RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
57 (5) 2127-2138, 2025
http://doi.org/10.54910/sabrao2025.57.5.33
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

PHYTOCHEMICAL ANALYSIS OF LOTUS (*NELUMBO NUCIFERA* L.) RHIZOMES IN JOTO RESERVOIR, EAST JAVA, INDONESIA

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SUMMARY

Lotus (*Nelumbo nucifera* L.) rhizomes are applicable in traditional medicine due to their therapeutic properties, which include compounds such as phenolics, tannins, alkaloids, and flavonoids. This study aimed to analyze the phytochemical properties, total flavonoid content, antioxidant activity, and bioactive compounds using gas chromatography-mass spectrometry (GC-MS) of *N. nucifera* rhizomes obtained from the Joto Reservoir in East Java, Indonesia. The results showed lotus rhizomes contain secondary metabolites, such as phenols, flavonoids, tannins, alkaloids, steroids, and terpenoids, with a total flavonoid content of 9.23 ± 0.04 mg/g QE. The DPPH (2,2-diphenyl-1-picrylhydrazyl) test indicated that the extract had a moderate antioxidant activity, with an IC_{50} of 57.88 ppm. The GC-MS profiling confirmed 238 identified compounds in the crude ethanolic rhizome extract of *N. nucifera*. The bioactive compounds included linoleic acid (5.90%), hexadecanoic acid (3.61%), octadecadienoic acid (2.62%), stigmasterol 3.β (2.13%), Z-7-hexadecenal (1.73%), 24-norursa-3,12-diene (1.53%), lanosterol (1.51%), campesterol (1.28%), beta-sitosterol (1.14%), betulin (0.82%), and rolipram (0.60%). Further studies revealed the ethanol extract of *N. nucifera* rhizomes is a valuable source of many bioactive compounds, supporting its beneficial traditional medicinal uses for health.

Keywords: Lotus (*N. nucifera* L.), antioxidant activity, GC-MS profiling, Joto reservoir, phytochemicals

Communicating Editor: Prof. Zahoor Ahmed Soomro

Manuscript received: January 24, 2025; Accepted: April 02, 2025.

Citation: Setiyowati PAI, v. Lim V, Hayati A, Maghfuroh L, Ramadani AH (2025). Phytochemical analysis of lotus (*Nelumbo nucifera* L.) rhizomes in Joto Reservoir, East Java, Indonesia. *SABRAO J. Breed. Genet.* 57(5): 2127-2138. http://doi.org/10.54910/sabrao2025.57.5.33.

Key findings: Phytochemical analysis showed the presence of flavonoid, alkaloid, and phenolic contents, which can enhance the efficacy of herbal medicines. The GC-MS profiling of lotus (*N. nucifera*) rhizome extract identified bioactive compounds, such as linoleic acid, stigmasterol, betulin, and rolipram.

INTRODUCTION

Nelumbo nucifera L., widely known as lotus, has been a cornerstone of traditional medicines and cultural practices across Asia for centuries, including in China, India, Vietnam, Japan, Thailand, and Indonesia. Lotus, known for its resilience and aesthetic appeal, is a symbol of purity, as well as a rich source of different bioactive compounds (Wang and Zhao, 2024; Seferli *et al*., 2024). Every part of the plant, especially its rhizomes, leaves, flowers, and seeds, contains phytochemicals with diverse therapeutic applications. These bioactive compounds attained recognition for their antioxidant, anti-inflammatory, hepatoprotective, anticancer, and antimicrobial properties (Assad *et al*., 2024).

The lotus widely spreads in wetland areas across Indonesia, such as Java, Sumatra, and Bali (Yang *et al*., 2024). The plant thrives in tropical and subtropical climates, making Indonesia an ideal location for its cultivation and sustainable utilization (Yang *et al*., 2024). Despite its prominence, the phytochemical diversity and pharmacological potential of Indonesian lotus cultivars remain underexplored. Investigating the therapeutic properties of different plant parts, i.e., flowers, stems, seeds, rhizomes, and leaves, can provide more understanding of their application in pharmaceutical and nutraceutical industries.

The lotus, especially the rhizomes, often consumed as food, is also crucial for its medicinal properties. The rhizomes are typical ingredients in Asian cuisines, such as in stir-fries, soups, and salads, particularly in China, Japan, India, and Vietnam (Dai *et al*., 2023). It is a rich source of dietary fibers, vitamins, and minerals, along with phytochemicals, tannins, flavonoids, and polyphenols (Indira *et al*., 2024). These bioactive compounds exhibit significant antioxidant and anti-inflammatory activities, contributing to the rhizomes' free radical scavenging potential, which makes them a functional ingredient for managing oxidative stress-related conditions (Solekha *et al*., 2024). Studies have demonstrated that in lotus, the phenolic and flavonoid contents have direct correlations with its antioxidant potential. For instance, Musyarofah *et al*. (2024) analyzed the total flavonoid and phenolic content of lotus flowers and reported IC_{50} values indicating a robust free radical scavenging activity.

Gas chromatography-mass spectrometry (GC-MS) is an advanced analytical technique essentially used for identifying and quantifying the complex array of bioactive compounds in plants (Seferli *et al*., 2024). The lotus rhizomes and leaf extracts exhibited significant antioxidant activity, with the flavonoids and phenolics playing a pivotal role. Past studies expressed that lotus has undergone cultivation in various Asian countries, with all its parts, particularly rhizomes, seeds, stems, leaves, and flowers, having incurred studies for their antioxidant properties (Yang *et al*., 2024). However, the secondary metabolites in lotus plants may vary based on their growing location, as well as influences from both biotic and abiotic factors.

The selection of the Joto Reservoir in Lamongan District, East Java, Indonesia, arose from this study due to its hypereutrophic conditions, marked by excessive nutrient levels that promote dense aquatic vegetation, including *N. nucifera* (Shaleh *et al.*, 2024). This study focuses on profiling *N. nucifera* rhizome metabolites via GC-MS, assessing bioactivity and antioxidant activity (IC_{50}), and quantifying flavonoids and phenolics for potential applications. These findings could contribute valuable insights into the potential application of *N. nucifera* rhizomes as functional food, pharmaceuticals, and nutraceutical formulations.

MATERIALS AND METHODS

Sample collection

The obtained *Nelumbo nucifera* rhizome samples came from the Joto Reservoir, Lamongan District, East Java, Indonesia (7°09′11″S 112°24′16″E) (Figure 1). Taxonomic identification, as conducted by the study, used a determination test by the Generasi Biologi Indonesia Foundation with certificate number BT.08.2482. The collected samples consisted of approximately 3 to 5 kg of fresh, mature rhizomes. The criteria of mature rhizome employed the description by Showkat *et al*. (2021). For subsequent analysis, the samples proceeded to the Laboratory of Biology, Airlangga University, Surabaya, East Java, Indonesia.

Sample extraction

The extraction process involved the washing, cutting, drying (40 °C), and grinding of 3 kg of fresh lotus rhizomes into powder. Then, 400 g of powder received mixing with 96% ethanol (1:10) for extraction. The extraction continued by using the maceration technique. The resulting extract solution reached evaporation using a rotary vacuum evaporator (Patel *et al.*, 2023). The forms of fresh, dried, powdered, and ethanolic extract of *N. nucifera* rhizomes appear in Figure 2.

Extract yield (%)

The percent yield determination of the extract employed calculating the ratio of dried extract weight obtained to the initial weight of the dried plant material used for the extraction, multiplied by 100. The following formula was typically applicable (Nazaruddin *et al*., 2024):
Extract yield (%) = Weight of extract obtained (g) / Weight of plant material used (g) × 100

Phytochemical analysis

The ethanolic extract of lotus rhizomes' analysis ensued for its qualitative phytochemical content to identify bioactive compounds, such as alkaloids, saponins, phenols, tannins, flavonoids, terpenoids, and steroids, following the methods as outlined by Khedr *et al*. (2024).

Antioxidant activity

The antioxidant activity of lotus rhizomes, when assessed, used the DPPH assay described by Ren and Yang (2022). Ethanolic extracts of *N. nucifera* rhizomes had prepared concentrations of 20, 40, 60, 80, and 100 ppm (Figure 3). The absorbance recording was at 517 nm using a spectrophotometer, with the percentage of inhibition calculated using the following formula:

Inhibition (%) = Absorbance (control) – Absorbance (sample) / Absorbance (control) x 100

Then, calculating the IC_{50} value succeeded by analyzing the correlation between the concentration and the percentage of inhibition using the equation y = ax + b, where the X-axis represents the sample concentration (ppm), and the Y-axis represents the percentage of inhibition. The standard used for comparison was ascorbic acid.

Flavonoid level analysis

The employment of a colorimetric method determined the flavonoid content in the extract by causing the sample to react with aluminum chloride ($AlCl_3$) and sodium acetate buffer. The absorbance measurement at 420 nm had the flavonoid concentration quantified using a calibration curve of quercetin (Solekha *et al*., 2024).

GC-MS profiling and phytocomponent identification

In the rhizome parts of the lotus plant, the GC-MS profiling of ethanol extract continued using a Perkin-Elmer GC Clarus 500 system, equipped with an AOC-20i auto-sampler and a gas chromatograph interfaced with a mass spectrometer (GC-MS). The analysis of phytocomponents took place according to Khobjai *et al*. (2021).

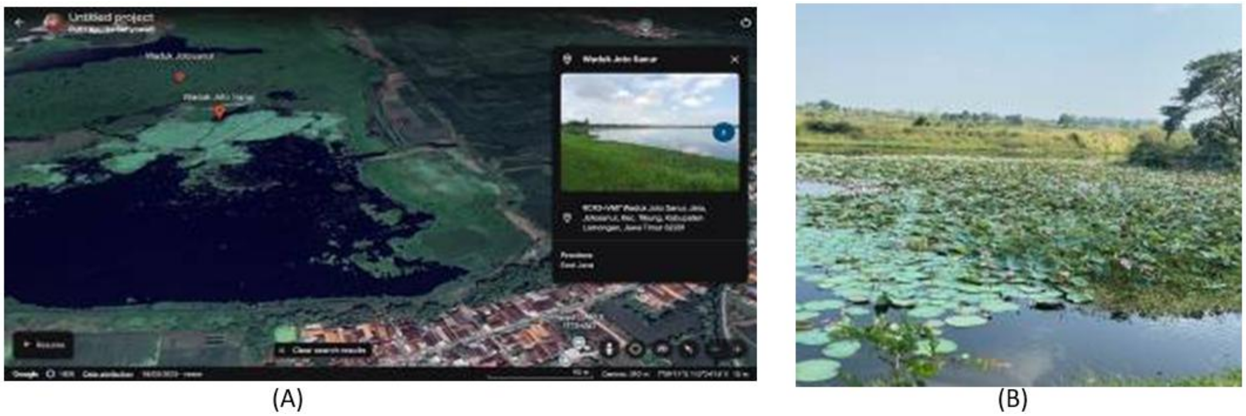

Figure 1. The location of *Nelumbo nucifera* plant in Joto Reservoir, East Java, Indonesia (Google Earth) (A). Close-up photograph of the *Nelumbo nucifera* population highlighting their growth in dense (B).

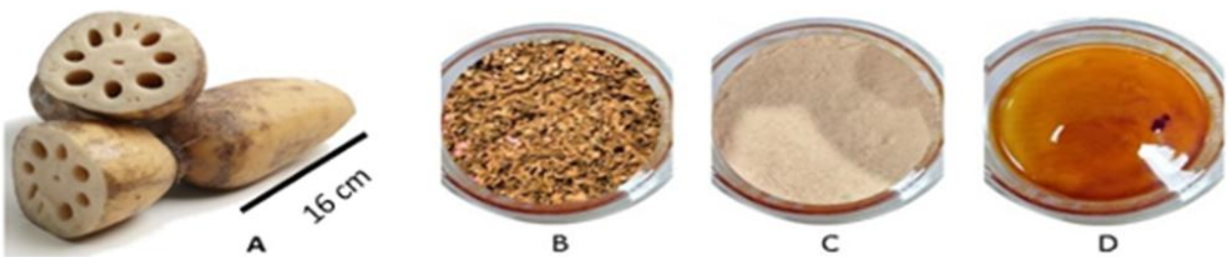

Figure 2. The fresh (A), dried (B), powdered (C), and ethanolic extract (D) of *Nelumbo nucifera* rhizomes. The dried lotus rhizome exhibits a brownish color and small flake-like shapes, the powder shows an ivory color with a flour-like texture, and the extract is characterized by a yellowish-brown color and a viscous texture.

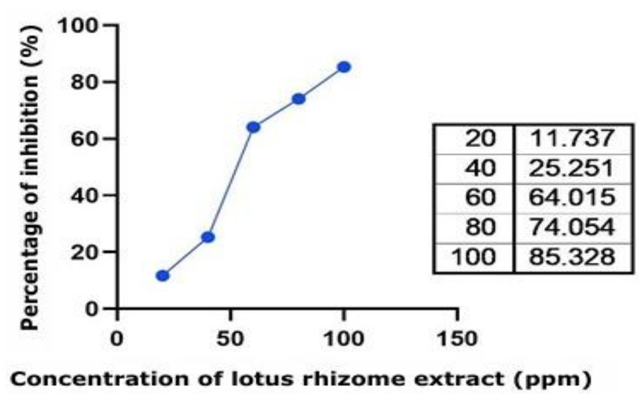

Figure 3. The relationship between the sample concentration of *Nelumbo nucifera* rhizomes ethanolic extract and percentage of inhibition. The graphics show positive correlation between the concentration of the lotus rhizome extract and the percentage of inhibition (antioxidant activity). The concentration of 100 ppm showed the best inhibition (85.328%).

Statistical analysis

The study expressed antioxidant activity (IC_{50}) and flavonoid content data as the mean ± relative standard deviation (RSD). The antioxidant data resulted from three replications, and performing statistical analysis used one-way analysis of variance (ANOVA), with confirmation engaging the SPSS software version 2.0.

RESULTS AND DISCUSSION

Extracted yield (%) and phytochemical analysis

The extraction yield percentage, defined as the ratio of the extract weight to the weight of the powdered *N. nucifera* rhizomes, is a crucial factor in determining the extract quality and its potential biological activity (Table 1). In this study, in lotus rhizomes, the extraction yield was 3.55%, which was slightly higher than the 2.29% yield reported previously for ethanol extraction (Ren and Yang, 2022). The discrepancy in extract yield can be due to several factors influencing the efficiency of the extraction process, such as variations in extraction conditions, solvent concentration, duration, and the inherent properties of the plant material.

The biological composition of lotus rhizomes significantly influenced the extraction yield. The rhizomes' varied water content can affect the concentration of bioactive compounds within the plant tissue, and more water reduces their availability for extraction. Additionally, the dense cellular structure of the rhizomes may hinder solvent penetration, further limiting extraction efficiency. These findings were consistent with those reported by Ngamkhae *et al*. (2022), who proposed that rhizomes with high water content and complex cellular matrices can restrict the release of bioactive compounds during the extraction process. Environmental factors, such as temperature, pressure, and solvent-to-plant material ratio, often with no standards to follow across studies, can also impact the extraction yield. Therefore, these parameters require careful control to ensure consistent and reliable results.

Although the extraction yield in lotus rhizomes was relatively low, it still reflected the complex interplay of factors, such as the rhizomes' water content, dense cellular structure, extraction time, and solvent choice. Dai *et al*. (2023) noted lotus rhizomes' dense structure requires longer extraction times and optimized solvents. Future studies should optimize conditions like extraction time and solvent type to improve yield and bioactive compound activity. This approach could improve the efficiency of the extraction process while also broadening the potential therapeutic application of lotus rhizomes.

The phytochemical screening results of the ethanol extract in lotus rhizomes revealed the flavonoids, terpenoids, phenolics, tannins, alkaloids, and steroids (Table 2), which align with the known biochemical profile of other plant extracts. In the presented study, the ability of 96% ethanol to extract a wide range of secondary metabolites was consistent with previous research emphasizing ethanol's efficiency as a solvent for polar compounds. Ethanol is an effective solvent for polyphenols, which bind to a polar fibrous matrix in plant materials. Moreover, ethanol's safety for human consumption makes it an ideal solvent for obtaining bioactive compounds suitable for therapeutic applications (Plaskova and Mlcek, 2023).

Polyphenols, a diverse group of biological compounds with aromatic rings and hydroxyl groups, are typical for their potent antioxidant properties. With over 10,000 compounds identified within this class (Zagoskina *et al*., 2023), their presence in the ethanol extract of lotus rhizomes was significant, suggesting the potential for antioxidant activity. The same attained further support from past studies, which often highlight polyphenols as key contributors to the biological activities of plant extracts (Zagoskina *et al*., 2023). The high polarity of ethanol allows it to efficiently solvate the hydroxyl groups found in polyphenolics compounds, facilitating their extraction from the fibrous matrix of the plant material.

Table 1. Percentage of yield extract in *Nelumbo nucifera* rhizomes.

Total of *N. nucifera* rhizomes powdered (g)	Total of *N. nucifera* rhizomes ethanolic extract (g)	Percentage of yield extract (%)
400	14.2	3.55

Table 2. Phytochemical screening of *Nelumbo nucifera* rhizomes extract based on qualitative secondary metabolites.

Secondary metabolites	Qualitative results
Alkaloid	Positive
Phenol	Positive
Flavonoid	Positive
Steroid	Positive
Terpenoid	Positive
Saponin	Negative
Tannin	Positive

Table 3. Antioxidant activity of *Nelumbo nucifera* rhizomes extract at various concentrations.

Concentration (ppm)	Percentage of inhibition (%) ± SD	IC_{50} (ppm)
20	11.74 ± 0.142*	
40	25.25 ± 0.203*	
60	64.02 ± 0.118*	57.88
80	74.05 ± 0.263*	
100	85.31 ± 0.117*	

* shows significantly different ($P < 0.05$).

Tannins and alkaloids are the key secondary metabolites identified in the ethanol extract, further demonstrating ethanol's versatility as a solvent. Although tannins are primarily water-soluble, their efficient extraction by ethanol is due to its ability to form hydrogen bonds with hydroxyl groups (Nazaruddin *et al*., 2024). Similarly, alkaloids dissolve in ethanol due to its interaction with the nitrogen-based functional groups found in alkaloid structures. These findings support the effectiveness of ethanol in extracting a wide spectrum of bioactive compounds in lotus rhizomes.

The identification of terpenoids and steroids in the ethanol extract is also noteworthy. The ethanol, typically considered a polar solvent, is familiar for extracting both polar and nonpolar compounds based on the concentration and specific interactions within the plant matrix. Terpenoids often comprise an association with essential oils, steroids, and lipid-soluble substances, which can still incur extraction from ethanol due to its relatively high polarity, especially at the concentration used in this study. These compounds' presence in the extract could contribute to the diverse biological activities of lotus rhizomes, such as anti-inflammatory, antimicrobial, and antioxidant effects (Khobjai, 2021).

Antioxidant activity and flavonoid total

In the present study, the observed IC_{50} value was 57.88 ppm for the ethanol-extracted lotus rhizomes, which reflects the considerable antioxidant potential of the isolate (<100 ppm) (Table 3). These findings agreed with previous research by Pebriani *et al.* (2024), who reported an IC_{50} value of 58.62 ppm for ethanol-extracted lotus rhizomes. The close alignment between these results further reinforces the reliability of the IC_{50} value and emphasizes the robust antioxidant properties of the extract.

Table 4. Flavonoid level of *Nelumbo nucifera* rhizomes extract.

Repetition	Flavonoid level (mg/g QE)	Average of flavonoid level (mg/g QE)
1	9.30	
2	9.26	9.23 ± 0.04
3	9.14	

In the extract, quantifying the presence of flavonoids resulted in 9.23 ± 0.04 mg/g QE (Table 4), which also plays a vital role in the observed antioxidant activity. Flavonoids are well-known for their ability to scavenge free radicals, including peroxynitrite, hydroxyl radicals, peroxyl radicals, and superoxide anions (Seferli *et al*., 2024). These compounds are potent antioxidants that protect cells from oxidative damage, as implicated in the development of various chronic diseases. In this study, the flavonoid content was higher than previous reports. For instance, Yamini *et al*. (2019) found a flavonoid content of 7.12 ± 0.45 mg/g QE in lotus rhizome extract. The difference in flavonoid content may refer to the variations in extraction conditions, such as solvent type and concentration, which eventually affect the solubility of bioactive compounds (Ngamkhae *et al*., 2022).

The consistency of results regarding the antioxidant activity and flavonoid content in the lotus rhizomes supports the hypothesis that ethanol is a highly effective solvent for extracting bioactive compounds, particularly polar flavonoids. Khedr *et al*.'s (2024) findings revealed that ethanol, a polar solvent, is particularly effective in extracting flavonoid compounds due to its ability to dissolve polar molecules. This previous research is in line with the results of the current study, where ethanol served to extract a significant amount of flavonoids from the lotus rhizomes, which likely contributed to the observed high antioxidant activity. Besides solvent factors, geographical and environmental conditions, including biotic and abiotic factors at Joto Reservoir, likely influenced the wild lotus antioxidant and flavonoid levels. The nutrient-rich soil, abundant in organic matter and minerals, may enhance flavonoid synthesis and fatty acid biosynthesis (Shaleh et al., 2024).

Moreover, studies have shown a strong link between flavonoid content and antioxidant activity in plant extracts. Ren and Yang (2022) found a significant positive correlation in lotus rhizomes, while Zhang *et al.* (2021) reported similar findings in ginger extracts. This connection further solidifies the interpretation of the latest study's results, where the high antioxidant activity was likely driven by the substantial flavonoid content in the extract.

The relevant findings corroborate previous research, further validating the potent antioxidant properties of ethanol-extracted lotus rhizomes and underscoring the pivotal contribution of flavonoids in mediating these effects. The results further suggested lotus rhizomes, particularly when extracted with ethanol, hold substantial promise as a functional food with therapeutic potential in mitigating oxidative stress-associated pathologies.

GC-MS profile of the lotus rhizome extract

The chromatogram of secondary metabolites in the lotus rhizome extract obtained from Joto Reservoir, East Java, Indonesia, incurred analysis by GC-MS (Figure 4, Table 5). The chromatogram of lotus rhizome extract displayed 283 peaks, consisting of secondary metabolite compounds in the groups of terpenoids, steroids, triterpenoids, phenols, fatty acids, aliphatic acid esters, and hydrocarbons. Several main components found in the ethanol extract of lotus rhizomes were linoleic acid (5.90%), hexadecanoic acid (3.61%), octadecadienoic acid (2.62%), stigmasterol 3.β (2.13%), Z-7-hexadecenal (1.73%), 24-norursa-3,12-diene (1.53%), lanosterol (1.51%), campesterol (1.28%), beta-sitosterol (1.14%), betulin (0.82%), and rolipram (0.60%). A previous study revealed that the secondary metabolites found in lotus rhizome extract include betulinic acid, lupeol, and some fatty acid compounds (Zhao *et al*., 2023).

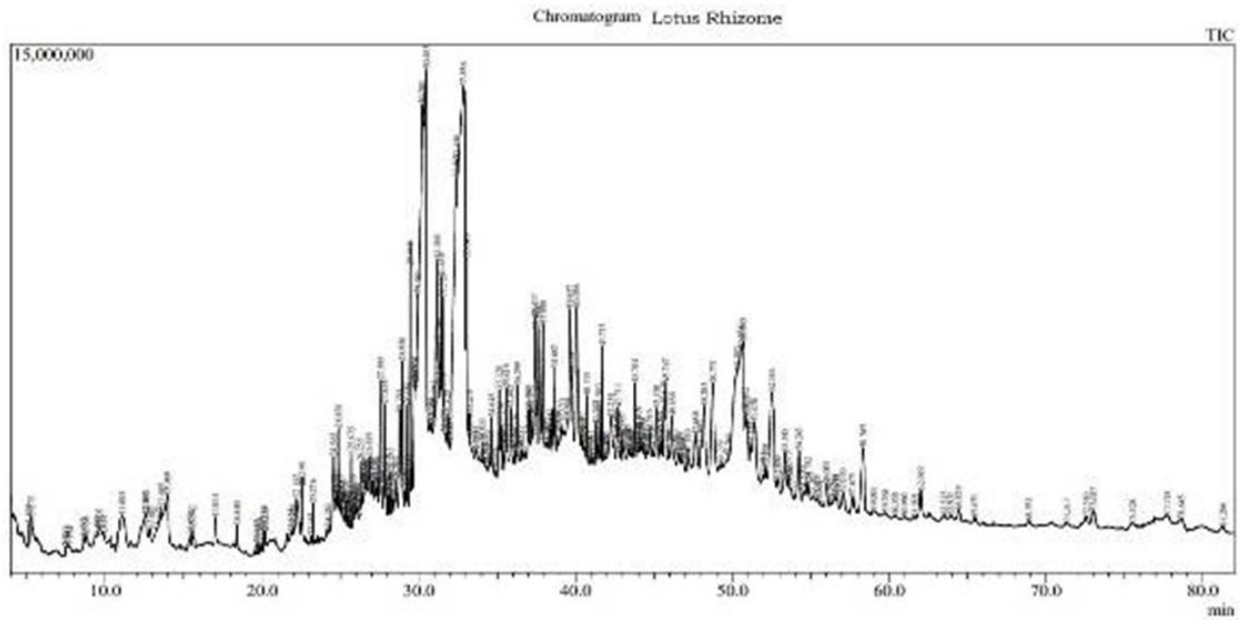

Figure 4. GC-MS chromatogram of *Nelumbo nucifera* rhizomes ethanolic extract obtained from Joto Reservoir, East Java, Indonesia. The high peak appeared at the 32.856 retention time (rt) reference to linoleic acid.

Table 5. Phytochemicals of *Nelumbo nucifera* rhizomes ethanolic extract obtained from Joto Reservoir, East Java, Indonesia.

Retention time (min)	Phytochemicals	Similarity Index (SI)	Peak Area (%)
5.275	5-methyl furfural	96	0.19
9.603	Isoxazole, trimethyl-	79	0.13
11,088	4H-Pyran-4-one, 2,3-dihydro-3,5	92	0.58
12.881	Ethyl hydrogen fumarate	90	0.12
24.565	Dodecanamide, N,N-bis(2-hydrox	94	0.45
30.483	Hexadecanoic acid	94	3.61
32.355	10E,12Z-Octadecadienoic acid	90	2.62
32.856	Linoelaidic acid	92	5,90
35.128	Oleyl alcohol, chlorodifluoroacetate	83	0.63
40.056	Z-7-Hexadecenal	83	1.73
42.254	Cinnamamide, N-(p-hydroxyphen	90	1.11
43.050	Rolipram	56	0.60
48.203	Campesterol	85	1.28
48.771	Stigmasta-5,23-dien-3.beta.-ol	88	1.75
50.307	Stigmast-5-en-3-ol, (3.beta)	84	2.13
50.554	beta-Sitosterol	66	1.14
51.420	24-Norursa-3,12-diene	79	1.53
52.516	Lanosterol	82	1.51
54.782	Lupeol	83	0.46
58.365	Betulin	87	0.82
60.358	2H-Benzo[F]oxireno[2,3-E]benzofuran-8	70	0.02
64.429	Nonacosanal	83	0.06
73.037	Eicosane	70	0.11
81.284	6-Nitro-cylohexadecane-1,3-dione	79	0.03

The results authenticated that the main compounds found in the ethanol extract of lotus rhizomes were dominant with linoleic, hexadecanoic, and octadecadienoic acids. These compounds are the fatty acids that may also exhibit antioxidant activity (Naik *et al*., 2021). Other triterpenoid compounds are lanosterol and betulin, which also have anti-inflammatory and antibacterial activities. Steroid compounds, such as betulin, are familiar for their antioxidant properties and their ability to enhance reproductive hormones and reduce cholesterol (Bishayee *et al*., 2022).

The identification of linoleic, hexadecanoic, and octadecadienoic acids as main components of the ethanol extract of lotus rhizomes, along with other bioactive compounds like lanosterol and betulin, underscores the plant's potential as a functional food and natural therapeutic agent. Further studies could explore the specific mechanism of action of these compounds and their potential in treating oxidative stress-related diseases, inflammation, and other health conditions.

Bioactivity of the lotus rhizome extract

In traditional Chinese medicine, the *N. nucifera* has been effective in treating inflammation, enhancing stamina, particularly in men, and promoting weight loss. Similarly, its other uses include making *N. nucifera* rhizome soup and brewing it with warm water (Wang and Zhao, 2024). The biological activity of some metabolite compounds in the lotus rhizome extract based on the GC-MS analysis appears in Table 5.

Linoleic acid (5.90%)

Linoleic acid is a nutrient derived from the carbonyl functional group ($C_{18}H_{32}O_2$). The human body utilizes linoleic acid as an energy source and as a precursor for synthesizing physiological regulators called eicosanoids, which include prostaglandins, prostacyclins, thromboxanes, and leukotrienes. Additionally, linoleic acid plays a crucial role as a structural component of cell membranes, influencing their properties, such as fluidity, flexibility, and permeability. Past studies showed that, in contrast to lower levels, the higher blood levels of linoleic acid correlated with a reduced risk of coronary heart disease, stroke, and type 2 diabetes mellitus (Jackson *et al*., 2024).

Stigmasterol (2.13%)

Stigmasterol ($C_{29}H_{48}O$, stigmasta-5,22-dien-3-ol) is a sterol compound found in plants and has various health benefits. Structurally, stigmasterol is similar to cholesterol; however, it is plant-based. The said biocompound has considerable pharmacological effects, including anti-inflammatory, anti-diabetic, antioxidant, and cholesterol-lowering properties. Stigmasterol can lower cholesterol levels by reducing its absorption in the digestive tract, which helps reduce the risk of heart disease and atherosclerosis. Additionally, this compound has anti-inflammatory effects by reducing the production of inflammatory cytokines and inhibiting enzymes associated with inflammation, such as COX-2 and iNOS (Goswami *et al*., 2023).

Beta-sitosterol (1.14%)

Beta-sitosterol ($C_{29}H_{50}O$), a plant sterol found in fruits, vegetables, rhizomes, and seeds, is chemically similar to cholesterol. It may help to lower cholesterol levels by reducing the amount of cholesterol absorbed by the body and can also alleviate swelling in the prostate and other tissues (Wang *et al*., 2023). Furthermore, beta-sitosterol has emerged to increase levels of sex hormones, such as estradiol in females and testosterone in males. Additionally, beta-sitosterol functions as an immune modulator by targeting specific T-helper (Th) lymphocytes and enhancing the activity of natural killer (NK) cells and T lymphocytes (Durrani *et al*., 2024).

Betulin (0.82%)

Betulin ($C_{30}H_{50}O_2$) is a pentacyclic triterpenoid that functions as a metabolite with multiple therapeutic properties, including antiviral, analgesic, anti-inflammatory, and antineoplastic effects. Past research demonstrated that betulin inhibits proinflammatory cytokines, NFκB, and MAPK, leading to a reduction in lung and liver injuries in septic rats (Hossain *et al*., 2022). Moreover, betulin helps alleviate hyperglycemia by inhibiting hepatic glucose. Betulin can also exhibit renal-protective effects by lowering profibrotic protein levels in kidney tissues of chronic kidney disease (CKD) in rats, including transforming growth factor β (TGF-β), connective tissue growth factor (CTGF), and fibronectin (Adepoju *et al*., 2023).

Rolipram (0.60%)

Rolipram is a member of the pyrrolidin-2-one class, known for its pivotal role as an antidepressant and an inhibitor of phosphoric diester hydrolases. It also functions as an immunomodulator by suppressing the release of proinflammatory cytokines. Additionally, rolipram works by inhibiting PDE4, an enzyme that breaks down cyclic adenosine monophosphate (cAMP) in tissues. By preventing this breakdown, rolipram increases cAMP levels, which, in turn, regulate processes, i.e., inflammation, immune responses, and neurotransmission. Elevated cAMP levels also activate protein kinase A (PKA), influencing cellular responses related to inflammation and cognitive functions (Dong *et al*., 2021).

CONCLUSIONS

The lotus (*N. nucifera* L.) rhizomes contain numerous bioactive compounds, including tannins, terpenoids, steroids, phenols, flavonoids, and alkaloids. Quantitative analysis revealed a total flavonoid content of 9.23 ± 0.04 mg/g QE, which could indicate the presence of antioxidant activity. The DPPH antioxidant activity's assessment resulted in the IC_{50} value of 57.88 ppm, indicating strong antioxidant activity. GC-MS analysis identified the key bioactive compounds such as linoleic acid, hexadecanoic acid, stigmasterol, and beta-sitosterol. These findings suggest that lotus rhizomes could be a valuable source of medicinal compounds.

ACKNOWLEDGMENTS

The authors express gratitude to the Indonesian Education Scholarship, Center for Higher Education Funding and Assessment, and Indonesian Endowment Fund for Education (Grant No. 00989/BPPT/BPI.06/9/2023) for funding and Majelis Diktilitbang Muhammadiyah Indonesia for institutional support through Hibah RisetMU batch VIII.

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