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Antioxidant Activity Test of The Aqueous Fraction of Tetanus Leaves (*Leea aequata* L.) Using The DPPH Method

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Abstract

Antioxidants play a crucial role in neutralizing free radicals, thereby helping to prevent cell damage and maintain overall health. This study aims to evaluate the antioxidant activity of the aqueous fraction of Tetanus (Leea aequata L.) leaf extract using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The extraction process was carried out through maceration using ethanol as a solvent, followed by aqueous fractionation. Antioxidant activity was measured based on the extract's ability to scavenge DPPH free radicals, expressed as the IC_{50} value. The results showed that the aqueous fraction of Tetanus leaf extract exhibited high antioxidant activity, with an IC_{50} value of 30.23 mg/L. The total phenolic and flavonoid contents in the aqueous fraction were 460.70 mg GAE/100g and 12,621.14 mg QE/100g, respectively. These findings align with pre vious studies indicating that the ethanolic extract of Leea angulata leaves also possesses strong antioxidant activity with a similar IC_{50} value. Therefore, the aqueous fraction of Tetanus leaf extract has the potential to be an effective natural antioxidant source and can be further developed as a raw material for health supplements or pharmaceutical products.

Abstrak

Antioksidan memainkan peran penting dalam menetralkan radikal bebas, sehingga membantu mencegah kerusakan sel dan menjaga kesehatan secara keseluruhan. Penelitian ini bertujuan untuk mengevaluasi aktivitas antioksidan fraksi air dari ekstrak daun Tetanus (Leea aequata L.) menggunakan metode 2,2-diphenyl-1-picrylhydrazyl (DPPH). Proses ekstraksi dilakukan melalui macerasi menggunakan etanol sebagai pelarut, diikuti dengan fraksinasi air. Aktivitas antioksidan diukur berdasarkan kemampuan ekstrak dalam menangkap radikal bebas DPPH, yang diekspresikan dalam nilai IC_{50}. Hasil penelitian menunjukkan bahwa fraksi air dari ekstrak daun Tetanus memiliki aktivitas antioksidan yang tinggi, dengan nilai IC_{50} sebesar 30,23 mg/L. Kandungan total fenolik dan flavonoid dalam fraksi air masing-masing adalah 460,70 mg GAE/100g dan 12.621,14 mg QE/100g. Temuan ini sejalan dengan penelitian sebelumnya yang menunjukkan bahwa ekstrak etanol dari daun Leea angulata juga memiliki aktivitas antioksidan yang kuat dengan nilai IC_{50} yang serupa. Oleh karena itu, fraksi air dari ekstrak daun Tetanus memiliki potensi untuk menjadi sumber antioksidan alami yang efektif dan dapat dikembangkan lebih lanjut sebagai bahan baku untuk suplemen kesehatan atau produk farmasi.

Keywords: *Antioxidants, DPPH, Leea aequata, Aqueous Fraction, Total Phenol, Total Flavonoid*

Kata kunci: *Antioksidan, DPPH, Leea aequata, Fraksi Air, Total Fenol, Total Flavonoid*

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INTRODUCTION

Free radicals are a well-known topic in the medical field. Numerous studies have shown that free radicals can cause various diseases in the body. With the rapid advancement of time an unregulated lifesty lesuch as consuming fast food without balancing it with exercise, along with exposure to air pollution from motor vehicles and cigarette smokecontri butes to the increase of free radicals in the body. If their levels exceed the normal capacity, this can lead to cell damage[1].

Free radicals are atoms or molecules that have unpaired electrons. This condition makes them highly reactive, as these atoms or molecules will seek to pair by attacking and binding electrons from surrounding molecules. If the bound electron comes from an ionic compound, the impact is not too dangerous. However, if the electron binds to a covalent compound, the consequences can be severe. This is due to the sharing of electrons within the outer orbital. Theoretically, free radicals are formed when covalent bonds are broken. Due to their active nature and

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irregular movement within living organisms, free radicals can trigger degenerative diseases such as cataracts, premature aging, rheumatism, liver disorders, and coronary heart disease[1].

In the human body, there is a defense mechanism capable of neutralizing free radicals through the antioxidant system. Antioxidants arecom pounds that function as electron donors, thereby inhibiting oxidation processes by binding to free radicals and highly reactive molecules without transforming into free radicals themselves[2].

Antioxidants are classified into two types: endogenous and exogenous.Endogenous antioxidants are naturally produced by the body, but their ability to neutralize free radicals is limited, making external intake of exogenous antioxidants necessary. Based on their sources, exogenous antioxidants are further categorized into natural and synthetic. Examples of synthetic antioxidants include butylated hydroxy anisole (BHA), butylated hydroxy toluene (BTH), and tertiary butyl hydro quinone (TBHQ)[3].

Meanwhile, natural antioxidants are generally derived from natural sources such as vegetables and fruits. Natural antioxidants are preferred over synthetic ones because they are free from chemical contamination and are easily accessible in the surrounding environment. Some examples of natural antioxidants include flavonoids, phenolic compounds, and folic acid[1].

Antioxidants are compounds that play a crucial role in protecting and stabilizing cells from damage caused by free radicals generated through oxidative stress. Additionally, antioxidants help convert free radicals into metabolic by products that are subsequently eliminated from the body. Consuming antioxidant rich fruits and vegetables can reduce the risk of diseases associated with free radicals [4]
Several plant species, including ginger, Jamaican cherry leaves, African leaves, bay leaves, dates, paprika (green and yellow vegetables), cabbage, strawberries, carrots, dark leafy vegetables, and bananas, are widely recognized for their antioxidant activity[4].

The use of natural-based medicine can serve as an alternative antioxidant source with fewer side effects and lower costs compared to conventional drugs. One plant family known for its potential in lowering blood glucose levels is the Leeaceae family. Several species from this family have been studied for their hypogly cemic effects, such as Leea indica, where its ethanol leaf extract has been proven to exhibit antioxidant properties[2].

Another study also revealed that Leea macrophylla has hypoglycemic effects. The ethanol extract from its leaves has shown potential in restoring damaged pancreatic cells in albino rats induced with streptozotocin[5].

Meanwhile one of the Leeaceae species still utilized in traditional medicine is *Leea aequata* L. In North Sumatra, this plant is locally known as Tetanus and is commonly used as herbal medicine by the local community. Therefore, this research aims to isolate active compounds with potential α-glucosidase inhibitory activity, which plays a significant role in blood glucose regulation[5].

This study aims to evaluate the antioxidant activity of the aqueous fraction of methanol extract from Tetanus leaves

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(*Leea aequata* L.) grown in North Sumatra. The evaluation is based on the polarity levels of the solvents used in the extraction process. To measure the antioxidant effectiveness of this fraction, the study employs the DPPH (2,2-diphenyl-1 picrylhydrazyl) assay, a commonly used spectrophotometric method for determinina compound's ability to scavenge free radicals. The results of this study are expected to provide further insights into the potential of natural antioxidants from Tetanus leaves and their contributions to the pharmaceutical and health care fields[4].

METHODOLOGY

This study aims to evaluate the antioxidant potential of the aqueous fraction of Tetanus (Lee aquata L.) leaves using the DPPH (2,2-diphenyl-1-picrylhydrazyl) me- thod. The research is conducted systematically to ensure the validity and accuracy of the obtained data and to provide scientific contri- butions to the development of natural antioxidant compounds[6].

Research Design

This study consists of several main stages that are carried out sequentially as follows:

Sample Collection and Preparation

The leaves of Tetanus (*Leea aequata* L.) are sourced from a predetermined location in North Sumatra. The samples are dried using the drayer cabinet method to maintain the stability of the active compounds. The dried leaves are then ground using a blender or grinder until a fine powder with a homogeneous particle size is obtained.[7]

Extraction and Fractionation

The leaf powder is extracted using the maceration method with distilled water as a solvent for 3 x 48 hours at room temperature with continuous stirring. The obtained filtrate is then concentrated and further fractionated using a liquid-liquid extraction method with an organic solvent to obtain the aqueous fraction[7].

Antioxidant Activity Assay Using the DPPH Method

A 0.1 mM DPPH solution in methanol is prepared as the test reagent. The aqueous fraction sample is tested at various concentrations to determine its antioxidant activity. The absorbance of the solution is measured using a UV-Vis spectrophotometer at a wavelength of 516 nm. The IC50 value is calculated as the main parameter to determine the antioxidant activity.

Data Analysis

The absorbance results from the DPPH assay are analyzed using linear regression to determine the IC50 value, which represents the sample concentration required to scavenge 50% of DPPH free radicals. The interpretation of the IC50 value follows a classification system based on antioxidant activity. An IC50 value of less than 50 µg/mL indicates very strong antioxidant activity. Values between 50 and 100 µg/mL are categorized as strong antioxidant activity. Values ranging from 100 to 200 µg/mL are considered to show moderate antioxidant activity, while values greater than 200 µg/mL suggest weak antioxidant activity. This classification helps assess the potency of the antioxidant properties of the sample[8].

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Tabel 1. Analytical Methods and Instruments Used

No	Test Parameter	Method	Instrument
1	Sample Concentration	Weighing	Digital Balance
2	DPPH Assay	Spectrophotometry	UV-Vis Spectrophotometer
3	Data Analysis	IC50 Calculation	Statistical Software

Tabel 2. Research Methodology Stage

No	Research Stage	Description
1	Sample Collection and Preparation	Tetanus leaves are collected, air-dried, and then ground into a fine powder.
2	Extraction with 96% Ethanol	The powdered leaves are extracted using maceration with 96% ethanol for 5 × 24 hours at room temperature with continuous stirring.
3	Fractionation (Liquid-Liquid Separation)	The extraction filtrate is fractionated using the liquid-liquid separation method with an organic solvent to obtain the aqueous fraction.
4	Antioxidant Activity Test using DPPH	The aqueous fraction sample is tested with a 0.1 mM DPPH solution in methanol, and the absorbance is measured using a UV-Vis spectrophotometer at a wavelength of 516 nm.
5	Data Analysis and Interpretation	The IC50 value is calculated to determine antioxidant capacity, and results are compared with standard antioxidants such as vitamin C or BHT.

RESULT AND DISCUSSION

Antioxidant Activity of Vitamin C, Aqueous Fraction of Tetanus Leaves (Leea aequata L.)

The antioxidant activity of Vitamin C, the aqueous fraction of Tetanus leaves (*Leea aequata* L.) was evaluated using the DPPH assay method. The inhibition percentage for each sample was analyzed through a linear regression equation to determine the IC_{50} values, which represent the concentration required to scavenge 50% of DPPH free radicals.

Tabel 3. Antioxidant Activity of Vitamin C, Aqueous Fraction, and Ethanolic Extract of *Leea aequata* L. Leaves

No	Sample	IC_{50} Value (ppm)	Antioxidant Categories
1	Vit C	5.84877533	Strong
2	Aqueous Fraction	30.238124	Moderate

Antioxidant Activity of Vitamin C

The inhibition curve for Vitamin C (Figure 1) follows the linear equation: **$y = 17.65x - 18.826$** ($R^2 = 0.9956$). The high R^2 value indicates a strong correlation between concentration and inhibition percentage, suggesting a reliable and predictable antioxidant response. As a reference antioxidant, Vitamin C exhibited strong free radical scavenging activity, with an IC_{50} value falling within the highly potent category ($IC_{50} < 50$ µg/mL). This demonstrates the exceptional antioxidant potential of Vitamin C, which is consistent with its well-known role as a powerful antioxidant in various biological systems.

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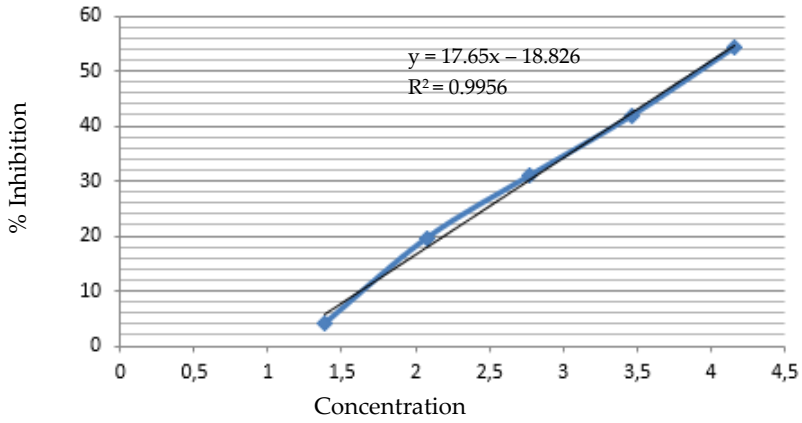

Figure 1. Vitamin C Curve

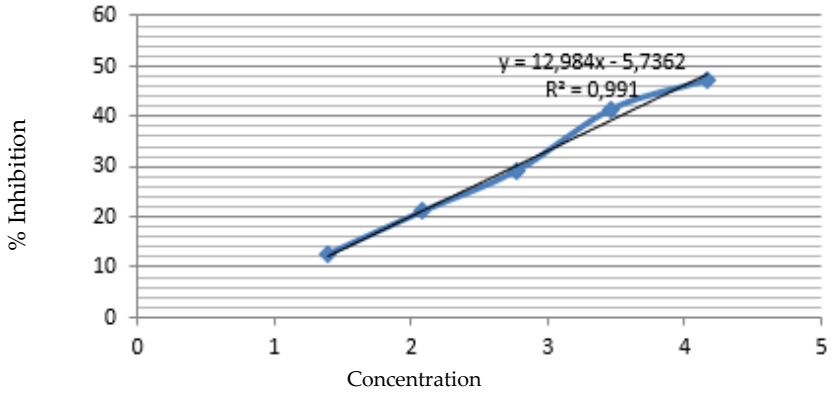

Figure 2. Aqueous Fraction Curve

Antioxidant Activity of Aqueous Fraction

The antioxidant potential of the aqueous fraction (Figure 2) is represented by the equation: **y = 12.984x - 5.7362** (R^2 = 0.991). The regression coefficient (R^2) suggests a highly linear relationship between concentration and inhibition, indicating that the extract's antioxidant activity is concentration-dependent. While the ethanolic extract's antioxidant activity is lower than that of Vitamin C, it still demonstrates considerable free radical scavenging capacity. Both the aqueous fraction of *Leea aequata* L. leaves exhibits antioxidant properties, although with varying levels of potency. These differences in antioxidant activity may be attributed to the distinct composition of bioactive compounds present in each extract. The aqueous fraction may contain a different profile of compounds, such as water-soluble antioxidants, that contribute to its activity[10]. The variation in potency between the two extracts suggests that the extraction method significantly influences the bioactive compound composition, which in turn affects the antioxidant potential. Therefore, further research is necessary to identify and quantify the specific bioactive compounds responsible for these antioxidant effects[7]

Despite these differences, the aqueous fraction shows promising antioxidant potential. This indicates that *Leea aequata* L. leaves, regardless of the extraction method, possess valuable antioxidant properties that may be harnessed for practical applications[6]. The moderate antioxidant activity observed in

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the aqueous fraction suggests it could be an effective natural source of antioxidants, suitable for use in food and pharmaceutical industries[9].

The findings of this study underscore the potential of *Leea aequata* L. leaf extracts as natural antioxidants. Given the growing interest in plant-based antioxidants for their health benefits and potential therapeutic effects, *Leea aequata* leaves may serve as a valuable resource for both the pharmaceutical and food industries[2]. However, to fully unlock the potential of these extracts, additional studies are needed. In vivo assays, compound isolation, and detailed bioactive compound profiling will help identify the specific compounds responsible for the observed antioxidant effects[5]. These studies will also be critical for understanding the mechanisms of action of these compounds in biological systems, ultimately providing a clearer picture of their therapeutic potential. Further research into the bioavailability and safety of these compounds will also be necessary to ensure their practical applications in health products. Moreover, exploring synergistic effects with other natural compounds could enhance their antioxidant efficacy. Additionally, assessing the stability of these compounds in various formulations is crucial for developing effective commercial products[11].

CONCLUSION

This study demonstrates that both the aqueous fraction and ethanolic extract of *Leea aequata* L. leaves exhibit antioxidant activity, as confirmed by the DPPH assay. Regression analysis showed a strong correlation between sample concentration and inhibition percentage, with Vitamin C serving as the positive control. Among the tested samples, Vitamin C exhibited the highest antioxidant activity, followed by the ethanolic extract. The IC_{50} values suggest that the antioxidant potential of the extracts is influenced by bioactive compounds such as polyphenols and flavonoids. Despite its moderate activity, the ethanolic extract holds potential as a natural antioxidant source for pharmaceutical and food applications. Further research is needed to isolate and identify the active compounds responsible for these effects and evaluate their biological activity.

REFERENCES

1. Anggarani, A. M., Ilmiah, M. & Nasyaya Mahfudhah, D. Antioxidant Activity of Several Types of Onions and Its Potensial as Health Supplements. *Indones. J. Chem. Sci.* **12**, 103–111 (2023).
2. Pharmacia, J., Waluya, M., Amaliah, R., Pusmarani, J. & Nasir, N. H. Skrining Fitokimia dan Uji Aktivitas Antioksidan Fraksi Air , Etil Asetat dan n - Heksan Kulit Batang Kayu Jawa (Lannea grandis (Dennst .) Engl) dengan Metode FRAP (Ferric Reducing Antioxidant Power) Phytochemical Screening and Antioxidant Activity A. **3**, (2024).
3. Retnaningtyas, Y., Wulandari, L. & Fauzan Arrozi. AKTIVITAS ANTIOKSIDAN FRAKSI AIR DAN FRAKSI ETER KOMBINASI EKSTRAK METANOL DAUN KOPI ARABIKA (Coffea arabica L.) DAN KULIT BATANG KAYU MANIS (Cinnamomum burmannii Nees ex Bl.). *J. Tumbuh. Obat Indones.* **16**, 11–18 (2023).
4. Pusmarani, J., Ulfa, U., Dewi, C. & Nasir, N. H. Aktivitas Antioksidan Fraksi Air, Etil Asetat dan N-Heksan Kulit Pisang Raja (Musa paradisiaca var. Sapientum). *J. Mandala Pharmacon Indones.* **8**, 275–283 (2022).
5. Sari, W. A. *et al.* UJI AKTIVITAS ANTIOKSIDAN EKSTRAK ETANOL AKAR

Pharmaceutical and Clinical Journal of Nusantara (PCJN)
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https://doi.org/10.58549/pcjn.v3i01.85

BAJAKAH TAMPALA (Spatholobus littoralis Hassk) MENGGUNAKAN. **2**, 374–384 (2024).

6. Wijaya, A., Rifki Widiastuti, N., Novi Rahmadani, A. & studi Diploma III Farmasi Akademi Farmasi Indonesia Yogyakarta, P. Antioxidant Activity of Water, Ethyl Acetate And Chloroform Fraction of Papaya (Carica papaya L.) Leaf Extract By DPPH Method. *J. Jamu Kusuma* **3**, 2798–0332 (2023).
7. Misfadhila, S., Azizah, Z. & Maisarah, L. Penggunaan Metode DPPH dalam Penentuan Aktivitas Antioksidan Ekstrak Metanol dan Fraksi Daun Sukun (Artocarpus Altilis (Parkinson Ex F. A. Zorn) Fosberg). *J. Farm. Higea* **11**, 75–82 (2019).
8. Harahap, U. *et al.* Antioxidant and antibacterial activities of ethanol extract of Vernonia amygdalina Delile. Leaves. in *AIP Conference Proceedings* vol. 2342 080011 (AIP Publishing LLC, 2021).
9. Dalimunthe, A. *et al.* The effect of extraction methods towards antioxidant activity of ethanol extract of Picria fel-terrae Lour. Herbs. in *IOP Conference Series: Earth and Environmental Science* vol. 1115 012040 (IOP Publishing, 2022).
10. Vrca, I. *et al.* Identification of Phenolic Compounds Present in Three Speedwell (Veronica L.) Species and Their Antioxidant Potential. *Antioxidants* **13**, 1–15 (2024).
11. Susilowati & Wulandari, S. Aktivitas Antioksidan Fraksi Etil Asetat dan Fraksi Air Ekstrak Etanol Daun Salam (Syzygium polyanthum (Wight .) Walp .) dengan Metode DPPH (1 , 1 Difenil-2 pikrilhidrazil. *Indones. J. Med. Sci.* **6**, 39–44 (2019).