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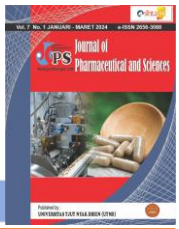

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Physicochemical Characterization and Antioxidant Activity of Ethanolic Extract of Wild Stinging Nettle (*Urtica dioica* L.)

Karakterisasi Fisikokimia dan Aktivitas Antioksidan Ekstrak Etanol Jelatang Liar (*Urtica dioica* L.)

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Abstract

Urtica dioica L. (wild stinging nettle) is a plant traditionally used as a food source and medicinal plant and is known to contain various bioactive compounds. This study aimed to characterize a 70% ethanolic extract of *U. dioica* based on quality parameters, total flavonoid content, and antioxidant activity. The roots, stems, and leaves of *U. dioica* were combined and extracted by maceration for 3 × 24 h. Extract characterization included the determination of moisture and ash contents using gravimetric methods, total flavonoid content using an aluminum chloride colorimetric method with quercetin as the reference standard, and antioxidant activity using the DPPH radical-scavenging assay. The extraction yielded 9.95% based on the dry weight of the simplicia. The extract showed a moisture content of 19.71 ± 0.29%, ash content of 32.13 ± 0.96%, and total flavonoid content of 1.8765 ± 0.0079 g quercetin equivalents (QE)/100 g sample, corresponding to 18.77 mg QE/g extract. The antioxidant assay showed 66.54 ± 0.11% inhibition of DPPH radicals under the experimental conditions used. These findings provide baseline information on the physicochemical and phytochemical characteristics of the combined root, stem, and leaf extract of *U. dioica*, supporting further research on extract standardization and identification of its bioactive constituents.

Keywords: Antioxidant Activity; Ethanolic Extract; Flavonoids; Extract Characterization; Urtica dioica.

Abstrak

Urtica dioica L. (jelatang liar) merupakan tanaman yang dimanfaatkan sebagai bahan pangan dan obat tradisional serta diketahui mengandung berbagai senyawa bioaktif. Penelitian ini bertujuan untuk mengkarakterisasi ekstrak etanol 70% *U. dioica* berdasarkan parameter mutu, kandungan flavonoid total, dan aktivitas antioksidan. Akar, batang, dan daun *U. dioica* dikombinasikan dan diekstraksi menggunakan metode maserasi selama 3 × 24 jam. Karakterisasi ekstrak meliputi penetapan kadar air dan kadar abu secara gravimetri, kadar flavonoid total menggunakan metode kolorimetri aluminium klorida dengan kuersetin sebagai standar, serta aktivitas antioksidan menggunakan metode penangkapan radikal DPPH. Ekstraksi menghasilkan rendemen sebesar 9,95% berdasarkan bobot simplisia kering. Ekstrak memiliki kadar air sebesar 19,71 ± 0,29%, kadar abu sebesar 32,13 ± 0,96%, dan kadar flavonoid total sebesar 1,8765 ± 0,0079 g QE/100 g sampel, setara dengan 18,77 mg QE/g ekstrak. Aktivitas antioksidan menunjukkan persentase inhibisi radikal DPPH sebesar 66,54 ± 0,11% pada kondisi pengujian yang digunakan. Temuan ini memberikan data dasar mengenai karakteristik fisikokimia dan fitokimia ekstrak kombinasi akar, batang, dan daun *U. dioica* yang dapat mendukung penelitian lebih lanjut mengenai standardisasi ekstrak dan identifikasi senyawa bioaktif.

Kata Kunci: Aktivitas Antioksidan; Ekstrak Etanol; Flavonoid; Karakterisasi Ekstrak; Urtica dioica.

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Introduction

Natural products constitute a major source of bioactive compounds derived from living organisms, including plants, fungi, animals, and microorganisms. In the field of pharmacognosy, natural products play a pivotal role as sources of drug candidates, health supplements, and phytopharmaceutical raw materials due to their diverse secondary metabolites with potential pharmacological properties. Medicinal plants continue to be an essential component of healthcare systems, particularly in developing countries. The World Health Organization estimates that nearly 80% of the population in developing regions still relies on traditional plant-based medicines to meet their primary healthcare needs [1,2].

One medicinal plant that has recently attracted considerable scientific interest is *Urtica dioica* L., commonly known as stinging nettle. This species is widely distributed across temperate regions of Asia, Europe, North America, and Africa. For centuries, *U. dioica* has been utilized as both a food source and a traditional remedy [3]. Nevertheless, in agricultural settings, the plant is often regarded as a weed because of its rapid growth, high adaptability, and extensive dispersal capacity [4].

The species epithet "*dioica*" refers to the dioecious nature exhibited by several subspecies of this plant, whereas the genus name *Urtica* originates from the Latin words *uro* and *urere*, meaning "to burn" and "to sting," respectively. These characteristics are believed to underlie the traditional use of stinging nettle to stimulate blood circulation and alleviate musculoskeletal disorders, such as arthritis and paralysis [5].

Numerous studies have demonstrated that *U. dioica* contains a wide range of bioactive constituents, including phenolic compounds, flavonoids, phenolic acids, terpenoids, sterols, alkaloids, lignans, carotenoids, vitamins, minerals, and other secondary metabolites [6,7]. These phytochemicals contribute to the plant's diverse biological activities, including antioxidant, anti-inflammatory, antidiabetic, antimicrobial, anticancer, and cardioprotective effects [8].

The biological activities of *U. dioica* are largely attributed to its ability to counteract oxidative stress. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are physiologically generated during cellular metabolism. However, excessive production of these reactive species may induce oxidative damage to essential biomolecules, such as DNA, proteins, and lipids, thereby contributing to the development of various degenerative disorders, including diabetes mellitus, atherosclerosis, cardiovascular diseases, cancer, and neurodegenerative diseases [9,10]. Antioxidant compounds, particularly flavonoids and phenolic constituents, are capable of scavenging free radicals through electron- or hydrogen-donating mechanisms, thereby reducing oxidative damage [11,12].

Despite the extensive evidence regarding the pharmacological potential of *U. dioica*, the quality and bioactivity of plant extracts are strongly influenced by extraction methods, solvent types, and the characteristics of the raw materials. Therefore, extract characterization represents a crucial step in the standardization of natural products to ensure their quality, safety, and consistency of biological activity. Parameters such as moisture content and ash content are commonly used as non-specific indicators of extract quality, providing important information on the stability, purity, and physicochemical properties of the extract. In contrast, total flavonoid content and antioxidant activity reflect the biological potential of the resulting extract [13,14] .

Based on these considerations, the present study aimed to characterize the 70% ethanolic extract of *Urtica dioica* by evaluating its moisture content, ash content, total flavonoid content, and antioxidant activity. The findings of this study are expected to provide a scientific basis for the standardization of *U. dioica* extracts as prospective raw materials for the development of herbal and phytopharmaceutical products.

Methods

Study Design

This study employed a laboratory-based experimental design to characterize the 70% ethanolic extract of wild stinging nettle (*Urtica dioica* L.) based on quality parameters, including moisture and ash contents, and phytochemical parameters, including total flavonoid content and antioxidant activity.

Sample Collection and Botanical Identification

Wild stinging nettle (*Urtica dioica* L.) was collected from Plemburan, Sleman Regency, Special Region of Yogyakarta, Indonesia. Prior to sample collection, botanical identification was performed at the Laboratory of Plant Systematics, Faculty of Biology, Universitas Gadjah Mada, to confirm the taxonomic identity of the plant material.

Sample Preparation and Extraction

The roots, stems, and leaves of *U. dioica* were collected and combined as the plant material for extraction. The three plant parts were homogenized prior to extraction. The use of the whole plant was based on considerations of material availability and efficient utilization of the collected plant material. The plant material was washed with running water, cut into small pieces, and dried in an oven at 50°C for 24 h. The dried material was subsequently ground using a mechanical grinder to obtain powdered simplicia.

Extraction was performed using the maceration method with 70% ethanol, following the procedure reported by Fadilah & Susanti (2020). The powdered simplicia was placed in a maceration vessel and pre-moistened with 70% ethanol for approximately 10 min. Additional 70% ethanol was then added until the simplicia was completely immersed. The maceration process was carried out at room temperature for 3 × 24 h, with replacement of the solvent every 24 h. The macerates obtained from each extraction cycle were collected and combined, then concentrated using a rotary evaporator under reduced pressure until a viscous ethanolic extract was obtained. The extraction yield was calculated as the percentage ratio between the weight of the resulting viscous extract and the initial weight of the dried simplicia.

Extract Characterization

The resulting *U. dioica* ethanolic extract was characterized at the Center for Food and Nutrition Studies, Universitas Gadjah Mada, under testing registration number PS/184/VI/2023. The characterization included determination of moisture content, ash content, total flavonoid content, and antioxidant activity. The analyses were conducted according to the analytical procedures applied by the testing laboratory.

Determination of Moisture Content

The moisture content of the *U. dioica* ethanolic extract was determined using a gravimetric method. Briefly, 1 g of the extract was accurately weighed into a previously dried and weighed dish. The sample was dried in an oven at 105°C for 5 h, cooled in a desiccator, and weighed. Drying and weighing were repeated until a constant weight was obtained. Moisture content was expressed as the percentage of weight loss relative to the initial sample weight.

Determination of Ash Content

Total ash content was determined using a gravimetric method. Approximately 2 g of the extract was weighed into a silica crucible that had previously been ignited and brought to a constant weight. The sample was incinerated in a muffle furnace at 600°C until the organic matter was completely removed. The crucible was subsequently cooled in a desiccator and weighed. The ignition and weighing process was continued until a constant weight was obtained. Ash content was expressed as the percentage of inorganic residue relative to the initial sample weight.

Determination of Total Flavonoid Content

Total flavonoid content was determined using the aluminum chloride ($AlCl_3$) colorimetric method. Briefly, 1 mg of *U. dioica* ethanolic extract was dissolved in 10 mL of 70% ethanol to obtain a solution with a concentration of 100 ppm. Subsequently, 0.5 mL of the sample solution was mixed with 0.2 mL of 10% $AlCl_3$ solution, 0.2 mL of 1 M acetic acid, and 3.0 mL of distilled water. The reaction mixture was incubated at room

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temperature for 30 min, and its absorbance was subsequently measured using a UV–Vis spectrophotometer at the maximum absorption wavelength.

A calibration curve was prepared using quercetin as the reference standard. The initial quercetin standard concentration was 0.249 mg/mL. Aliquots of 0.1, 0.2, 0.3, 0.4, and 0.5 mL were used to obtain concentrations of 0.0249, 0.0498, 0.0747, 0.0996, and 0.1245 mg/mL, respectively. The calibration curve produced a linear regression equation of $y = 8.1687x + 0.0146$, with a coefficient of determination of $R^2 = 0.9988$.

Total flavonoid content was calculated from the resulting regression equation and expressed as grams of quercetin equivalents per 100 g of sample (g QE/100 g), equivalent to percentage (%).

Determination of Antioxidant Activity

Antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging assay according to the analytical procedure applied by the Center for Food and Nutrition Studies, Universitas Gadjah Mada. Following the reaction and incubation procedure, the absorbance of the sample and control solutions was measured using a UV–Vis spectrophotometer at the appropriate wavelength.

Antioxidant activity was expressed as the percentage of DPPH radical inhibition (% inhibition), calculated based on the difference in absorbance between the control and sample solutions.

Data Analysis

All measurements were performed in at least duplicate. The data were analyzed descriptively and are presented as mean ± standard deviation (SD).

Results and Discussion

Sample Identification

Botanical identification was performed at the Laboratory of Plant Systematics, Faculty of Biology, Universitas Gadjah Mada. The identification confirmed that the plant material used in this study was *Urtica dioica* L., registered under specimen number 0172/STb./XI/2022. Based on the botanical identification, the plant was classified within the Division Magnoliophyta, Class Liliopsida, Order Urticales, Family Urticaceae, Genus *Urtica*, and Species *Urtica dioica* L., locally known as wild stinging nettle.

Characteristics of the Ethanolic Extract of Wild Stinging Nettle (*Urtica dioica*)

The roots, stems, and leaves of *U. dioica* were combined and homogenized before extraction. A total of 8,950 g of fresh plant material yielded 870 g of dried simplicia. Maceration with 70% ethanol for 3 × 24 h yielded 86.6 g of a dark green viscous extract, corresponding to an extraction yield of 9.95% based on the dry weight of the simplicia.

The use of the whole plant was intended to maximize the utilization of the available plant material and improve the efficiency of sample preparation, given the limited availability of *U. dioica* at the collection site. However, because the roots, stems, and leaves were combined, the present results represent the characteristics of a whole-plant extract and cannot be used to determine the contribution of individual plant organs to the measured parameters. The characteristics of the resulting ethanolic extract are presented in Table 1.

Table 1. Characteristics of the ethanolic extract of *Urtica dioica*

Parameter	Content (%)		
	Replicate I	Replicate II	Mean ± SD
Moisture content	19.92	19.50	19.71 ± 0.29
Ash content	31.45	32.81	32.13 ± 0.96
Total flavonoid content	1.8709	1.8821	1.8765 ± 0.0079
Antioksidan activity	66.4670	66.6167	66.54185 ± 0.11

The extraction yield of 9.95% indicates that a measurable fraction of the dried plant material was extracted using 70% ethanol. The extraction efficiency may be influenced by solvent polarity, extraction duration, plant matrix characteristics, and the distribution of soluble constituents within different plant tissues. The use of 70% ethanol provides an intermediate-polarity extraction medium that can facilitate the recovery of a broad range of polar and moderately polar phytochemicals.

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Moisture Content

The moisture content of the *U. dioica* ethanolic extract was 19.71 ± 0.29%. This value exceeds the maximum moisture content of 10% specified in the General Standard Parameters for Medicinal Plant Extracts issued by the Ministry of Health of the Republic of Indonesia [16]. A relatively high moisture content may compromise extract stability by providing favorable conditions for microbial growth and potentially accelerating the degradation of susceptible constituents through hydrolytic reactions [17].

Nevertheless, the moisture content of viscous plant extracts may vary depending on the plant species, plant parts, extraction procedure, and concentration or evaporation conditions. Moisture contents ranging from approximately 5% to 30% have been reported for viscous extracts [18]. In the present study, the relatively high moisture content may partly be associated with the use of 70% ethanol, which contains a substantial proportion of water, together with the characteristics of the whole-plant matrix.

From a quality-control perspective, the observed moisture content indicates that the extract did not meet the aforementioned 10% criterion and therefore warrants further optimization of the drying or concentration process before being considered for standardized preparation. Additional drying approaches, such as vacuum-assisted drying or freeze-drying, may be considered in future studies to improve extract stability. However, the suitability of a particular drying method should be experimentally evaluated because excessive heat or prolonged processing may also affect thermolabile bioactive compounds.

Ash Content

The total ash content of the *U. dioica* ethanolic extract was 32.13 ± 0.96%. Ash content reflects the inorganic residue remaining after incineration and may originate from naturally occurring minerals in the plant as well as extrinsic materials such as soil, dust, or other inorganic contaminants [19].

The ash content obtained in the present study was higher than values reported in previous studies. Tarasevičienė *et al.* (2023) reported ash contents of 18.86% and 18.41% for *U. dioica* leaves and roots, respectively, whereas other studies reported values of 16.21% and 17.67% [21,22]. The higher value observed in the present study may partly reflect the use of roots, stems, and leaves as a combined raw material. Differences in mineral accumulation among plant organs, together with variations in soil characteristics, nutrient availability, geographical location, and cultivation or collection conditions, may contribute to differences in ash content [23–26] .

However, total ash analysis does not distinguish between intrinsic plant minerals and extrinsic inorganic contaminants. Therefore, the high ash value observed in this study should be interpreted as an indicator of total inorganic residue rather than as evidence of a specific mineral composition. Further elemental analysis would be required to identify and quantify individual minerals.

Total Flavonoid Content

The total flavonoid content of the *U. dioica* ethanolic extract was 1.8765 ± 0.0079 g quercetin equivalent (QE)/100 g sample, corresponding to 18.77 mg QE/g extract. This value was slightly higher than that reported for *U. dioica* leaf extract prepared using the same ethanol concentration, which contained 16.03 mg QE/g extract [20]. The difference may reflect variations in plant material and extraction conditions between the two studies, particularly because the present study used a combination of roots, stems, and leaves.

Solvent composition is an important factor affecting flavonoid recovery. Hydroalcoholic solvents such as 70% ethanol provide an intermediate-polarity medium that can facilitate the extraction of phenolic and flavonoid compounds with different solubility characteristics [20,27]. Extraction duration may also influence compound recovery. Haido, Matti and Taher (2024) reported that increasing extraction time can enhance mass transfer until equilibrium is reached between the plant matrix and extraction solvent. However, prolonged extraction may increase the possibility of degradation of susceptible bioactive compounds through exposure to oxygen and light [28,29]. Thus, the 3 × 24 h maceration period used in this study should not be interpreted as an optimal extraction duration for all *U. dioica* preparations.

The phytochemical composition of *U. dioica* varies among plant organs. Rutin has been identified as an important flavonoid constituent in the leaves, while the concentrations of rutin and 3-caffeoylquinic acid have been reported to differ between roots and aerial parts [31,32]. These differences highlight the importance of specifying the plant material when comparing flavonoid content across studies. Therefore, the flavonoid content obtained in this study represents the composite extract of the roots, stems, and leaves rather than the flavonoid content of any individual plant organ.

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Antioxidant Activity

The antioxidant activity of the *Urtica dioica* ethanolic extract was evaluated using the DPPH radical scavenging assay and exhibited an inhibition value of 66.54 ± 0.11%. This result indicates that the wild stinging nettle extract possesses considerable antioxidant capacity at the tested concentration.

The antioxidant activity of *U. dioica* is likely associated with the presence of hydroxyl groups in flavonoid molecules, which are capable of donating hydrogen atoms or electrons to stabilize DPPH free radicals [28]. Consequently, higher flavonoid concentrations are generally associated with greater antioxidant potential and enhanced protection against oxidative stress [33]. However, the antioxidant activity of a plant extract should not be attributed exclusively to its total flavonoid content because multiple phytochemical classes may contribute to radical-scavenging activity. The present study did not identify individual antioxidant compounds or quantify total phenolic content; therefore, the contribution of flavonoids to the observed DPPH inhibition cannot be quantitatively established.

The antioxidant activity of plant extracts is influenced by several factors, including the plant parts used and the extraction solvent. In the present study, extraction was performed using the entire plant and 70% ethanol as the solvent. Previous studies have shown that methanolic extracts of *U. dioica* roots exhibit slightly higher antioxidant activity than ethanolic extracts, with inhibition percentages of 46.71% and 45.03%, respectively [34].

Another study evaluating *U. dioica* leaf extracts using FRAP, DPPH, and ABTS assays reported a FRAP value of 7.50 mM Fe(II)/g dry weight and IC_{50} values of 31.38 μg/mL and 23.55 μg/mL for the DPPH and ABTS assays, respectively [35]. Lower IC_{50} values indicate stronger antioxidant activity. These differences illustrate that antioxidant activity values obtained using different plant parts, extraction solvents, concentrations, and assay systems should not be interpreted as directly equivalent.

The antioxidant activity observed in this study is likely related to the relatively high flavonoid content of the extract. Flavonoids and other phenolic compounds are known to neutralize free radicals by donating electrons or hydrogen atoms, thereby inhibiting oxidative stress. The use of 70% ethanol as the extraction solvent may have contributed to the relatively high antioxidant activity because solvents of intermediate polarity are more effective in extracting phenolic compounds [36,37].

The addition of water to alcoholic solvents may also promote the swelling of plant tissues, thereby increasing the contact area between the solvent and the plant matrix and enhancing extraction efficiency [38,39]. Nevertheless, several studies have reported that aqueous extracts may exhibit higher antioxidant activity in specific assays, such as the ABTS assay, in which inhibition values as high as 91.83% have been observed [28]. Recent advances in extraction technologies, including high-pressure extraction, have also been shown to enhance antioxidant activity and improve protection against DNA damage [40]. These findings suggest that optimization of extraction conditions may be an important direction for future research.

A limitation of the present study is that antioxidant activity was evaluated as percentage inhibition at a single test concentration, and the concentration of the extract used in the DPPH assay was not documented in the available experimental records. Consequently, an IC_{50} value could not be calculated, and the observed inhibition value should not be interpreted as an intrinsic potency measure or directly compared with IC_{50} values reported in other studies. In addition, no positive control was included in the original DPPH assay. Future studies should evaluate a series of extract concentrations, include an appropriate reference antioxidant such as ascorbic acid or quercetin as a positive control, and determine the IC_{50} value to provide a more robust assessment of antioxidant potency. Furthermore, quantitative analysis of total phenolic content and individual phenolic/flavonoid constituents would help clarify their contribution to the antioxidant activity of *U. dioica* extract.

Conclusions

The 70% ethanolic extract of wild stinging nettle (*Urtica dioica* L.), obtained by maceration for 3 × 24 h, yielded 9.95% based on the dry weight of the simplicia. The extract showed a moisture content of 19.71 ± 0.29% and an ash content of 32.13 ± 0.96%, while the total flavonoid content was 1.8765 ± 0.0079 g quercetin equivalents (QE)/100 g sample, equivalent to 18.77 mg QE/g extract. The DPPH assay demonstrated 66.54 ± 0.11% radical inhibition under the experimental conditions used. These findings indicate that the extract contains measurable flavonoids and exhibits DPPH radical-scavenging activity; however, its antioxidant potency cannot be quantitatively determined from the present data because the extract concentration used in

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the assay was not documented and an IC_{50} value could not be calculated. The moisture content exceeding the applicable 10% criterion also indicates the need for further optimization of extract drying or concentration before standardized preparation. Overall, this study provides baseline information on the physicochemical and phytochemical characteristics of the combined root, stem, and leaf extract of *U. dioica* and supports further investigation into its standardization and bioactive constituents.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article. The authors have no financial, personal, or professional relationships that could be construed as influencing the research design, data collection, analysis, interpretation of the results, or the decision to publish the findings presented in this study.

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