00 | 4.16 |
| 60.093 | Stigmasterol | $C_{29}H_{48}O$ | 412.70 | 0.80 |
| 61.651 | γ-Sitosterol | $C_{29}H_{50}O$ | 414.70 | 3.00 |
| 65.414 | Lupeol | $C_{30}H_{50}O$ | 426.72 | 2.95 |

**Table 4.** Phytochemicals in *L. leucocephala* var. Taramba silage

| R. Time | Name of the Compound | Molecular Formula | Molecular weight | Peak Area % |
|---|---|---|---|---|
| 29.725 | Hexadecanoic acid, methyl ester | $C_{17}H_{34}O_2$ | 270.45 | 1.12 |
| 31.125 | *n*-hexadecenoic acid | $C_{16}H_{32}O_2$ | 256.42 | 12.82 |
| 33.882 | 9,12, 15-Octaswcatrienoic acid | $C_{18}H_{30}O_2$ | 278.43 | 1.70 |
| 34.422 | Phytol | $C_{20}H_{40}O$ | 296.53 | 10.34 |
| 35.932 | 9,12-Octadecadienoic acid | $C_{18}H_{32}O_2$ | 280.44 | 9.12 |
| 36.868 | Octadecanoic acid | $C_{18}H_{36}O_2$ | 284.48 | 3.29 |
| 38.204 | 3,7,11,15-Tetramethylhexadec | $C_{20}H_{40}$ | 280.50 | 0.82 |
| 41.641 | Linoelaidic acid | $C_{18}H_{32}O_2$ | 280.45 | 0.86 |
| 43.012 | Phthalic acid | $C_6H_4(CO_2H)_2$ | 166.14 | 1.05 |
| 47.734 | α-Tocospiro B | $C_{29}H_{50}O_2$ | 430.71 | 0.83 |
| 50.481 | 9,12,15-Octadecatrienoic acid | $C_{19}H_{32}O$ | 292.00 | 6,21 |
| 53.052 | 1,2-Benzenedicarboxylic acid | $C_{24}H_{38}O_4$ | 390.00 | 6.74 |
| 58.659 | Vitamin E | $C_{29}H_{50}O_2$ | 430.71 | 3.04 |
| 60.351 | Campesterol | $C_{28}H_{48}O$ | 400.70 | 1.27 |
| 60.529 | Stigmasterol | $C_{29}H_{48}O$ | 412.70 | 1.07 |
| 62.081 | Betta sitosterol | $C_{29}H_{50}O$ | 414.00 | 5.13 |
| 65.008 | Lupeol | $C_{30}H_{50}O$ | 426.72 | 6.79 |

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The comparison of fresh and silage samples reveals notable differences in the chemical composition of the two varieties of plants (Table 1-4). The Wondergraze variety includes additional components such as *n*-hexadecanoic acid, pentadecanoic acid, and phytol, along with other compounds like octadecanoic acid and 2-thiophenecarboxylic acid. Notably, it also contains squalene, tetratetracontane, 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester, and tetratetracontane. On the other hand, the Taramba variety showcases a different range of compounds, including squalene, phthalic acid, and 2,6,10-dodecatrien-1-ol, as well as 2-methyl-3-methyls-butenyl and β-amyrin, alongside 1,2-benzenedicarboxylic acid. It is important to note that during the ensiling process, some volatile compounds may be lost due to evaporation or microbial activity, which can impact on the overall content.

In this study, some important compounds have been identified, such as stigmasterol, which is an unsaturated sterol produced in the mevalonate pathway. It accumulates in plants under stress and plays a key role in stress responses [11]. Stigmasterol has several therapeutic effects, including antioxidant and anti-inflammatory benefits, as well as impacts on the nervous system [12], supporting brain development, memory, and neurological health. Its production involves key enzymes such as SMT1 and SSR2, which convert cycloartenol into plant sterols, including stigmasterol and sitosterol [13]. Squalene, a triterpene produced by both plants and animals, serves as a precursor for sterols and other compounds, and also acts as a carbon source for microorganisms in fermentation. It exhibits antioxidant and anticancer properties [10]. γ-Sitosterol, a member of the stigmastane group, inhibits the complement C1 complex, indicating its potential as a bioactive compound [14]. β-Amyrin offers pharmacological benefits, while phytol from *L. leucocephala* is useful in treating arthritis and inflammatory diseases [15]. Phytol also possesses anticancer, anti-inflammatory, and antimicrobial properties [16].

## CONCLUSION

This study successfully identified and characterised the volatile constituents of the leaves of two *L. leucocephala* varieties, Taramba and Wondergraze, through GC-MS analysis. The findings demonstrate that both varieties possess unique profiles of beneficial compounds with potential applications in nutrition and health. The discovery of distinct chemical compositions in fresh and silage samples further emphasises the importance of these varieties in contributing to dietary health and wellness. Future research could explore the therapeutic benefits of these compounds and their practical applications in various fields, including agriculture and medicine.

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## AUTHOR'S CONTRIBUTION

Firstca Aulia Rachma conducted the research and drafted the article. The supervisory team, Agustono Wibowo, Nurain Aziman, Norizan Ahmat, and Iswan Budy Suyub designed the experimental work and guided the research progress. Neni Kartini Che Mohd Ramli contributed to the sample collection, while Suhaidi Ariffin handled the GC-MS analysis. Finally, Megat Ahmad Kamal Megat Hanafiah

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and Mohd Nazrul Hisham Daud reviewed and approved the article revisions for submission.

## CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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