

Volatile Profile and Antioxidant Activity of *Lemna aequinoctialis* Hydrosol Revealed by HS-SPME-GC-MS

Wacharakorn Patumanon^a, Ratchaphon Lertchaiyongphanit^a, Phatcharalak Pankratok^b,
Tharinee Saleepochn^b, Peerapat Roongsattham^c, Witcha Imaram^{a,*}

^a Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand

^b Department of Chemistry, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand

^c Department of Genetics, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand

Abstract

Lemna aequinoctialis is a rapidly growing duckweed species with a high capacity for nutrient uptake from aquatic environments, making it of considerable interest across a wide range of research fields. Despite its widespread distribution and potential applications, studies on the chemical constituents of this species—particularly its volatile profile—remain limited. Therefore, this study presents the first investigation of volatile compounds in *L. aequinoctialis* hydrosol obtained by steam distillation. Volatile analysis using headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME–GC–MS) revealed the presence of several major volatile compounds, including (E)-2-pentenylfuran, β -cyclocitral, β -ionone, β -homocyclocitral, dihydropseudoionone and 2-pentylfuran. In addition to chemical characterization, the antioxidant activity of the hydrosol was evaluated using the DPPH radical scavenging assay, which showed an inhibition of $30.02 \pm 2.99\%$ and a Trolox equivalent antioxidant capacity (TEAC) of $0.019 \pm 0.0016 \mu\text{mol TE/mL}$ hydrosol. These findings indicate that *L. aequinoctialis* hydrosol contains bioactive volatile compounds with moderate antioxidant activity. Overall, this study provides fundamental chemical and functional evidence that supports further investigations into the potential applications of *L. aequinoctialis* hydrosol.

Introduction

Lemna aequinoctialis, is a fast-growing duckweed species belonging to the family Lemnaceae. Duckweeds are recognized as protein-rich plants and have been proposed as sustainable feed resources for poultry and livestock. In particular, *L. aequinoctialis* contains approximately 32–37% protein and is rich in essential amino acids such as leucine, lysine, and valine (Song et al., 2025). Despite its nutritional relevance and abundance, metabolomic and chemical studies on *L. aequinoctialis* remain limited. Previous investigations on volatile and metabolite profiles in other *Lemna* species have reported the presence of various volatile organic compounds and secondary metabolites, including flavonoids and carotenoids.

Beyond nutritional applications, hydrosols derived from plant materials have gained increasing attention as value-added products (Almeida et al., 2024). Hydrosols, also referred to as aromatic waters, are aqueous by-products obtained during steam distillation and contain trace amounts of essential oils and water-soluble compounds that contribute to their aroma and biological activity. Compared with essential oils, hydrosols are generally considered safer, less toxic, and more economical to produce (D'Amato et al., 2017). However, information regarding the chemical composition, bioactivity, and potential applications of hydrosols derived from *L. aequinoctialis* is currently lacking.

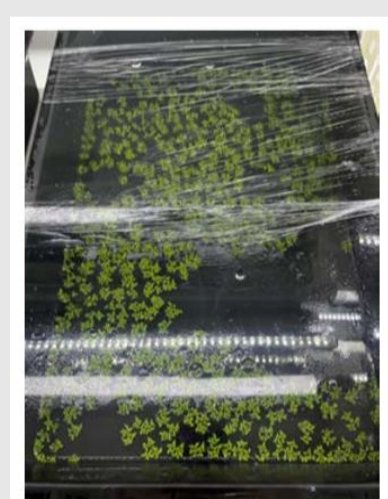
Materials and Methods

Cultivation of *Lemna aequinoctialis*

Lemna aequinoctialis was cultivated in black plastic containers containing 4 L of deionized water supplemented with NPK fertilizer (16:16:16, 4 g per 4 L). Plants were maintained at room temperature for two weeks.



1 week



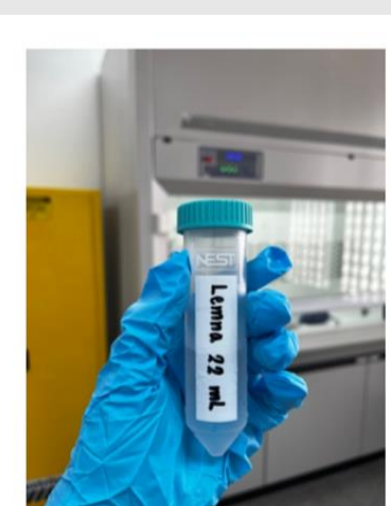
2 week

Hydrosol extraction by steam distillation

Freeze-dried *L. aequinoctialis* powder (1.2 g) was suspended in 25 mL of distilled water and subjected to steam distillation at 100 °C in a 100 mL round-bottom flask equipped with a standard distillation apparatus and magnetic stirring. A total of 22 mL of hydrosol was collected and stored at –20 °C until analysis.



Distillation



Hydrosol

Volatile compounds in the hydrosol were analyzed using HS-SPME-GC-MS, and antioxidant activity was evaluated using the DPPH radical scavenging assay with Trolox as the reference standard

Results and Discussion

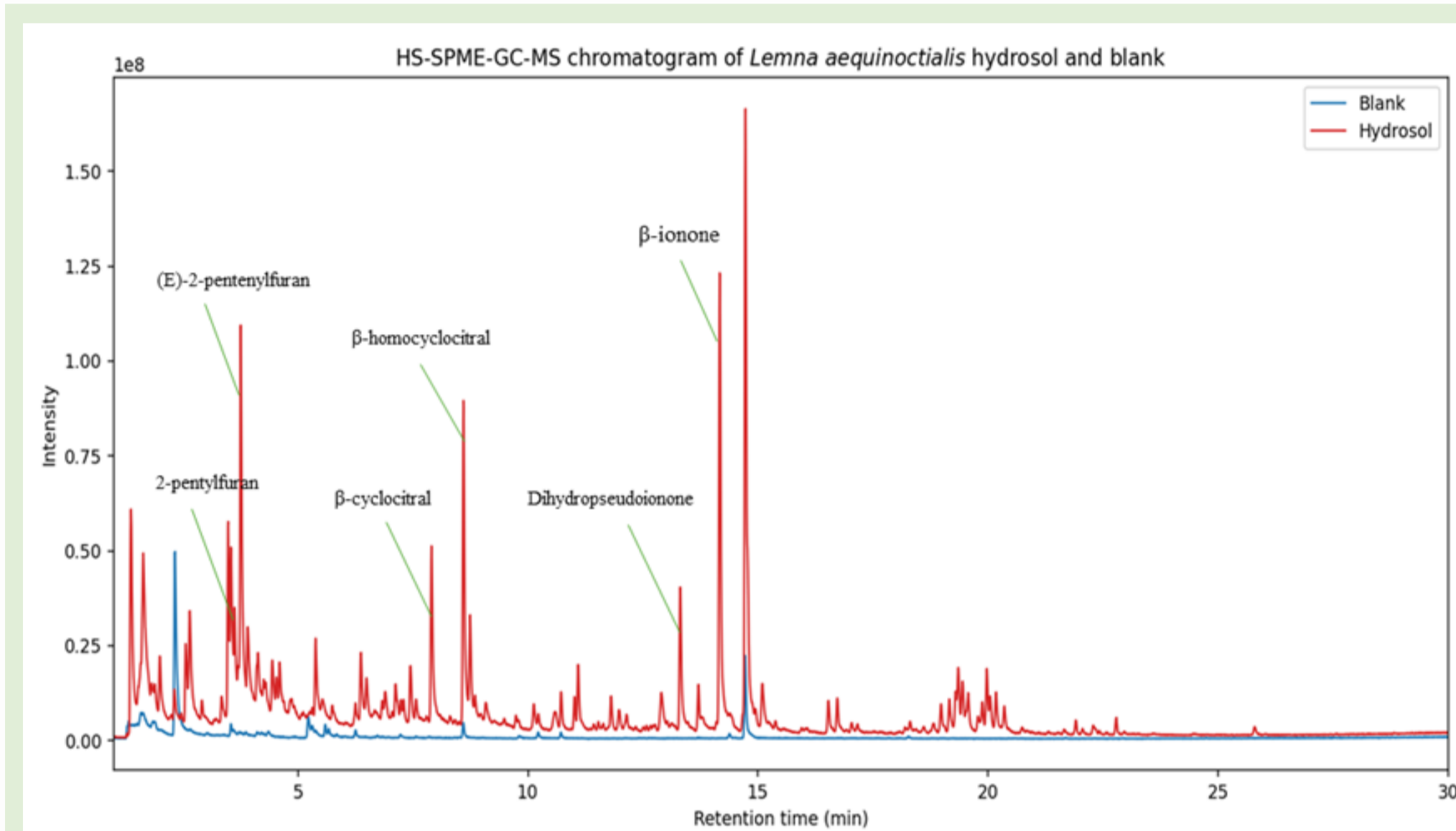


Figure 1. HS-SPME-GC-MS Chromatogram of *Lemna aequinoctialis* Hydrosol and Blank

Table 1. Major compounds in *Lemna aequinoctialis* Hydrosol

No.	Retention time	Name compounds	SI	RSI	%Relative
1	3.6266	2-pentylfuran	938	938	0.25
2	3.7567	(E)-2-pentenylfuran	904	924	1.58
3	7.9044	β -cyclocitral	957	958	0.79
4	8.7401	β -homocyclocitral	952	956	0.38
5	13.3103	Dihydropseudoionone	926	926	0.64
6	14.1695	β -ionone	956	956	2.03

SI: Similarity index; RSI: Reverse similarity index

Table 2. Antioxidant activities of *Lemna aequinoctialis* Hydrosol

Antioxidant assay		
Antioxidant Assays	%inhibition	Trolox equivalent
DPPH	$30.02 \pm 2.99\%$	0.019 ± 0.0016285 $\mu\text{mol TE/mL Hydrosol}$

Values are presented as mean \pm standard deviation (n = 3)

Conclusion

Hydrosol obtained from *Lemna aequinoctialis* was chemically characterized for the first time using HS-SPME–GC–MS. Six major volatile compounds—(E)-2-pentenylfuran, β -cyclocitral, β -homocyclocitral, dihydropseudoionone, β -ionone, and 2-pentylfuran—were tentatively identified in the hydrosol. In addition, the hydrosol exhibited moderate antioxidant activity, with a DPPH radical scavenging activity of $30.02 \pm 2.99\%$ and a TEAC value of $0.019 \pm 0.0016 \mu\text{mol TE/mL}$. These findings provide fundamental chemical and functional insights into *L. aequinoctialis* hydrosol and support further investigations into its potential applications as a value-added plant-derived product.

Acknowledgements

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References

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