International Journal of Drug Research in Clinics

Int J Drug Res Clin 2026;4:e9113
doi:10.34172/ijdrc.9113
http://ijdrug.com

Original Article

Evaluation of the Antioxidant Activity of Ethanol Extracts of *Amaranthus spinosus* and *Hedyotis corymbosa* Using the 2,2-Diphenyl-1-Picrylhydrazine Radical Scavenging Assay

Tatang Irianti[1*], Sandricka Eleanor[1], Dionisius Setyo Wibowo[1], Putri Kusmawati[1], Airin Khairunnisa Prima Rejeki[1], Winda Rakhmadani[1]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gadjah Mada University, Daerah Istimewa Yogyakarta 55281, Indonesia

Article History:
Received: January 15, 2026
Revised: February 20, 2026
Accepted: April 15, 2026
ePublished: May 15, 2026

***Corresponding Author:**
Tatang Irianti,
Email: intanti@ugm.ac.id

Abstract

Introduction: Free radicals are highly reactive molecules, so they can react with molecules around them. The use of antioxidants is one of the methods to reduce the effect of free radicals. *Amaranthus spinosus* and *Hedyotis corymbosa* (L.) are traditional medicinal plants with potential antioxidant properties. This study aimed to identify bioactive compounds and evaluate the antioxidant activity of ethanol extracts from these two medicinal plants using the DPPH method.

Methods: Extraction was performed via maceration using 70% ethanol. In addition, total phenolic content (TPC) and total flavonoid content (TFC) were measured using the Folin-Ciocalteu and aluminum chloride methods, respectively. Finally, antioxidant activity was analyzed colorimetrically using a UV-Vis spectrophotometer.

Results: The ethanol extract of *A. spinosus* demonstrated a TPC of 42.76 mg GAE/g and a TFC of 2.12 mg QE/g, with moderate antioxidant activity (the half-maximal inhibitory concentration [IC_{50}] = 207.84 µg/mL). Moreover, the ethanol extract of *H. corymbosa* contained 36.11 ± 0.48 mg GAE/g of phenolics and 11.26 mg QE/g of flavonoids, exhibiting moderate antioxidant activity with an IC_{50} value of 137.86 µg/mL.

Conclusion: These findings indicated that both plants hold promise as natural antioxidant sources for further development.

Keywords: Antioxidant activity, 2,2-diphenyl-1-picrylhydrazine, *Amaranthus spinosus*, *Hedyotis corymbose*, Radical scavenging

Please cite this article as follows: Irianti T, Eleanor S, Wibowo DS, Kusmawati P, Rejeki AKP, Rakhmadani W. Evaluation of the antioxidant activity of ethanol extracts of *Amaranthus spinosus* and *Hedyotis corymbosa* using the 2,2-diphenyl-1-picrylhydrazine radical scavenging assay. Int J Drug Res Clin 2026;4:e9113. doi:10.34172/ijdrc.9113

Introduction

Antioxidants are compounds that can prevent the formation of free radical reactions in lipid oxidation.[1] They are classified into endogenous and exogenous types. In addition, exogenous antioxidants are divided into natural and synthetic types. Butyl hydroxyl anisole , α-butyl hydroxyl toluene, and tetrabutyl hydroxyl quinone are synthetic antioxidants. Meanwhile, natural antioxidants, such as flavonoids, phenols, and folic acid, can be obtained from vegetables and fruits.[2,3]

The compound 2,2-diphenyl-1-picrylhydrazine (DPPH) is stable and radical, and antioxidant activity is measured using the DPPH method in vitro. More precisely, this activity is estimated based on the transfer of electrons by the antioxidant compound. When a DPPH solution is mixed with a compound that can donate hydrogen atoms, a color change occurs from purple to yellow because the DPPH is reduced to diphenylpicrylhydrazine.[4] In addition, absorbance can be determined using a UV-Vis spectrophotometer at a wavelength of 517 nm, and the absorbance value is proportional to the number of received electrons.[4]

Research on the identification of active compounds in medicinal plants with antioxidant activity continues to advance in support of developing herbal medicines as alternatives or adjuncts to synthetic therapies. The use of medicinal plants has long been a part of Indonesia's traditional healing heritage, passed down through generations.[5] The country's rich biodiversity presents vast opportunities to explore a wide range of plant species with therapeutic potential. Among them are *Amaranthus spinosus* and *Hedyotis corymbosa* (*Oldenlandia*

corymbosa), which contain flavonoid and phenolic compounds that have antioxidant activity.[6]

A. spinosus, a member of the Amaranthaceae family, has been reported in several studies to possess significant antioxidant activity.[7] Its flavonoid compounds are known to scavenge DPPH free radicals.[7] Meanwhile, *H. corymbosa*, a species from the Rubiaceae family, has been widely used in traditional herbal medicine. All parts of the plant, leaves, stems, flowers, and roots are known to contain active compounds, such as flavonoids and phenolic compounds, including catechol and gallic acid, which function as antioxidants.[6]

Based on this background, the present study seeks to identify bioactive compounds and evaluate the antioxidant activity of ethanol extracts from *A. spinosus* and *H. corymbosa* using the DPPH method. To achieve this objective, the research includes several steps, ranging from plant extraction to antioxidant activity analysis.

Materials and Methods

Materials

The materials used in this study included the leaves of *A. spinosus* and the whole plant of *H. corymbosa*, 70% technical ethanol, ethanol (p.a.), methanol (p.a.), butanol (p.a.), chloroform, distilled water, and glacial acetic acid. The other materials were Folin–Ciocalteu reagent, 1% sodium hydroxide, gallic acid, quercetin, 10% aluminum chloride ($AlCl_3$), and 1 M sodium acetate, DPPH, ethanol, silica gel 60 F_{254} plates, $AlCl_3$, ferric chloride ($FeCl_3$), and Dragendorff's reagent.

Methods

Ethanol Extraction Method

The ethanol extraction procedure was performed separately on the dried powdered samples of *A. spinosus* leaves and *H. corymbosa*. Approximately 400 g of the *A. spinosus* powder was macerated with 4 L of 70% ethanol for 24 hours, followed by two additional macerations using 2 L and 1 L of 70% ethanol, respectively, each for 24 hours. The filtrates from all three extractions were filtered using a Büchner funnel under vacuum, then concentrated using a rotary evaporator and water bath at 50–60°C until a viscous extract was obtained. For *H. corymbosa*, 280 g of the powder was macerated with 2.8 L of 70% ethanol for 24 hours, with stirring for 5 minutes every hour during the first 4 hours. Eventually, the residue was re-extracted using the same procedure, and all filtrates were combined and concentrated to obtain a viscous extract.

Phytochemical Identification by Thin-Layer Chromatography

Each extract (0.2 g) was dissolved in 10 mL of absolute ethanol and sonicated in a weighing bottle. In addition, silica gel 60 F_{254} plates were activated in an oven at 100°C for 10 minutes, and elution chambers were pre-saturated with mobile phases. Moreover, a solvent system of butanol:acetic acid:water (5:1:4, v/v) with rutin as standard was used for flavonoid identification. Furthermore, methanol:water (7:3, v/v) with gallic acid as standard was employed for phenolic compounds. Samples and standards were spotted in parallel on the plate, dried, and developed to a solvent front of 8 cm. Spots were visualized under visible UV light at 254 nm and 366 nm, and after spraying with $AlCl_3$ for flavonoids and $FeCl_3$ for phenolics. It should be noted that identification was based on comparison with standard compounds.

Determination of Total Phenolic Content

TPC was determined using the Folin–Ciocalteu method, as described in the Indonesian Herbal Pharmacopoeia (2nd Edition). Using gallic acid as the standard solution, with absorbance measured at 730 nm using a UV-Vis spectrophotometer. In addition, a blank was prepared similarly without the sample. Ultimately, a calibration curve was constructed using the standard, and results were expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g).

Estimation of Total Flavonoid Content

TFC using standard quercetin solution (10 mg) was dissolved in ethanol (p.a.). The absorbance was measured at the maximum wavelength using a spectrophotometer. Additionally, a blank was prepared without $AlCl_3$, and a calibration curve was constructed using the standard. The results were expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g).

Antioxidant Activity by the 2,2-Diphenyl-1-Picrylhydrazine Radical Scavenging Method

Antioxidant activity was evaluated using the DPPH radical scavenging assay. A 0.1 mM DPPH solution was prepared by dissolving 3.9 mg of DPPH in 100 mL and 15.8 mg of ethanol for *A. spinosus* and *H. corymbosa*, respectively, and then homogenized and stored in a dark bottle. The maximum absorption wavelength was determined by mixing 2 mL of the DPPH solution with 2 mL of ethanol and scanning with a spectrophotometer (400–600 nm for *A. spinosus* and 200–800 nm for *H. corymbosa*).

The *A. spinosus* extract was tested at concentrations of 125–350 µg/mL. The *H. corymbosa* extract was prepared from an 8,000-ppm stock solution and diluted as necessary. Moreover, vitamin C was used as a reference standard at 1–4 µg/mL (*A. spinosus*) and 1 mg/mL (*H. corymbosa*). For each assay, the test or standard solution was mixed with 2 mL of DPPH and ethanol to a total volume of 4 mL, homogenized, and incubated in the dark for 30 minutes. The Absorbance was estimated at the previously determined maximum wavelength using ethanol as blank. In addition, a negative control was prepared without the sample. All assays were separately conducted for each extract.

Results

Ethanol Extraction

Extraction was performed to obtain the active compounds from the dried plant material using 70% ethanol as the

solvent through the maceration method. The resulting extract was a thick (viscous) extract, and its yield was calculated to assess the efficiency of the extraction process. The extraction results and yields of each plant are presented in Table 1.

Thin-Layer Chromatography

The chromatogram of *A. spinosus* observed before and after spraying with $AlCl_3$ was examined under visible light, UV_{254} nm, and UV_{366} nm (Figures 1 and 2).

TLC was performed for phenolic compounds using gallic acid as a reference standard, due to its stability and relatively low cost. The resulting chromatograms are displayed in Figures 3 and 4.

The antioxidant activity of ethanol extracts from *A. spinosus* and *H. corymbosa* was evaluated using TLC with the DPPH reagent. Chromatograms were observed before and after DPPH spraying (Figure 5 for *A. spinosus*) and under UV 366 nm, UV 254 nm, and visible light (Figure 6 for *H. corymbosa*).

Total Phenolic Content

The TPC of the *H. corymbosa* ethanol extract was determined using the Folin–Ciocalteu method with gallic acid as the reference standard. The extract exhibited a phenolic content of 36.11 ± 0.48 mg GAE/g extract. This result was further supported by TLC analysis using the $FeCl_3$ spray reagent, which produced dark blue to black spots, indicating the presence of phenolic compounds.

Total Flavonoid Content

The TFC of the *H. corymbosa* ethanol extract was determined by a colorimetric method using $AlCl_3$ and quercetin as the reference standard. The measured flavonoid content was 11.26 ± 0.59 mg QE/g extract. The presence of flavonoids was also confirmed by TLC analysis, in which $AlCl_3$ spraying produced yellowish-green fluorescent spots under UV 366 nm, which is consistent with the characteristics of flavonoid compounds.

2,2-Diphenyl-1-Picrylhydrazine Antioxidant Activity Assay

The antioxidant activity of ethanol extracts from *A. spinosus* and *H. corymbosa* was evaluated using the DPPH free radical scavenging method. The half-maximal inhibitory concentration (IC_{50}) value, representing the concentration required to inhibit 50% of DPPH radicals, was used as the main parameter. A lower IC_{50} value indicates stronger antioxidant capacity.

Discussion

Thin-Layer Chromatography

The phytochemical profiling analysis of the ethanol extracts of *A. spinosus* and *H. corymbosa* was performed using TLC with silica gel 60 F254 as the stationary phase. In addition, mobile phases were selected based on the targeted compound groups. Visualization was conducted under visible light, UV254, and UV366, and after spraying with $AlCl_3$, $FeCl_3$, Dragendorff's, and DPPH reagents.[8]

In *A. spinosus*, a flavonoid spot was observed at Rf 0.59, closely matching rutin (Rf 0.625), appearing yellow under visible light and blue under UV366, representing flavonols, flavones, and chalcone glycosides.[9] Post-$AlCl_3$ spraying, the spot turned yellow-green, confirming the presence of flavonoids.[10,11] The $FeCl_3$ test revealed a black spot at Rf 0.79, indicating phenolics.[12] Moreover, DPPH spraying produced a yellow spot, suggesting antioxidant activity.[13,14]

In *H. corymbosa*, flavonoids were detected at Rf 0.562 (close to rutin, Rf 0.587), appearing yellow to brown under visible light.[15] UV 254 displayed fluorescence quenching at Rf 0.375, 0.562, and 0.938. Furthermore, UV366 illustrated yellowish-green fluorescence intensified after $AlCl_3$ spraying, which is typical for flavonoids.[11] Additionally, phenolic compounds, identified by comparison to gallic acid, showed dark blue to black spots at Rf 0.438, 0.563, and 0.75, and darkening at Rf 0.35 and 0.938.[16] Based on the results, Dragendorff's reagent failed to produce alkaloid spots, implying low or undetectable alkaloid levels.[17,18] Ultimately, DPPH test demonstrated yellow spots at Rf 0.812 and 0.875, confirming antioxidant compounds, especially flavonoids.[19,20]

Total Phenolic Content

TPC was estimated using the Folin–Ciocalteu method,[21] with gallic acid as the standard. Gallic acid was selected for its stability, natural origin, and affordability.[22] It is noteworthy that this method relies on the reduction of phosphotungstate-phosphomolybdate reagents by phenolic groups, producing a blue color measured at 768 nm.[23]

The *A. spinosus* extract contained 42.76 mg GAE/g at a concentration of 1000 µg/mL (Table 2). A coefficient of variation below 5% indicated good accuracy.[24] In comparison, higher phenolic content (194.21±9.22 mg GAE/g), using Soxhlet extraction dan 100% methanol as the solvent..[25] The *H. corymbosa* extract analyzed using the same method yielded a standard curve equation of $y = 0.0091x - 0.0603$ ($R^2 = 0.992$). In addition, the average absorbance at 1,500 ppm was 0.433, resulting in a phenolic content of 36.11 ± 0.48 mg GAE/g, indicating a substantial phenolic concentration.

Total Flavonoid Content

Total flavonoids were determined using the $AlCl_3$

Table 1. Ethanol Extraction Yield of *Amaranthus spinosus* and *Hedyotis corymbosa*

Plant Species	Weight of Dried Material (g)	Weight of Viscous Extract (g)	Yield (%)	Extract Color
Amaranthus spinosus	400	96.82	24.21	Dark green
Hedyotis corymbosa	280	56.05	20.02	Brown

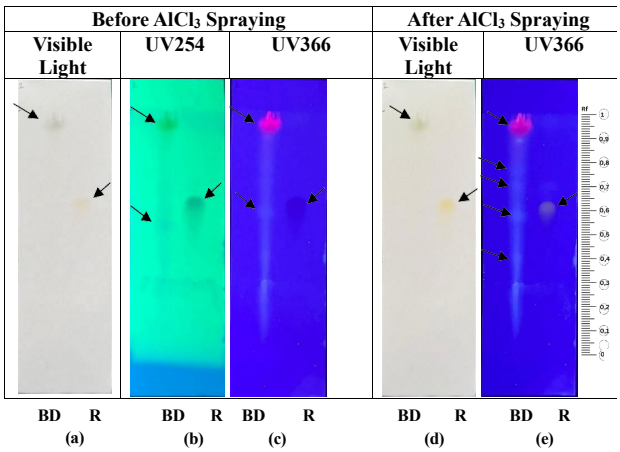

Figure 1. TLC Chromatogram of Flavonoids in *Amaranthus spinosus*
Note. TLC: Thin-layer chromatography. BD: The *Amaranthus spinosus* extract; R: Rutin standard

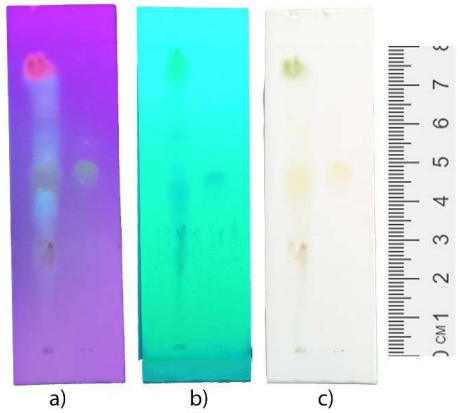

Figure 2. Thin-Layer Chromatography Profile of *Hedyotis corymbosa* After $AlCl_3$ Visualization Observed Under **(a)** UV 366 nm, **(b)** UV 254 nm, and **(c)** Visible Light
Note. $AlCl_3$: Aluminum chloride

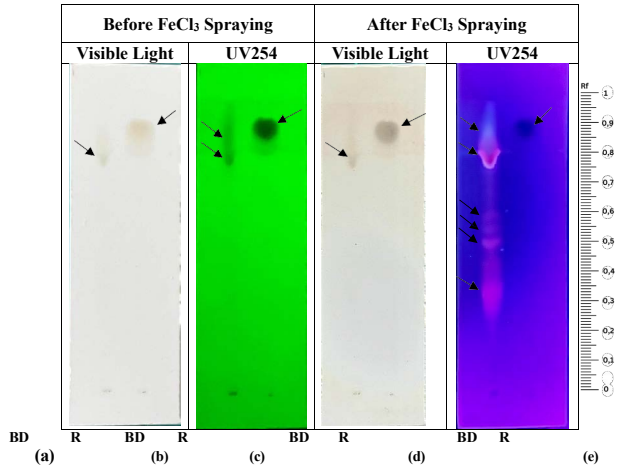

Figure 3. TLC Chromatogram of Phenolic Compounds in *Amaranthus spinosus*
Note. TLC: Thin-layer chromatography; BD: The *Amaranthus spinosus* extract; R: Rutin standard

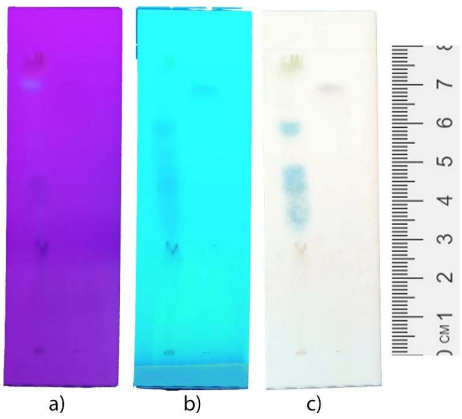

Figure 4. Thin-Layer Chromatography Profile of *Hedyotis corymbosa* After $FeCl_3$ Visualization Observed Under **(a)** UV 366 nm, **(b)** UV 254 nm, and **(c)** Visible Light
Note. $FeCl_3$: Feric chloride

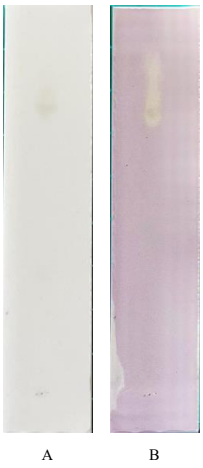

Figure 5. TLC Chromatogram of Ethanol Extract of *Amaranthus spinosus* Leaves Observed **(a)** Before and **(b)** After Spraying With DPPH
Note. TLC: Thin-layer chromatography; DPPH: 2,2-diphenyl-1-picrylhydrazine

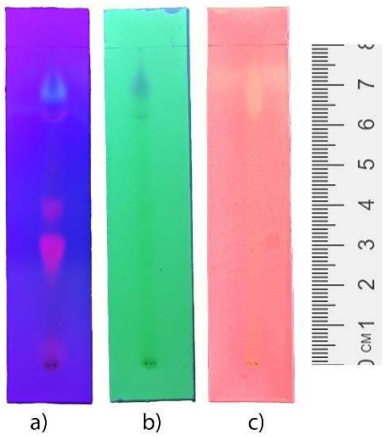

Figure 6. Thin-Layer Chromatography Profile of *Hedyotis corymbosa* After DPPH Visualization Observed Under **(a)** UV 366 nm, **(b)** UV 254 nm, and **(c)** Visible Light
Note. DPPH: 2,2-diphenyl-1-picrylhydrazine

colorimetric method,[21] with quercetin as the reference standard. Quercetin forms stable complexes with hydroxyl and carbonyl groups in flavonoid structures,[26] resulting in a yellow color measured at 433 nm.[27]

The standard curve for *A. spinosus* was y = 0.0067x – 0.016 (R^2 = 0.9945), showing good linearity. Accordingly, the TFC was 2.12 mg QE/g (Table 3), which is lower than the 27.63 ± 2.13 mg QE/g,[25] likely due to different extraction methods. *H. corymbosa* showed a quercetin calibration curve of y = 0.0059x + 0.021 (R^2 = 0.992), and

Table 2. Total Phenolic Content of *Amaranthus spinosus* Ethanol Extract

Replicate	Absorbance	Average Concentration (mg/mL)	Total Phenolic Content (mg GAE/g)
	0.33		
1	0.33	0.04	42.78
	0.33		
	0.33		
2	0.33	0.04	43.11
	0.33		
	0.32		
3	0.32	0.04	42.38
	0.32		

Average ± SD: 42.76 ± 0.33

Note. SD: Standard deviation.

Table 3. Total Flavonoid Content of *Amaranthus spinosus* Ethanol Extract

Replicate	Absorbance	Average Concentration (mg/mL)	Total Flavonoid Content (mg GAE/g)
	0.26		
1	0.26	0.04	2.06
	0.26		
	0.28		
2	0.27	0.04	2.17
	0.27		
	0.27		
3	0.27	0.04	2.12
	0.27		

Table 4. IC_{50} Values and Antioxidant Activity Classification

Sample	IC_{50} (µg/mL)	Antioxidant Activity Category
Amaranthus spinosus	207.84	Moderate
Hedyotis corymbosa	137.86	Moderate
Vitamin C (control)	3.91	Very strong

Note: IC_{50}: The half-maximal inhibitory concentration.

the measured absorbance displayed a flavonoid content of 11.26 ± 0.59 mg QE/g. This value was higher than the one reported by Divya et al at 26.44 ± 0.91 mg QE/g, which is possibly influenced by environmental factors, such as temperature, humidity, light exposure, and nutrients, affecting secondary metabolite synthesis.[28]

Antioxidant Activity (2,2-Diphenyl-1-Picrylhydrazine Assay)

The antioxidant activity was evaluated using the DPPH radical scavenging assay, which is widely used for its simplicity, speed, and sensitivity.[4] The principle involves DPPH reduction by antioxidant compounds, indicated by a decrease in absorbance at 516 nm. A greater decrease reflects stronger free radical neutralization.

In this study, 0.1 mM DPPH was prepared in ethanol, and vitamin C was used as a positive control due to its potent radical-scavenging capacity.[29] In addition, antioxidant activity was expressed as IC_{50}, the concentration required to inhibit 50% of DPPH radicals.[30] The IC_{50} of the *A. spinosus* extract was 207.84 µg/mL (Table 4),), categorized as moderate antioxidant activity.[31] This was lower than with IC_{50} values of 56.81 and 83.45 µg/mL using 100% and 80% methanol, respectively.[7,32] It should be noted that differences may arise from solvent polarity and extraction efficiency for phenolics and flavonoids.[33]

Similarly, the *H. corymbosa* extract exhibited moderate antioxidant activity with an IC_{50} of 137.86 µg/mL, comparable to the 133.49 ± 1.7 µg/mL.[34] In contrast, vitamin C showed strong activity with an IC_{50} of 3.91 µg/mL [4] (Table 4). The antioxidant capacity of *H. corymbosa* is likely attributed to its phenolic and flavonoid content, which function as hydrogen donors, free radical reducers, and singlet oxygen quenchers.[35]

Conclusion

The findings of this study demonstrated that the ethanol extract of *A. spinosus* L. (spiny amaranth) contained 42.76 mg GAE/g of total phenolics, 2.12 mg QE/g of total flavonoids, and an IC_{50} value of 207.84 µg/mL. Meanwhile, that of *H. corymbosa* (snakeweed) depicted a TPC of 36.11 ± 0.48 mg GAE/g, TFC of 11.26 ± 0.59 mg QE/g, and an IC_{50} value of 137.86 µg/mL. Based on these results, both extracts fall within the moderate category of antioxidant activity.

Variations in antioxidant capacity are influenced by the levels of phenolic and flavonoid compounds, which act as electron donors and free radical scavengers, indicating the potential of both plants as the natural sources of antioxidants. Accordingly, further research is warranted to isolate the active constituents, evaluate their in vivo effects, and explore their development as therapeutic candidates.

Acknowledgements

The authors would like to thank the RTA UGM program 2020 for its financial support of the present research.

Authors' Contribution

Conceptualization: Tatang Irianti
Data Curation: Sandricka Eleanor
Formal Analysis: Dionisius Setyo Wibowo
Funding Acquisition: Putri Kusmawati
Investigation: Airin Khairunnisa Prima Rejeki
Methodology: Winda Rakhmadani
Project Administration: Dionisius Setyo Wibowo and Sandricka Eleanor
Resources: Putri Kusmawati
Software: Dionisius Setyo Wibowo
Supervision: Airin Khairunnisa Prima Rejeki
Validation: Tatang Irianti
Visualization: Winda Rakhmadani
Writing–Original Draft: Tatang Irianti and Airin Khairunnisa Prima Rejeki
Writing–Review & Editing: Tatang Irianti

Competing Interests

The authors declare that they have no conflict of interests.

Consent for Publication

Not applicable.

Data Availability Statement
All generated or analyzed data are included in this article.

Ethical Approval
Not applicable.

Funding
This research received financial support from the RTA UGM program 2020 batch 1.

References
1. Lung JKS, Destiani DP. Uji aktivitas antioksidan vitamin A, C, E dengan metode DPPH. Farmaka 2017;15(1):53–62. doi:10.24198/jf.v15i1.12805
2. Ilmiah M, Anggarani MA, Mahfudhah DN. Literature Review of Antioxidant Activity of Several Types of Onions and Its Potensial as Health Supplements. Indones J Chem Sci 2023;12(1):103–11.
3. Oliveira GL da S, Oliveira FRDAM, Alencar MVOB de, Junior ALG, Souza AA de, Cavalcante AA de CM, et al. Evaluation of antioxidant capacity of the aqueous extract of Cynara scolymus L . (Asteraceae) in vitro and in Saccharomyces cerevisiae. African J Pharm Pharmacol 2014;8(5):136–47.
4. Molyneux P. The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol 2004;26(2):211–9.
5. Olivera A, Moore TW, Hu F, Brown AP, Sun A, Liotta DC, et al. Inhibition of the NF-κB signaling pathway by the curcumin analog, 3,5-Bis(2-pyridinylmethylidene)-4-piperidone (EF31): anti-inflammatory and anti-cancer properties. Int Immunopharmacol 2012;12(2):368–77. doi:10.1016/j.intimp.2011.12.009
6. Endrini S. Antioxidant activity and anticarcinogenic properties of " rumput mutiara " { Hedyotis corymbosa (L .) Lam .} and " pohpohan " { Pilea trinervia (Roxb .) Wight }. J Med Plant Res 2011;5(16):3715–8.
7. Al-Tamimi A, Alfarhan A, Al-Ansari A, Rajagopal R. Antioxidant, enzyme inhibitory and apoptotic activities of alkaloid and flavonoid fractions of Amaranthus spinosus. Physiol Mol Plant Pathol 2021;116:101728. doi:10.1016/j.pmpp.2021.101728
8. Cintya H, Chan M., Purba A, Kokita T, Destinyie F, Bernardi W. IIsolation of Curcumin from White Turmeric Using Maceration and Thin Layer Chromatography (TLC) Methods. J Pro-Life 2021;8(3):205–17. doi:10.33541/pro-life.v8i3.3335
9. Murwanto PE, Santoso D. Antioxidant activity analysis of Cynara scolimus l., Artemisia china l., Borreria repensdc., Polygala paniculata l. from taman nasional gunung merapi using dpph (2,2-difenil-1-pikrilhidrazil) radical scavenging analysis. Maj Obat Tradis 2012;17(3):53–60. doi:10.22146/tradmedj.8011
10. Ahmed F, Iqbal M. Antioxidant activity of Ricinus Communis. Org Med Chem 2018;5(3):1–6. doi:10.19080/OMCIJ.2018.05.555667
11. Theodora C, Gunawan IW, Swantara IM. Isolasi dan identifikasi golongan flavonoid pada ekstrak etil asetat daun gedi (Abelmoschus manihot L.). J Kim 2019;13(2):131–8. doi:10.24843/JCHEM.2019.v13.i02.p02
12. Harborne J. Metode Fitokimia: Penuntun Cara Modern Menganalisa Tumbuhan. kedua. Institut Teknologi Bandung; 1987.
13. Putri AO, Hati MC, Ishanti NP, Srivaliana H. Identifikasi Senyawa Flavonoid pada Beberapa Jenis Tanaman dengan Kromatografi Lapis Tipis: Literature Review. PHARMADEMICA : J Kefarmasian dan Gizi 2024;3(2):45–54. doi:10.54445/pharmademica.v3i2.40
14. Febriani A, Hidayati AR, Suryani D. Antibacterial Testing of Moringa oleifera L. Fruit Extract Against The Growth of The Bacteria Staphylococcus aureus. J Biol Trop 2023;23(1):307–14. doi:10.29303/jbt.v23i1.6149
15. Anwar HU, Andarwulan N, Yuliana ND. Identifikasi Komponen Antibakteri Pada Ekstrak Buah Takokak Menggunakan Kromatografi Lapis Tipis. J Mutu Pangan. 2017;4(2):59–64.
16. Windyaswari AS, Purba JP, Nurrahmah SS, Ayu IP, Imran Z, Amin AA, et al. Phytochemical profile of sea grass extract (Enhalus acoroides): A new marine source from Ekas Bay, East Lombok. IOP Conf Ser Earth Environ Sci 2019;278(1):012081. doi:10.1088/1755-1315/278/1/012081
17. Karmilah, Musdalipah, Daud NS, Reymon, Fauziah Y. Identification of sea urchin gonads chemical compounds using thin-layer chromatography from Bokory island, Southeast Sulawesi. J Phys Conf Ser 2021;1899(1):012050. doi:10.1088/1742-6596/1899/1/012050
18. Raal A, Meos A, Hinrikus T, Heinämäki J, Romāne E, Gudienė V, et al. Dragendorff's reagent: Historical perspectives and current status of a versatile reagent introduced over 150 years ago at the University of Dorpat, Tartu, Estonia. Pharmazie 2020;75(7):299–306. doi:10.1691/ph.2020.0438
19. Handayani V, Ahmad AR, Sudir M. Uji Aktivitas Antioksidan Ekstrak Metanol Bunga dan Daun Patikala (Etlingera elatior (Jack) R . M . Sm) Menggunakan Metode DPPH. Pharm Sci Res 2014;1(2):86–93.
20. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci 2016;5:e47. doi:10.1017/jns.2016.41
21. Kementerian Kesehatan RI. Farmakope Herbal Indonesia Edisi II: Suplemen I. II. Jakarta: Kementerian Kesehatan RI; 2022.
22. Mongkolsilp S, Pongbupakit I, Sae-Lee N, Sitthithaworn W. Radical Scavenging Activity and Total Phenolic Content of Medicinal Plants Used in Primary Health Care. SWU J Pharm Sci 2004;9(1):32–5.
23. Vermerris W, Nicholson R. Biosynthesis of Phenolic Compounds. In: In Phenolic Compound Biochemistry. Springer Netherlands; 2006. p. 63–149.
24. Savitri A, Megantara S. Metode KLT-densitometri sebagai penetapan kadar bahan aktif sediaan farmasi. Farmaka 2019;17(2):455–63.
25. Cheerakuzhy NH, Puthenparampil D, Malayil D, Narayanankutty A. Variation in the polyphenol composition, antioxidant, and anticancer activity among different Amaranthus species. South African J Bot 2020;135:408–12. doi:10.1016/j.sajb.2020.09.026
26. Kamtekar S, Keer V, Patil V. Estimation of Phenolic content , Flavonoid content , Antioxidant and Alpha amylase Inhibitory Activity of Marketed Polyherbal Formulation. J Appl Pharm Sci 2014;4(9):61–5.
27. Lindawati NY, Ma'ruf SH. Penetapan kadar total flavonoid ekstrak etanol kacang merah (Phaseolus vulgaris L.) secara spektrofotometri visibel. Jurnal Ilmiah Manuntung: Sains Farmasi Dan Kesehatan 2020;6(1):83–91.
28. Metlen KL, Aschehoug ET, Callaway RM. Plant behavioural ecology: dynamic plasticity in secondary metabolites. Plant Cell Environ 2009;32(6):641–53. doi:10.1111/j.1365-3040.2008.01910.x
29. Gordon MH. 4 - Measuring antioxidant activity. In: Pokorny J, Yanishlieva N, Gordon M, editors. Antioxidants in Food: Woodhead Publishing; 2001. p. 71–84. doi:10.1016/9781855736160.1.71
30. Setiawan F, Yunita O, Kurniawan A. Uji Antioxidant Activity Test of Ethanol Extract of Secang Wood (Caesalpinia sappan) Using DPPH, ABTS, and FRAP Methods. Media Pharm Indones 2018;2(2):82–9.
31. Widyanto RM, Safira L, Sofian NF, Mardhiyati SA, Pradiptasari P, Dini CY, et al. Studies on the Antioxidant and Cytotoxic Potentials of the Peel Extract of Dacryodes rostrata. BIO Web Conf 2021;41:07005:1-5.
32. Amabye TG. Evaluation of Physiochemical , Phytochemical , Antioxidant and Antimicrobial Screening Parameters of Amaranthus spinosus Leaves. Nat Prod Chem Res 2016;4(1):3–

7.
33. Boeing JS, Barizão EO, Silva BC, Montanher PF, de Cinque Almeida V, Visentainer JV. Evaluation of solvent effect on the extraction of phenolic compounds and antioxidant capacities from the berries: application of principal component analysis. Chem Cent J 2014;8(1):48. doi:10.1186/s13065-014-0048-1
34. Divya M, Shanti G, Amalraj S, Amiri-Ardekani E, Gurav S, Ayyanar M. Evaluation of in vitro enzyme inhibitory, anti-inflammatory, antioxidant, and antibacterial activities of Oldenlandia corymbosa L. and Oldenlandia umbellata L. whole plant extracts. Pharmacol Res - Mod Chinese Med 2023;8:100286. doi:10.1016/j.prmcm.2023.100286
35. Zeb A. Concept, mechanism, and applications of phenolic antioxidants in foods. J Food Biochem 2020;44(9):e13394. doi:10.1111/jfbc.13394