Jurnal Pharmascience, Volume 13, Issue 1, March 2026, page: 207-219
ISSN-Print 2355 – 5386
ISSN-Online 2460-9560
https://dx.doi.org/10.20527/jps.v13i1.24618
Research Article

Metabolite Profiling of The Antioxidant Properties of The Aquatic Plant *Cyperus involucratus* Employing UHPLC-HRMS

Profil Senyawa Antioksidan dari Tanaman Air Tawar Cyperus involucratus melalui Pendekatan UHPLC-HRMS

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ABSTRACT

The genus *Cyperus* is widely recognized as decorative flora. Empirically, *Cyperus* plants have been utilized to treat wounds, inflammation, and digestive disorders. While the genus is reported to exhibit antioxidant properties, scientific data regarding *Cyperus involucratus* remains limited. This research aimed to identify antioxidant compounds in *C. involucratus* using Ultra-High-Performance Liquid Chromatography-High Resolution Mass Spectrometry (UHPLC-HRMS). The *C. involucratus* methanolic extract was obtained through maceration using a 70% methanol solvent. Separation was performed using a UHPLC gradient system with a flow rate of 0.3 mL/min , while compound identification was conducted using a Quadrupole-Orbitrap MS detector in positive mode. The radical scavenging activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The analysis identified 175 metabolites within the methanolic extract. Based on mzCloud library matching with confidence scores of 97.4–100%, 12 primary bioactive compounds were identified: oleamide, α -linolenic acid, kaempferol, scutellarin, hexadecanamide, 1-linoleoyl glycerol, 1-stearoy-rac-lglycerol, adenine, adenosine, L-tyrosine, stearamide and nootkatone. The extract possessed a moderate radical scavenger with an IC_{50} value of 57.94 µg/mL. These findings provide a scientific foundation for further investigation of *C. involucratus* as a potential natural antioxidant, although further *in vivo* studies are required to validate its broader pharmacological outcomes.

Keywords: Cyperus involucratus, **UHPLC-HRMS, Antioxidant, DPPH, Profiling**

ABSTRAK

Tanaman air tawar marga Cyperus secara luas dikenal sebagai flora hias. Secara empiris, berbagai spesies dari tanaman ini dimanfaatkan untuk mengobati luka, peradangan dan gangguan pencernaan. Marga Cyperus dilaporkan memiliki aktivitas antioksidan, namun informasi ilmiah mengenai rumput jekeng (Cyperus involucratus) masih terbatas. Penelitian ini bertujuan untuk mengidentifikasi profil metabolit dan aktivitas antioksidan pada ekstrak metanol C. involucratus menggunakan Ultra High Performance Liquid Chromatography-High Resolution Mass Spectrometry (UHPLC-HRMS) dan penentuan aktivitas penangkapan radikal bebas terhadap radikal bebas 2,2 difenil-1-pikrilhidrazil (DPPH). Tahap awal adalah pembuatan ekstrak dengan maserasi menggunakan pelarut metanol 70% hingga diperoleh ekstrak metanol C. involucratus. Proses pemisahan menggunakan kromatografi cair UHPLC dengan sistem fase gerak gradien, laju alir 0,3 mL/menit. Identifikasi senyawa menggunakan detektor MS Quadrupole-Orbitrap mode positif. Analisis profil metabolit berhasil mengidentifikasi 175 metabolit dalam ekstrak metanol C. involucratus. Berdasarkan pencocokan pustaka mzCloud dengan skor kepercayaan 97,4-100%, sebanyak 12 senyawa bioaktif mayor teridentifikasi meliputi oleamide, α-linolenic acid, kaempferol, scutellarin, hexadecanamide, 1-linoleoyl glycerol, 1-stearoy-rac-lglycerol, adenine, adenosine, L-tyrosine, stearamide dan nootkatone. Hasil penelitian menunjukkan bahwa ekstrak metanol C. involucratus memiliki kemampuan menengah dalam penangkapan radikal bebas terhadap DPPH (IC_{50} =57,94 μg/mL). Informasi ilmiah ini merupakan landasan awal ke arah pengujian in-vivo untuk pengembangan C. involucratus sebagai kandidat antioksidan alami yang potensial.

Kata Kunci: Cyperus involucratus, UHPLC-HRMS, Antioksidan, DPPD, Eksplorasi

I. INTRODUCTION

Indonesia ranks second globally in terrestrial biodiversity after Brazil and contains approximately 10% of the world's flowering plants, including 30–40% of Asia's plant species (Borelli et al., 2024). The *Cyperaceae* family is extensively dispersed globally, including polar regions, temperate zones, tropical areas, and arid climates in both the northern and southern hemispheres. This family can thrive in both terrestrial and marine habitats (Bezzera et al., 2022; Jailson et al., 2019). One of the abundant species, *C. involucratus* is a ligneous aquatic herbaceous plant. Wood comprises cellulose (54-68%) and lignin (24-32%) (See Fig.1) (Dávid et al., 2021; Hassanein et al., 2014).

Figure 1. *C. involucratus*

Empirically, genus *Cyperus* serving as a raw material for paper in Greek and Roman civilizations, as well as for food and medicinal purposes (Khalifa, 2021; Lawal et al., 2016). Its application has been documented in the management of digestive diseases, as an anti-inflammatory agent, an antioxidant, a diuretic, and for irregular periods (Babiaka et al., 2021; Dávid et al., 2021; Hassanein et al., 2014; Selma-Royo et al., 2022).

Research indicates that multiple species within the genus *Cyperu*s possess a range of chemical constituents, notably including terpenoids such as monoterpenes, sesquiterpenes, and triterpenoids, as well as steroids, polyphenols—specifically tannins and flavonoids—and saponins (Dávid et al., 2021; Nassar et al., 2015). Several isolates from this group have been documented to possess antioxidant, anti-inflammatory, antipyretic, analgesic, antibiotic, anticancer, bronchodilator, vasodilator, antidepressant, anti-arthritis, anti-obesity, spasmolytic, along with cytoprotective effects (Dharmono et al., 2022; Hamad, 2023; Taheri et al., 2021).

Indeed, investigations into the species *C. involucratus* remain limited, although multiple species within the same genus have been documented as possessing potential for development as candidates in herbal medicine.

The main purpose of the research was to investigate the phytochemical constituents of the methanolic extract of *C. involucratus* utilizing HR-LCMS approach and determine the antioxidant capacity toward DPPH.

II. METHOD

A. Sample Preparation

The plant selection was conducted via a randomized sampling method. The specimens of *C. involucratus* were collected from Pamijahan, Bogor, West Java, Indonesia and were authenticated at the Faculty of Fisheries and Marine Sciences, Institut Pertanian Bogor (IPB) - University (Identification No: 1709/IT3.F2.02/PT.01.09/M/B/2025).

Following collection, the specimens underwent thorough cleansing and wet sorting. The plant material was then finely sliced and dried in an oven at 40°C for 72 hours. After completing the dry sortation, the dried *C. involucratus* was turned into a powder.

B. Plant Extraction

A total of 150 g of the *C. involucratus* dried powder was macerated with 70% MeOH at 1:3 ratio for 24 hours. This was done three times over three-days period. The MeOH extracts were obtained after pooling the resultant filtrates and concentrating them via rotary evaporation.

The yield for each extract was also determined.

C. Metabolite Profiling

1. Sample preparation for analysis

An approximate mass of 5 to 10 mg of the extract was dissolved into 1 mL of analytical grade methanol and placed into a 1.5 mL vial. The solution was then sonicated for 30 minutes before being filtered and injected through a 0.22 µm PTFE syringe filter.

2. UHPLC parameters

A Vanquish™ UHPLC Binary Pump system (Thermo Scientific™, Germany) was used for the analysis.

Separation was achieved on a Thermo Scientific™ Accucore™ Phenyl-Hexyl column (100 mm × 2.1 mm ID × 2.6 µm) maintained at 40°C. The injection volume was 3 µL.

Solvent system: a binary solvent system was utilized with two solvents, whereas solvent A: 0.1% formic acid in water and solvent B: 0.1% formic acid in MeOH. The elution gradient was delivered at a flow rate of 0.3 mL/min. The gradient progressed from 5% to 90% Solvent B over 0–16 minutes, followed by a 4-minute hold, before returning to the initial conditions (5% B) by the 25-minute mark. (Table I).

Chromatographic conditions: separation was carried out on a Thermo Scientific™ Accucore™ Phenyl-Hexyl column (100 mm × 2.1 mm ID × 2.6 µm) maintained at 40°C. The injection volume was set at 3 µL.

Mobile phase: the binary system consisted of 0.1% formic acid in water (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B), both MS-grade. The flow rate was set at 0.3 mL/min.

Gradient program: the concentration of solvent B was increased from 5% to 90% in 16 minutes. When the concentration of solvent B reached 90%, the flow was extended for 4 minutes before returning to 5% until the 25 minute-mark (Table I).

Table I. UHPLC gradient elution

Time (min)	Flow Rate (mL/min)	Solvent A (%)	Solvent B (%)	Description
0.0 – 16.0	0.3	95.0 → 10.0	5.0 → 90.0	Linear Gradient
16.0 – 20.0	0.3	10.0	90.0	Isocratic Hold
20.0 – 25.0	0.3	95.0	5.0	Re-equilibration

3. HRMS detection

Compound detection was performed on a Q Exactive™ Hybrid Quadrupole-Orbitrap™ HRMS (Thermo Scientific™, Germany) equipped with an ESI source. Data acquisition was performed in full MS/dd-MS2 mode using XCalibur 4.4 software. The gas source was nitrogen with a flow of 32 AU (sheath), 8 AU (auxiliary), and 4 AU (sweep). The spray voltage was set at 3.30 kV, the capillary temperature was set at 320°C, and the auxiliary gas heater was set at 30°C. The resolution was set at 70,000 for full MS and 17,500 for dd-MS2, scanning the range 66.7–1000 m/z.

4. Data processing and identification

Raw data processing was performed using Compound Discoverer™ 3.2. Raw data analysis was performed based on comparison with raw data with solvent blanks. Data identification was performed using the mzCloud™ spectral library, where criteria for positive identification include a mass accuracy range of -5 to 5 ppm and MS2 preferred ions matching.

D. Metabolite Categorization

The mzCloud databases were utilized for comparing mass spectra and fragmentation patterns for identification of metabolites. The Relative Peak Area (%) helps to elucidate the semi-quantitative distribution of the identified compounds. Relative Peak Area (%) is calculated by the formula (Individual Peak Area / Total Integrated Area of Identified Compounds) x 100%. This method represents the relative ion abundance within the specific analytical conditions (ESI positive mode) and is intended for qualitative profiling.

E. DPPH Assay

The radical scavenging activity of the *C. involucratus* methanolic extract was evaluated using the DPPH assay according to a modified method (Windyaswari et al., 2022).

Briefly, various concentrations of the extract (5-50 μg/mL) were prepared. A 1 mL aliquot of each sample concentration was mixed with 1 mL of 0.1 mM DPPH solution in methanol. The mixture was shaken vigorously and incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Ascorbic acid was used as a positive control. The percentage of inhibition was determined using the following Equation 1.

$$DPPH\ assay = \frac{A0 - A1}{A0} \times 100\% \ldots (\mathbf{1})$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample. Results were reported as IC_{50} (μg/mL), representing the concentration

required to scavenge 50% of DPPH radicals, was determined by linear regression analysis (Wahdaningsih & Najini, 2025).

III. RESULTS AND DISCUSSION

A. Metabolite Profiling of *C. involucratus* Methanolic extract

The untargeted metabolite profiling of *C. involucratus* methanolic extract using UHPLC-HRMS/MS identified a total of 175 metabolites. Compound annotation was performed based on mzCloud library matching with similarity scores ranging from 97.4–100% and mass accuracy within ±5 ppm. (Nurlely et al., 2025).

Based on their chemical ontology from mzCloud databases, these constituents were categorized into primary and secondary metabolites. As shown in Figure 2, primary metabolites dominated the total ion chromatogram (89.69%) of the relative peak area, primarily represented by fatty acids, fatty amides, nucleobase and nucleosides, while secondary metabolites comprised 10.30 % consist of flavonoids and terpenoids. This distribution reflects the high relative ion abundance of structural lipids and fatty amides in the methanolic extract under ESI positive mode conditions.

Among the detected constituents, twelve major bioactive compounds were prioritized based on their high library matching scores (97.4–100%) and documented biological significance. These compounds are detailed in Table II, ordered by their Relative Peak Area 9(%).

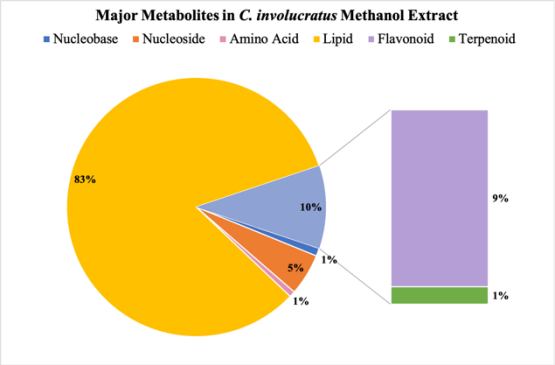

Figure 2. Distribution of Metabolites in *C. involucratus* Methanolic extract

The phytochemical profile of *C. involucratus* methanolic extract reveals a complex assembly of secondary metabolites beyond the flavonoid class. While flavonoids like kaempferol and scutellarin are prominent, the HRMS data also identified significant non-flavonoid constituents, such as the sesquiterpene nootkatone and various fatty acid amides including oleamide, stearamide, and hexadecanamide. From a technical perspective, the predominance of these specific classes in our results may be influenced by the ionization parameters and the extraction solvent used. This study employed a positive ionization mode (ESI+), which is highly sensitive for certain nitrogen-containing compounds and flavonoids but may have limitations in detecting other classes, such as highly acidic saponins or certain polyphenols that

are better ionized in negative mode (Wojtanowski & Mroczek, 2018). Additionally, the use of 70% methanol as a solvent primarily targets semi-polar to polar metabolites (Tu et al., 2025). Nevertheless, the detection of these diverse compounds is consistent with the metabolic complexity found in the *Cyperaceae* family, suggesting that the extract's radical scavenging activity results from a multi-target synergistic effect involving multiple chemical classes rather than flavonoids alone.

Table II. Qualitative profiling of major bioactive compound in *C. involucratus* methanolic extract via UHPLC-HRMS

No	Metabolite Name	Molecular Formula	Molecular Weight (Da)	RT (min)	Relative Peak Area (%)*	Classes	Pharmacological Activity
1	Oleamide	$C_{18}H_{35}NO$	281.27	14.55	15.64	Fatty Amide	Hypnotic, anti inflammatory
2	α -Linolenic acid	$C_{18}H_{30}O_2$	278.22	14.53	10.22	Unsaturated Fatty Acid	Omega-3, antioxidant
3	Kaempferol	$C_{15}H_{10}O_6$	286.04	7.60	12.15	Flavonoid (Flavonol)	Antioxidant, anticancer
4	Scutellarin	$C_{21}H_{18}O_{12}$	462.08	5.85	8.42	Flavonoid Glycoside (Flavonoid)	Anti inflammatory, neuroprotective
5	Hexadecanamide	$C_{16}H_{33}NO$	255.25	14.50	6.88	Fatty Amide	Antiinflammatory, antimicrobial
6	1-Linoleoyl glycerol	$C_{21}H_{38}O_4$	354.27	14.22	5.30	Monoglyceride (Lipid)	Antimicrobial
7	1-Stearoyl-rac-glycerol	$C_{21}H_{42}O_4$	358.30	15.66	4.90	Monoglyceride (Lipid)	Emollient, antimicrobial
8	Adenine	$C_5H_5N_5$	135.05	1.10	4.85	Nucleobase (Purin)	Metabolic signaling, energy transfer
9	Adenosine	$C_{10}H_{13}N_5O_4$	267.09	1.10	3.12	Nucleoside	Vasodilator, anti-inflammatory
10	L-Tyrosine	$C_9H_{11}NO_3$	181.07	1.10	2.75	Amino acid	Neurotransmitter precursor
11	Stearamide	$C_{18}H_{37}NO$	283.28	15.52	2.21	Fatty Amide	Biological signaling
12	Nootkatone	$C_{15}H_{22}O$	218.16	13.63	2.12	Sesquiterpenoid (Terpenoid)	Anti inflammatory, anti obesity.

*) *Relative Peak Area (%) represents the proportional distribution of ion intensities and does not reflect absolute concentration (w/w)*

The presence of flavonoids (kaempferol and scutellarin) in the methanolic extract of *C. involucratus* is associated with free radical scavenging activity. Windyaswari et al. (2022) reported that flavonoids are responsible for antioxidant activity through an electron donor mechanism. Furthermore, the content of sesquiterpenes, nucleosides, unsaturated fatty acids, and fatty amides in the methanolic extract of *C. involucratus* is reported to have a synergistic correlation with antioxidant activity through free radical scavenging and chain reaction termination (Bouyahya et al., 2024; Rauf et al., 2024; Wojtanowski & Mroczek, 2018). This creates a significant opportunity for the use of *C. incolucratus* as a natural antioxidant to be developed in the future.

In the methanolic extract of *C. involucratus*, lipids represent the predominant metabolite group, including fatty amides and fatty acid amides. While this study primarily validates the antioxidant potential of the extract, literature reports suggest that fatty amides may exhibit anti-inflammatory properties, and fatty acid amides have been noted for antibacterial and cytotoxic activities in other biological models. (Olteanu et al., 2025). The fatty amide and fatty acid amides gives significant contribution to the overall radical scavenging capacity through hydrogen donation and synergistic effects with phenolic compounds (Kurhaluk et al., 2025; Lahreche et al., 2023).

The presence of adenine, adenosine, and L-tyrosine in the *C. involucratus* methanolic extract as primary metabolites contributes functionally to its antioxidant profile (Furuhashi, 2020). Nucleosides and nucleobases participate in cytoprotective mechanisms and potential metal chelation, whereas L-tyrosine possesses a phenolic group capable of donating hydrogen atoms to stabilize free radicals (Furuhashi, 2020; Neyra Recky et al., 2021). The presence of these compounds, alongside key flavonoids like kaempferol, suggests a synergistic effect that enhances the overall DPPH radical scavenging capacity while maintaining the chemical integrity of the extract against oxidative degradation (Bouyahya et al., 2024; Rauf et al., 2024).

Concurrently, the secondary metabolite profile of the *C. involucratus* methanolic extract revealed a flavonoid and terpenoids of with flavones and flavonols as the predominant constituents. In general, flavonoids have strong antibacterial and antioxidant properties, while the sesquiterpenes which has cyclic structure and double bonds facilitate effective electron delocalization, which can terminate lipid peroxidation chain reactions and maintain cell membrane integrity against oxidative damage (Jha et al., 2022; Masyita et al., 2022).

B. Correlation with Antioxidant Activity (DPPH Assay)

The antioxidant capacity of *C. involucratus* methanolic extract was evaluated using the DPPH radical scavenging assay and ascorbic acid as control (Table III). The DPPH free radical, commonly employed to assess the capacity of compounds to function as free-radical scavengers and hydrogen donors, provides a rapid, straightforward, and cost-effective approach for evaluating antioxidant properties. In vitro, various plant extractions have demonstrated the ability to counteract DPPH radical scavenging activity (Mentari & Wahyudi, 2025; Wahdaningsih & Najini, 2025). The IC_{50} value of *C. involucratus* methanolic extract 57.94 μg/mL indicating moderate activity (Baliyan et al., 2022; Phongpaichit et al., 2007).

This biological activity is closely linked to the metabolite profile presented in Table II.

Table III. DPPH Assay of *C.involucratus* methanolic extract

Sample	Absorbance (516 nm)	IC_{50} (μg/mL)	Category
Positive control: Ascorbic acid	0.715	3.33±0.18	Strong
C.involucratus methanolic extract	0.608	57.94±0.31	Moderate

Notably, the presence of key flavonoids such as kaempferol and scutellarin provides a robust biochemical basis for the observed activity. Their antioxidant mechanism is primarily attributed to the presence of hydroxyl groups which facilitate hydrogen atom donation to stabilize DPPH radicals (Baliyan et al., 2022; Del Mondo et al., 2022).

In addition to flavonoids, the sesquiterpenoid identified in *C. involucratus* contributes to the antioxidant capacity by stabilizing free radicals through electron or hydrogen atom donation (Jha et al., 2022; Masyita et al., 2022).

The presence of this sesquiterpene suggests a synergistic interaction with other phenolic constituents and supporting the observed IC_{50} value (Kurhaluk et al., 2025)

Furthermore, the detection of unsaturated fatty acids like α -linolenic acid suggests a synergistic effect that enhances the extract's overall capacity to mitigate oxidative stress, though these indirect mechanisms require further direct validation (de Jorge Gouvêa et al., 2023)

IV. CONCLUSION

The results of this study provide comprehensive characterization information on the metabolite profile of *C. involucratus* methanolic extract using UHPLC-HRMS. Of the 175 identified

metabolites, 12 major metabolites were identified as a combination of primary metabolites (lipids and amino acids) and secondary metabolites (flavonoids and terpenoids). All of these major compounds were reported to correlate with the initial antioxidant potential of the extract observed in the DPPH radical scavenging assay. Although the identified metabolites indicate that *C. involucratus* is a promising source of natural antioxidants, these results are preliminary. Further investigations involving target quantification and in vivo biological models are needed to ensure the validity of the activity and safety of *C. involucratus* for its use in the pharmaceutical field.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

This work was supported by The Institute of Research Community and Services (LPPM) at Universitas Jenderal Achmad Yani (Hibah Internal LPPM UNJANI 2025, No Skep 188/UNJANI/VI/2025). The author would like to thank the Department of Biology Pharmacy, Faculty of Pharmacy, Universitas Jenderal Achmad Yani and EBM SciTech for the facilities that has been provided.

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