Extract
2026, Vol. 1, No. 1, pp. 16–21

Kognia Prisma Pustaka

Original Article

Antioxidant Activity of Ethanol Extract of Stems and Leaves of *Euphorbia hirta*

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Abstract

Euphorbia hirta L. is a medicinal plant traditionally used for various ailments, yet the differential bioactive potential of its specific organs requires elucidation. This study aimed to comparatively evaluate the extraction yield, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities of the ethanol extracts from the stems and leaves of *E. hirta*. The plant material was extracted via maceration using 96% ethanol. Antioxidant activity was assessed using the ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays, while the phytochemical contents were determined spectrophotometrically. The results revealed significant organ-specific variation. The leaf extract exhibited a substantially higher extraction yield (17%) and TPC (226.669 mg GAE/g) compared to the stem (4% yield; 52.270 mg GAE/g). Conversely, the stem extract demonstrated higher TFC (26.049 mg QE/g) than the leaves (20.604 mg QE/g). In antioxidant testing, the leaf extract displayed remarkably superior radical scavenging activity (DPPH IC_{50} = 6.538 µg/ml; ABTS IC_{50} = 4.832 µg/ml), approaching the potency of the quercetin standard, while the stem extract showed higher ferric reducing power (FRAP value of 533.315 µmol Fe^{2+}/g). The impact of this research is significant, as it scientifically validates the traditional preference for *E. hirta* leaves and identifies them as a highly potent, accessible source of natural antioxidants for managing oxidative stress-related conditions. Furthermore, it highlights the distinct phytochemical profiles of different plant parts, guiding the selective utilization of *E. hirta* parts for targeted nutraceutical and pharmaceutical applications.

Keywords

Antioxidant, *Euphorbia hirta*, Free radicals, Leaf, Stem.

1. Introduction

Oxidative stress is a condition of imbalance between the production of reactive oxygen species (ROS) and the ability of a biological system to neutralize them. ROS are reactive molecules that can damage various cellular components, including lipids, proteins, and DNA. ROS production occurs naturally in the body as a result of normal metabolism, primarily through the oxidative phosphorylation pathway in mitochondria (Midah et al., 2021). However, exposure to external factors such as air pollution, ultraviolet radiation, cigarette smoke, and the consumption of unhealthy foods can excessively increase ROS levels in the body (Sharifi-Rad et al., 2020). This imbalance can lead to oxidative damage to tissues and cells, contributing to various degenerative diseases such as cancer, diabetes mellitus, hypertension, atherosclerosis, and neurodegenerative diseases like Alzheimer's and Parkinson's

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Received: 25-02-2026; **Accepted:** 01-03-2026; **Published:** 09-04-2026

(Setiawan et al., 2019). Therefore, efforts to reduce oxidative stress are crucial in preventing and managing various diseases associated with free radicals.

The potential of medicinal plants as a source of natural antioxidants has gained increasing attention from the scientific community in recent decades, primarily as a safe and effective alternative for addressing modern health problems, especially those caused by oxidative stress (Kumar et al., 2021). The main advantage of medicinal plants lies in the complexity of their phytochemicals, which work synergistically and have minimal side effects compared to synthetic drugs (Karimi et al., 2015). This multi-target mechanism of action makes medicinal plants highly potential for treating complex diseases involving various pathological pathways, including degenerative diseases triggered by oxidative stress (Muscolo et al., 2024).

Euphorbia hirta L. is a small plant from the Euphorbiaceae family, widely distributed in tropical and subtropical regions (Al-Snafi, 2017). This plant is included in the Indonesian herbal pharmacopoeia list, containing the active compound quercetin, and has been known in traditional Chinese medicine, where the crude extract of the entire *E. hirta* plant is used to treat diarrhea due to enteritis in pigs, cattle, horses, sheep, and fish, as well as to cure gonorrhea and hematuria (Ghosh et al., 2019). This plant has long been used in traditional medicine to treat various ailments, including respiratory tract infections, digestive disorders, and inflammatory diseases (Nugroho et al., 2021; Tran et al., 2020). Its secondary metabolite content, especially phenolic compounds and flavonoids, has been reported to possess significant antioxidant activity. Various studies indicate that extracts from this plant can scavenge free radicals, thus having the potential to be used as a therapeutic agent in preventing or reducing the impact of oxidative stress on the body (Sahertia et al., 2023).

The differences in plant parts often influence the secondary metabolite content and their biological activities. Several studies have explored the influence of extraction methods, solvents, and plant parts on antioxidant potential. For instance, research conducted by Puspitasari et al. (2023) found that the ethanol extract of mature *E. hirta* leaves exhibited significant antioxidant activity compared to young leaves. In a study conducted by Suarjana et al. (2025) found that solvent polarity had a substantial influence on the antioxidant activity of *E. hirta* extracts, with ethanol being an optimal solvent for the extraction procedure. The findings of the FRAP, DPPH and ABTS tests exhibited that the *E. hirta* ethanol extract had a substantially greater antioxidant activities in comparison to ethyl acetate and n-hexane extracts. However, to date, no study has specifically compared the antioxidant activity between the stem extract and the leaf extract of *E. hirta*. Given that factors like leaf maturity and solvent polarity influence *E. hirta*'s antioxidant activity, we hypothesized that the plant part (stem vs. leaf) would also be a critical determining factor. Therefore, this study aims to quantitatively compare the *in vitro* antioxidant activity and total phenolic/flavonoid content of *E. hirta* stem and leaf extracts to identify the most potent source of natural antioxidants for potential pharmaceutical applications. The results of this study are expected to provide more comprehensive information regarding the plant part with more optimal antioxidant activity and to support the utilization of this plant in the health and pharmaceutical fields.

2. Methods

2.1. Materials

The plant sample used in this study was *Euphorbia hirta* L. obtained from the rice field area of Lukluk Village, Mengwi District, Badung Regency, Bali. Ethanol 96% (technical grade) and distilled water were purchased from CV. Saba Kimia, Denpasar, Bali. Methanol, Mayer's reagent, Dragendroff's reagent, Wagner's reagent, Magnesium powder, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from PT. Smart Lab Indonesia, Banten, Indonesia. 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), quercetin, acetate buffer (300 mM) pH 3.6, H_2SO_4, Folin-Ciocalteu reagent, gallic acid, concentrated acetic anhydride, Na-acetate, and ascorbic acid were purchased from Sigma-Aldrich, Missouri, USA. $FeCl_3$, $FeSO_4.7H_2O$, potassium persulfate, HCl, sodium acetate, $AlCl_3$, NaOH, chloroform, and sodium acetate were purchased from Merck Millipore, Massachusetts, USA.

2.2. Extract preparation

The preparation of *E. hirta* plant extract was carried out using the maceration method. The solvent used in this extraction process was 96% ethanol. A total of 200 grams each of the powdered simplicia from *E. hirta* leaves and stems were macerated with 1,000 mL of ethanol at room temperature for 3 days. The filtrate was filtered using a Buchner funnel to separate the filtrate from the residue. Subsequently, the filtrate was evaporated using a vacuum rotary evaporator at 50°C to separate the extract from the solvent, resulting in a viscous extract. The extract was then stored in a closed glass container.

2.3. Determination of total phenolic content

A total of 1 mL of the ethanol extract solution from *E. hirta* stems was taken, and the stock solution was then diluted 10-fold. Subsequently, 5 mL of 7.5% Folin-Ciocalteu reagent was added, the mixture was vortexed, and allowed to stand for 8 minutes. Next, 4 mL of 1% NaOH solution was added, vortexed until homogeneous, and left to stand for 30 minutes. The absorbance of the solution was measured at a wavelength of 739 nm. A similar procedure was performed on the ethanol extract of *E. hirta* leaves. The total phenolic content of the ethanol extracts from *E. hirta* leaves and stems was expressed in mg GAE/g extract (Putra et al., 2021).

2.4. Determination of total flavonoid content

A total of 0.5 mL of the stem extract solution was placed into a test tube, followed by the addition of 1.5 mL of methanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of sodium acetate,

and 2.8 mL of distilled water. The mixture was vortexed and allowed to stand for 30 minutes, after which its absorbance was measured using a UV-Vis spectrophotometer at 430 nm. A similar procedure was performed on the ethanol extract of *E. hirta* leaves. The total flavonoid content in the ethanol extracts of *E. hirta* leaves and stems was expressed as mg QE/g extract (Putra et al., 2021).

2.5. The FRAP assay

A total of 200 µL of the extract solution was placed into a cuvette, followed by the addition of 1.3 mL of methanol and 500 µL of FRAP reagent. The mixture was incubated for 15 minutes. Its absorbance was measured at a wavelength of 597 nm. The reducing power of the extract, in converting Fe^{3+} to Fe^{2+}, was expressed as the FRAP value (Putra & Suarjana, 2025).

2.6. The DPPH assay

A total of 2 mL of *E. hirta* stem extract solution at various concentrations was placed into a cuvette and mixed with 2 mL of DPPH. The mixture was then incubated for 15 minutes in a dark room. Its absorbance was measured at a wavelength of 516 nm. The same procedure was also performed on *E. hirta* leaf extract and quercetin as a positive control. A mixture of 2 mL of DPPH and 2 mL of methanol was used as a blank. The DPPH radical scavenging activity was expressed as the IC_{50} value (Putra et al., 2022).

2.7. The ABTS assay

A total of 1 mL of *E. hirta* stem extract solution at various concentrations was placed into a cuvette and mixed with 1 mL of $ABTS^+$ solution. The mixture was then incubated for 6 minutes in a dark room. Its absorbance was measured at a wavelength of 745 nm. The same procedure was also performed on *E. hirta* leaf extract and quercetin as a positive control. The $ABTS^+$ radical scavenging activity was expressed as the IC_{50} value (Putra et al., 2025).

2.8. Data analysis

Statistical analysis was performed using an independent t-test to compare the total phenolic content, total flavonoid content, and FRAP values between sample groups. Meanwhile, DPPH and $ABTS^+$ radical scavenging activities were analyzed using one-way ANOVA, followed by Tukey's test to identify significant differences among the tested sample groups.

3. Results and Discussion

The *Euphorbia hirta* L. plant used in this study was identified at the Botanical Characterization Laboratory, Plant Determination/Plant Identification, National Research and Innovation Agency. The purpose of identification is to accurately identify the plant under study to avoid errors when collecting research materials. Based on the yield calculation results of the extract from the stems and leaves of patikan kebo (*Euphorbia hirta* L.), the yield for each extract was obtained as follows (Table 1). The results of the calculation of the yield of ethanol extract of patikan kebo stems were 4% and ethanol extract of patikan kebo leaves was 17%. Extraction yield was calculated based on the ratio of the initial weight (weight of the simplicia) to the final weight (weight of the extract) multiplied by 100%. The research data shown in Table 1 indicate that the leaf extract produced a higher yield compared to the stem extract. The high yield obtained from the leaf sample is thought to be due to the sample being ground during the extraction process. This is supported by the previous finding that the finer the sample during the extraction process, the more effective and efficient the extraction becomes. This is further supported by Ismail (2018), who stated that reducing the particle size by blending/grinding the sample into powder results in a higher extract yield.

Table 1. Extraction yield

Sample	Mass of simplicia (g)	Mass of extract (g)	Yield (%)
Stem	200	8	4
Leaf	200	34	17

The results of the total phenolic content (TPC) test of the ethanol extract of the stem and leaves of *E. hirta* can be seen in Table 2. Based on the calculations of the TPC in Table 2, it is observed that the total phenolic content of the stem extract of *E. hirta* is 52.270 ± 1.066 mg GAE/g extract, while that of the leaf extract is 226.669 ± 5.035 mg GAE/g extract. This indicates that the highest total phenolic content was found in the ethanol extract of the leaves of *E. hirta*. Leaves typically produce more phenolics than stems due to their role as the plant's primary interface with the environment, requiring a robust chemical defense system against higher levels of abiotic stress such as UV radiation and biotic threats like herbivory. This frontline position necessitates the synthesis of phenolic compounds like flavonoids, which act as a sunscreen to mitigate UV damage and as feeding deterrents (Xu & Wang, 2025). This functional requirement is supported by a strong tissue-specific biosynthetic regulation; for instance, research on *Syzygium cumini* confirms that leaves exhibit significantly higher phenolic content and antioxidant activity compared to stems (Imran et al., 2025). Furthermore, studies on tea plants reveal that the accumulation pattern is governed by the expression of specific structural and regulatory genes in the phenylpropanoid pathway, with many genes showing higher relative expression in the leaves to drive this specialized metabolism (Jiang et al., 2013).

The results of the total phenolic content analysis (Table 2)

Table 2. TPC and TFC of ethanol extract of *E. hirta* stem and leaf

Sample	TPC (mg GAE/g extract)*	TFC (mg QE/g extract)*
Stem	52.270 ± 1.066	26.049 ± 0.754
Leaf	226.669 ± 5.035	20.604 ± 1.301

* = significantly different at $p<0.05$ using the independent t-test; TPC = total phenolic content; TFC = total flavonoid content; GAE = gallic acid equivalent; QE = quercetin equivalent.

Flavonoids are the largest group of naturally occurring The results of the total flavonoid content calculation based on Table 2 show that the total flavonoid content of the stem extract of *E. hirta* is 26.049 ± 0.754 mg QE/g extract, while that of the leaf extract is 20.604 ± 1.301 mg QE/g extract. This indicates that the highest total flavonoid content was found in the ethanol extract of the stems of *E. hirta* ($p < 0.05$). The findings of the present study reveal important insights into the distribution of bioactive flavonoids in different parts of *Euphorbia hirta* L. The total flavonoid content (TFC) in the stem extract (26.049 ± 0.754 mg QE/g extract) was notably higher ($p < 0.05$) than that in the leaf extract (20.604 ± 1.301 mg QE/g extract). This organ-specific variation in flavonoid accumulation contributes to the growing body of knowledge on this medicinally important plant species. The TFC values obtained in this study are consistent with, though somewhat lower than, recent findings from other research groups. A 2025 investigation by Suarjana et al. (2025) reported substantially higher total flavonoid content in ethanol extracts of *E. hirta* aerial parts, measuring 28.507 ± 0.464 mg QE/g extract . This difference may be attributed to variations in extraction methodology, plant phenological stage, or geographical origin of the plant material. The same study confirmed that ethanol is an optimal solvent for flavonoid extraction due to its appropriate polarity, which aligns with our choice of ethanol as the extraction solvent.

The results of the antioxidant activity test of the ethanol extract of the stem and leaves of *E. hirta* can be seen in Table 3. Based on the calculation of the FRAP values from Table 3, it is observed that the FRAP value of the stem extract is 533.315 ± 33.188 µmol Fe^{2+}/g extract, while that of the leaf extract is 407.921 ± 26.452 µmol Fe^{2+}/g extract. This indicates that the highest antioxidant activity using the FRAP method was found in the ethanol extract of the stems of *E. hirta* ($p < 0.05$). The observation that the ethanol extract of *E. hirta* stems exhibits superior ferric reducing antioxidant power (FRAP) compared to its leaves provides valuable insight into the differential distribution of secondary metabolites within the plant. The stem might require more structural antioxidants to prevent oxidative damage in vascular tissues. The plant could be storing these compounds in the stem for transport or later use.This finding aligns with the established premise that antioxidant capacity is directly correlated with the concentration and class of phytochemicals present, such as phenolic acids, flavonoids, and tannins, which are potent electron donors (Akporhuarho et al., 2022; Mahomoodally et al., 2020). The higher FRAP value in the stem suggests a greater accumulation of these polar, reducing compounds in the stem tissue relative to the leaves.

Table 3. The antioxidant activity of ethanol extract of *E. hirta* stem and leaf

Sample	FRAP value (µmol Fe^{2+}/g extract)*	Scavenging activity, IC_{50}		Category
		DPPH (ppm)	ABTS (ppm)	
Stem	533.315	70.508 ± 0.910^{c}	59.556 ± 2.120^{c}	Strong
Leaf	407.921	6.538 ± 0.136^{b}	4.832 ± 0.234^{b}	Very strong
Quercetin	–	1.383 ± 0.016^{a}	1.120 ± 0.013^{a}	Very strong

* = significantly different based on independent t-test ($p < 0.05$); letters a – c indicates significant differences based on the one way ANOVA test followed by the Tukey post hoc test ($p < 0.0001$).

Based on the DPPH assay, the methanol extract of The DPPH radical scavenging activity test showed that the IC_{50} values for the stem and leaf extracts of Euphorbia hirta were 70.508 ± 0.910 µg/ml and 6.538 ± 0.136 µg/ml, respectively. As a comparison, the quercetin used in this study had an IC_{50} value of 1.383 ± 0.016 µg/ml. The substantial difference in antioxidant activity between the leaf and stem extracts of *E. hirta* observed in this study, with leaf extract demonstrating a potency over ten times greater than the stem extract, underscores the significant influence of the plant part on phytochemical composition and bioactivity. This finding is consistent with previous research documenting varying DPPH scavenging capacities across different parts of *E. hirta*, a phenomenon typically attributed to the differential accumulation of phenolic compounds and flavonoids which are principal contributors to antioxidant potential (Tripathi et al., 2021). The leaf extract"s marked activity aligns with reports that leaves often contain higher total phenolic and flavonoid contents compared to stems, leading to superior radical scavenging (Tran et al., 2020). Furthermore, the potency of the leaf extract approaches that of the quercetin standard (IC_{50} = 1.383 µg/ml) and compares favorably with IC_{50} values reported for other potent *E. hirta* extracts, such as the ethyl acetate fraction (IC_{50} = 10.33 µg/ml) (Tran et al., 2020) and ethanol extracts (IC_{50} = 95.1 µg/ml) (Rupha et al., 2022), reinforcing the therapeutic promise of this plant. These results contribute to the growing body of evidence supporting *E. hirta*, particularly its leaves, as a valuable source of natural

antioxidants for managing oxidative stress-related conditions.

Based on the ABTS$^+$ radical scavenging test (Table 3), the IC_{50} values obtained for the stem and leaf extracts were 59.556 ± 2.120 µg/ml and 4.832 ± 0.234 µg/ml, respectively. As a comparison, the quercetin used in this study had an IC_{50} value of 1.120 ± 0.013 µg/ml. The pronounced disparity in ABTS$^+$ radical scavenging activity between the leaf and stem extracts of *E. hirta* provides compelling evidence for the differential accumulation of antioxidant phytochemicals across plant tissues, with the leaf demonstrating potency an order of magnitude greater than the stem. This finding is consistent with established research documenting that leaf extracts of *E. hirta* consistently exhibit superior radical scavenging capacity compared to stems, a phenomenon directly correlated with their higher total phenolic and flavonoid content. The leaf extract's IC_{50} value compares favorably with, and is substantially more potent than, previously reported ABTS scavenging activities for *E. hirta* extracts, such as whole plant extracts (IC_{50} = 205 µg/ml) and purified flavonoids (IC_{50} = 60.7 µg/ml) , while approaching the activity of the quercetin standard (IC_{50} = 1.120 µg/ml). The strong performance of the leaf extract reinforces the understanding that the aerial parts, particularly leaves, are rich sources of phenolic compounds including flavonoids like quercetin derivatives and gallic acid, which are known to possess potent electron-donating and radical-scavenging capabilities (Sharma et al., 2014). These results not only corroborate the traditional medicinal use of *E. hirta* leaves but also highlight their potential as a valuable and accessible source of natural antioxidants for mitigating oxidative stress-related disorders.

4. Conclusion

In conclusion, this study demonstrates a significant variation in the phytochemical composition and bioactivity between the stem and leaf extracts of *E. hirta*, highlighting the critical importance of organ-specific selection for therapeutic applications. While the leaf extract exhibited a substantially higher extraction yield and total phenolic content, which correlated with its exceptional and significantly more potent radical scavenging activity in the DPPH and ABTS assays, the stem extract conversely displayed a higher total flavonoid content and superior ferric reducing antioxidant power (FRAP). These findings underscore that the leaves are a remarkably rich source of potent antioxidants, validating their traditional medicinal use and positioning them as a promising candidate for scavenging free radicals, whereas the distinct phytochemical profile of the stems may offer alternative applications requiring specific reducing capacities.

Supplementary Material

No supplementary materials are associated with this manuscript.

Acknowledgments

Not Applicable.

Author Contributions

Amelia Christania: conceptualization, data curation resources, and writing–original draft; **Ayun**: data curation, methodology, validation, and supervision; **I Made Gde sudyadnyana sandhika**: methodology, software, formal analysis, investigation, writing–review & editing, and supervision.

Funding

This work is not supported by any external funding.

Conflicts of Interest

The authors declare no conflicts of interest.

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