Pharmaceutical and Clinical Journal of Nusantara (PCJN)
Volume 03, No. 01, March 2025, Page 21-25
E-ISSN 2985 – 4040 (Online Media)
https://nusantarascientificjournal.com/index.php/pcjn/index
https://doi.org/10.58549/pcjn.v3i01.88

Antioxidant Activity Test of The Ethyl Acetate Fraction of Tetanus Leaf (*Leea aequata* L.) Using The DPPH Method

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Abstract

Degenerative diseases, such as diabetes, stroke, and cancer, are increasingly common in modern society, with free radicals as one of the main causes. Both natural and artificial sources of antioxidants are available. However, natural antioxidants are a better choice due to concerns over the negative effects of synthetic antioxidants. This study aims to evaluate the antioxidant activity of ethanol extract from titanus (leea aequata L.) leaves using DPPH (2,2-diphenyl-1-picrylhydrazyl) method. titanus leaves are known to contain secondary metabolites that have potential as antioxidants, including flavonoids and phenols. This type of research is an experimental method. The extraction process was carried out by maceration method using 96% ethanol, followed by fractionation using solvents with different polarities to obtain ethyl acetate fraction. The results showed that the ethyl acetate fraction showed the ability as a free radical antidote with an IC_{50} value of 11.309 ppm, which indicates significant antioxidant activity. These results indicate that titanus leaf extract has the potential to be used as a source of natural antioxidants in the prevention of degenerative diseases.

Keywords: Antioxidant, Tetanus leaf (*leea aequata* L.), DPPH, Extraction, Fractionation

Abstrak

Penyakit degeneratif, seperti diabetes, stroke, dan kanker, semakin umum dalam masyarakat modern, dengan radikal bebas sebagai salah satu penyebab utama. Sumber antioksidan alami dan buatan tersedia. Namun, antioksidan alami merupakan pilihan yang lebih baik karena kekhawatiran akan efek negatif dari antioksidan sintetis. Penelitian ini bertujuan untuk mengevaluasi aktivitas antioksidan ekstrak etanol dari daun titanus (leea aequata L.) menggunakan metode DPPH (2,2-difenil-1-pikrilhidrazil). Daun titanus diketahui mengandung metabolit sekunder yang memiliki potensi sebagai antioksidan, termasuk flavonoid dan fenol. Jenis penelitian ini adalah metode eksperimental. Proses ekstraksi dilakukan dengan metode maserasi menggunakan etanol 96%, diikuti dengan fraksinasi menggunakan pelarut dengan kepolaran yang berbeda untuk memperoleh fraksi etil asetat. Hasil penelitian menunjukkan bahwa fraksi etil asetat menunjukkan kemampuan sebagai penangkal radikal bebas dengan nilai IC_{50} sebesar 11,309 ppm, yang menunjukkan aktivitas antioksidan yang signifikan. Hasil ini menunjukkan bahwa ekstrak daun titanus memiliki potensi untuk digunakan sebagai sumber antioksidan alami dalam pencegahan penyakit degeneratif.

Kata kunci : *Antioksidan, Daun Titanus (leea aequata L.), DPPH, Ekstraksi, Fraksinasi*

Received: 06 March 2025 | *Revised: 08 April 2025* | *Accepted: 10 April 2025* | *Publish: 01 Mei 2025*

INTRODUCTION

In the context of health, degenerative diseases such as diabetes, stroke, coronary heart disease, obesity, cardiovascular disorders, liver dysfunction, cataracts, and cancer are often mentioned these days[1]. Free radicals are one of the main causes of these disorders, which are often caused by the body's cell functions declining from normal[2].

Although the human body has an enzyme-based antioxidant system, the amount is often insufficient to neutralize free radicals that enter the body. Antioxidants are substances that can inhibit oxidation reactions by donating electrons to free radicals, thus acting as a defense system against these free radicals[3]. Antioxidants are defined as inhibitors that inhibit oxidation by reacting with reactive free radicals to form unreactive and stable free radicals [2].

Both natural and artificial sources of antioxidants are available. However, natural antioxidants are a better choice due to concerns over the negative effects of synthetic antioxidants[4]. Natural antioxidants can stop lipid peroxidation in food, reduce degenerative disorders, and

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protect organisms from the harm of reactive oxygen species[5].

Antioxidant activity test can be done by using DPPH (*2,2-Diphenyl-1-Picrylhydrazyl*) method. DPPH is a free radical that can accept one hydrogen electron, so it is stable. The DPPH test method is one of the most widely used techniques to evaluate the effectiveness of a compound as an antioxidant. This test measures the ability of antioxidant compounds to neutralize free radicals. The free radical used in this test is *2,2-diphenyl-1-picrylhydrazyl* (DPPH), which is a synthetic free radical, stable at room temperature, and soluble in polar solvents. The stability of this free radical is due to the delocalized electrons in its molecule, making it unreactive like other free radicals. This method was chosen due to its advantages of being simple, easy to perform, fast, sensitive, and requiring only a small amount of materials[6].

Based on previous research, phytochemical screening of ethanol extracts of titanus leaves contains secondary metabolite compounds such as flavonoids, phenols, tannins, quinones, saponins, alkaloids, and steroids/triterpenoids. [7].

The tetanus plant (*leea aequata* L) is one source of natural materials that show antioxidant potential. These compounds help protect cells from free radical damage by neutralizing oxidants and preventing oxidative stress in the body, so tetanus leaves have secondary metabolites that have potential as antioxidants so it is necessary to isolate compounds to determine the active compounds [8]. The purpose of this study was to test the antioxidant activity of the ethyl acetate fraction contained in tetanus leaf extract.

METHODOLOGY

Materials and Tools

The samples used were obtained from the Patumbak area, namely tetanus leaves. The chemicals used were ethanol 96%, methanol, ethyl acetate, n-hexane, distilled water, *2,2-diphenyl-1-picrylhydrazyl* (DPPH), vitamin c and quercetin. The equipment used in the study was a set of extraction tools, rotary evaporator, uv vis spectrophotometer, and separating funnel.

Sample Preparation

Samples of tetanus leaves (*leea aequata* L) that have been collected are wet sorted, then washed thoroughly using running water. After drying in a drying cabinet, the simplisia was pulverized with a blender and stored in a tightly closed container at room temperature in a dry place. Dry sorting is then carried out to remove foreign objects that still remain[9,10].

Extraction and Fractionation

A total of 200 g of simplisia was extracted by maceration using 96% ethanol for 1 x 24 hours. The process was carried out for 3 days and then concentrated with a rotary evaporator and waterbath. The yield was calculated based on the percentage of weight (b/b) between the yield and the weight of the simplisia powder used by weighing[11].

The fractionation method used is liquid-liquid extraction, the method used is the charos paris method, namely thick ethanol extract dissolved with hot water filtered then extract it gradually using n-hexane and ethyl acetate, is the fractionation technique used. Concentrated fractions of water, n-hexane, and ethyl

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acetate were then obtained by filtering and concentrating each fraction[7].

Antioxidant Testing

This test was conducted to measure quantitative antioxidant activity using the DPPH test. Samples of extract, tetanus leaf fraction, vitamin c, and quercetin with concentration variations of 4, 8, 16, 32, and 64 ppm each as much as 2 ml, added with 2 ml of 100 ppm DPPH solution and placed in a dark room during the incubation time. Then the absorbance was measured at the maximum wavelength. The percentage of DPPH inhibition was calculated and the results were expressed as IC_{50} value (ppm)[12].

RESULTS AND DISCUSSION

After sorting, washing, chopping, drying, dry sorting, grinding, and sieving, tetanus (*leea aequata* L.) leaves are reduced to powdered simplisia, which can weigh up to 200 g. To make dry simplisia, tetanus leaves are made by wet sorting, washing with clean running water, and cutting. Then put into a simplisia drying cabinet with a temperature of 40°C[13].

Simplisia tetanus leaves as much as 200 mg produced a thick 96% ethanol extract of 130 grams so that the yield value of thick tetanus leaf extract was 26%. Ethanol 96% is suitable as a solvent because it is a universal solvent that can attract polar and nonpolar molecules[14]. Ethanol can dissolve molecules with polar, semi-polar, and non-polar properties because it is non-toxic, selective, has a high dissolving capacity, and has good absorption quality [15]. The 96% ethanol solvent has the properties of universality, polarity, ease of acquisition, and the ability to penetrate sample cell walls and produce concentrated extracts[16].

This study uses a liquid - liquid extraction fractionation method by shaking using a tool in the form of a separatory funnel. The principle of separation is based on the difference in polarity and specific gravity of the three fractions used. Therefore, three types of solvents are used that have different levels of polarity and do not mix with each other such as water which is polar, ethyl acetate which is semi-polar, and n-hexane which is non-polar. The results of the ethyl acetate fraction that has been obtained are concentrated using a tool in the form of a waterbath[17].

After that, the percentage of yield was calculated and the result of the ethyl acetate fraction was 25%.

Antioxidant Testing

The ethyl acetate fraction of the plant and the comparator were tested for their ability to reduce free radicals using *2,2-diphenyl-1-picrylhydrazyl* (DPPH) radicals. DPPH radical is a stable free radical with a wavelength of 515-517 nm that can be dissolved in ethanol or methanol. The ability of extracts, fractions, or positive controls in the form of quercetin and vitamin c is demonstrated by this procedure. These antioxidants have the ability to combat free radicals by converting purple free radicals into yellow non-radicals by giving hydrogen to DPPH[18]. There is a color shift from purple to yellow in the test results when extracts, fractions, or positive controls in the form of vitamin c and quercetin are operated. This is because the antioxidants in the sample have reduced the free radical molecules by

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giving electrons to DPPH, which causes the formation of picryl groups[19].

Antioxidant activity test obtained the final result in the form of IC_{50} value which is used as a parameter to see the magnitude of the ability of compounds that have potential as antioxidants. This study obtained results in the form of IC_{50} values on vitamin C of 5.848 ppm, quercetin 10.086 ppm, extract 39.484 and ethyl acetate fraction 11.309 ppm. These results are in line with previous research which shows the IC_{50} value of the extract of 6.09 ppm and ethyl acetate fraction of 5.11 ppm which means the sample has very strong antioxidant activity[20].

Where the smaller the IC_{50} value, the greater the antioxidant activity of a sample, and vice versa, the greater the IC_{50} value, the smaller the antioxidant activity of a sample[21].

The following figure shows that the ethyl acetate fraction (FEA) has the ability to counteract DPPH free radicals. Compared to positive controls such as vitamin c and quercetin, the ethyl acetate fraction showed promising results, although its IC_{50} value was higher than vitamin c (5.848 ppm) and quercetin (10.086 ppm), indicating that the lower the IC_{50} value, the stronger the antioxidant activity[22].

So it can be concluded that the ethyl acetate fraction of tetanus leaves has a very strong antioxidant activity value because the IC_{50} value obtained is below 50 ppm[21].

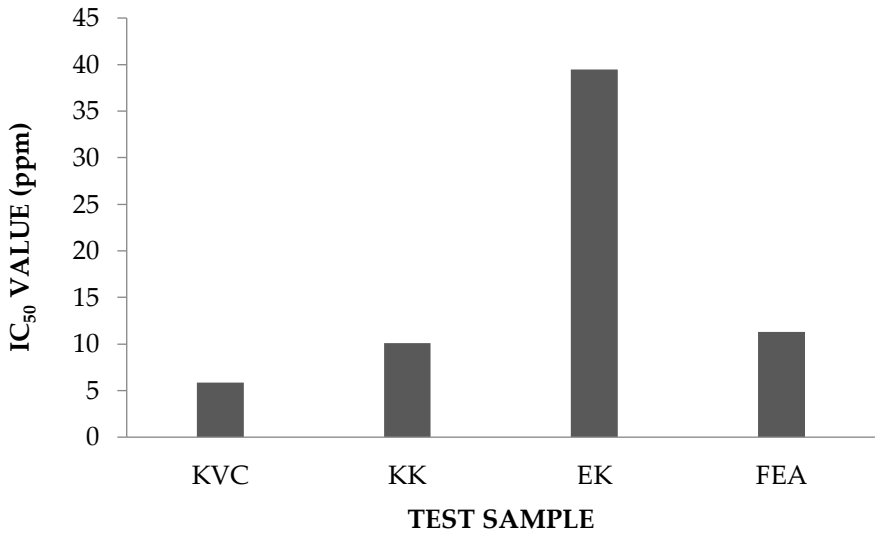

Figure1. DPPH free radical scavenging activity of vitamin c control (KVC), quercetin control (KK), viscous extract of tetanus leaves (EK) and ethyl acetate fraction of tetanus leaves (FEA).

CONCLUSIONS

From the results of this study it can be concluded that antioxidant activity testing using the DPPH method shows the ethyl acetate fraction of tetanus leaf extract has a good ability to neutralize free radicals. The test results show the IC_{50} value of the ethyl acetate fraction is 11.309 ppm, indicating that this fraction has significant antioxidant activity.

REFERENCE

1. Estevania dan Yusmarlisa. Uji Aktivitas Kandungan Antioksidan Pada Daun Bangun-bangun (Plectranthus amboinicus) Secara Spektrofotometri Ultraviolet-Visible. *J. Farm. Jfm* **1**, 16–20 (2018).
2. Kesuma, Y. *Antioksidan Alami Dan Sintetik*. (2015).

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https://nusantarascientificjournal.com/index.php/pcjn/index
https://doi.org/10.58549/pcjn.v3i01.88

3. Lubis, M. F., Syahputra, H., Illian, D. N. & Kaban, V. E. Antioxidant activity and nephroprotective effect of Lansium parasiticum leaves in doxorubicin-induced rats. *J. Res. Pharm.* **26**, 565–573 (2022).
4. Hamdi, O. A. A. *et al.* Neuroprotective and antioxidant constituents from Curcuma zedoaria Rhizomes. *Rec. Nat. Prod.* **9**, 349–355 (2015).
5. Mid, A. A., Syaima, H. & Hindryawati, N. Prosiding Seminar Nasional Kimia dan Terapan III 2023 eISSN 2987-9922 Jurusan Kimia FMIPA UNMUL. 37–39 (2024).
6. Ningsih, S. K. W. 聂倩 1， 郝志华 1， 王忠丽 1， 郜玉兰 1. **8**, 1149–1151 (2016).
7. Indrianto, R., Nastira, K., Emran, R. & Insanu, M. Current Research on Biosciences and Isolation of α -glucosidase enzyme inhibitor from titanus (Leea aequata L .). **6**, 41–47 (2024).
8. Mahdalena, D., Armaita & Maifita, Y. Analisis Aktivitas Anti Oksidan Flavonoid Daun Ekor Naga (Rhapidhophora pinnata Schott) dengan Dosis Bertingkat terhadap Hematokrit dan Kadar HB Mus Musculus Bablc Albino Jantan yang Diberi Papapran Asap Rokok. *-Shiha J. Med. Researsh* **1**, 12–20 (2020).
9. Etanol, E. & Kluwih, D. SOKHLETASI TERHADAP KADAR FLAVONOID TOTAL. **10**, 133–140 (2024).
10. Estefania, V. *et al.* ORIGINAL ARTICLE The effect of turmeric parent extract gel (Curcuma longa Linn) on incision wound healing in male white rats (Rattus norvegicus) Pengaruh gel ekstrak induk kunyit (Curcuma longa linn) terhadap penyembuhan luka sayat pada tikus putih . 616–627 (2024).
11. Satria, D. *et al.* Synergistic Antibacterial Effect of Ethyl Acetate Fraction of Vernonia amygdalina Delile Leaves with Tetracycline against Clinical Isolate Methicillin-Resistant Staphylococcus aureus (MRSA) and Pseudomonas aeruginosa. *Adv. Pharmacol. Pharm. Sci.* **2023**, 1–11 (2023).
12. Hasanah, N., Dahlia, A. A. & Handayani, V. Uji Aktivitas Antioksidan Ekstrak Daun Kedondong Laut (Nothopanax fructicosum (L.) Miq) Dengan Metode Peredaman Radikal Bebas DPPH. *Makassar Nat. Prod. J.* **1**, 10 (2023).
13. Suwandi, D. W., Imaroh, S. & Kurniati, N. F. AKTIVITAS ANTIINFLAMASI INFUSA BAWANG PUTIH SEGAR DAN BAWANG HITAM (Allium sativum L.) PADA TIKUS PUTIH (Rattus norvegicus L.)YANG DIINDUKSI KARAGENAN. *Acta Pharm. Indones.* **47**, 1–8 (2022).
14. Ridwanto, R. *et al.* Indonesian Journal of Pharmaceutical Antibacterial Activity Test of Malacca Leaf Extract (Phyllanthus emblica) Against Staphylococcus aureus and Staphylococcus epidermidis Bacteria. **07**, 22–27 (2024).
15. Suci, N., Khairunnisa, L., Nasri, N. & Kaban, V. E. Indonesian Journal of Pharmaceutical and Pengembangan Formulasi Krim Pelembab Kulit Berbasis Sari Buah Mangga (Mangifera indica L .): Pendekatan Fungsional dan Kosmetika Alami. **07**, 59–69 (2024).
16. Wendersteyt, N. V., Wewengkang, D. S. & Abdullah, S. S. UJI AKTIVITAS ANTIMIKROBA DARI EKSTRAK DAN FRAKSI ASCIDIAN Herdmania momus DARI PERAIRAN PULAU BANGKA LIKUPANG TERHADAP PERTUMBUHAN MIKROBA Staphylococcus aureus, Salmonella typhimurium DAN Candida albicans. *Pharmacon* **10**, 706 (2021).
17. Makalunsenge, M. O., Yudistira, A. & Rumondor, E. Uji Aktivitas Antioksidan Ekstrak dan Fraksi dari Callyspongia aerizusa yang Diperoleh dari Pulau Manado Tua. *Pharmacon* **11**, 1679–1684 (2022).
18. Satria, D. *et al.* Phytochemicals, proximate composition, minerals and volatile oil analysis of Zanthoxylum acanthopodium DC. fruits. *F1000Research* **12**, 227 (2023).
19. Devi Reskita Cahyani, Tamrin & RH.Fitri Faradilla. Evaluasi Metode In Vitro Pada Analisis Aktivitas Antioksidan Beberapa Buah Tropis: Studi Kepustakaan [Evaluation In Vitro Method Analysis In Antioxidant Activities of Some Tropical Fruits:A Review]. *J Sains Dan Teknol. Pangan* **5**, 3466–3480 (2020).
20. Mostofa, M. G. *et al.* Apoptosis-inducing anti-proliferative and quantitative phytochemical profiling with in silico study of antioxidant-rich Leea aequata L. leaves. *Heliyon* **10**, (2024).
21. Pratiwi, A. R., Yusran, Islawati & Artati. Analisis Kadar Antioksidan pada Ekstrak Daun Binahong Hijau Anredera cordifolia (Ten.) Steenis. *Bioma J. Biol. Makassar* **8**, 66–74 (2023).
22. Pratiwi, A. & Salimah, I. Aktivitas Antioksidan dan Antimikroba Minyak Atsiri Kembang Leson. *Al-Kauniyah J. Biol.* **13**, 139–146 (2020).