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RESEARCH ARTICLES

Potential Study of Ethyl Acetate Extract of Gagatan Harimau Leaves (*Ampelocissus thyrsiflora* (Blume) Planch

Kajian Potensi Ekstrak Etil Asetat Daun Gagatan Harimau (Ampelocissus thyrsiflora (Blume) Planch)

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Abstract

North Sumatra is home to diverse biodiversity, including promising medicinal species such as *Ampelocissus thyrsiflora* (Gagatan Harimau), known for its phenol and flavonoid content. This research focuses on the antioxidant potential of the ethyl acetate extract of this plant through antioxidant assays as well as determining the total phenol and flavonoid content. Simplicia were extracted by maceration method using ethyl acetate after defatting using n-hexane. Furthermore, phenolic content was analyzed using the Folin-Ciocalteu method which was determined in mg gallic acid equivalent (GAE) per gram of extract, while flavonoid content was analyzed through the aluminum chloride ($AlCl_3$) test and expressed in mg quercetin equivalent (QE) per gram of extract. Evaluation of antioxidant activity was carried out through the ABTS radical capture method with IC_{50} value parameter. The results showed a total phenolic content of 92.89 ± 1.28 mg GAE/g extract and flavonoid content of 95.12 ± 1.97 mg QE/g extract. The results of antioxidant activity analysis stated strong potential with IC_{50} of 46.03 ± 0.09 µg/mL. Antioxidant activity increased as the concentration of the extract increased, showing a strong linear correlation (R^2 = 0.9984). These results indicate that the ethyl acetate extract of *Ampelocissus thyrsiflora* leaves has the potential to be developed as a source of natural antioxidants in the pharmaceutical and functional food fields.

Keywords: ABTS, *Ampelocissus thyrsiflora* , antioxidant activity, ethyl acetate extract

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Abstrak

Sumatera Utara merupakan rumah bagi keanekaragaman hayati, termasuk spesies obat yang menjanjikan seperti Ampelocissus thyrsiflora (Gagatan Harimau), yang dikenal karena kandungan fenol dan flavonoid. Riset ini berfokus pada potensi antioksidan dari ekstrak etil asetat dari tanaman ini melalui uji antioksidan serta menentukan kandungan total fenol dan flavonoid. Simplisia diekstraksi dengan metode maserasi menggunakan etil asetat setelah defatting dengan n-heksana. Selanjutnya, kandungan fenolik dianalisis menggunakan metode Folin–Ciocalteu yang ditetapkan dalam mg asam galat ekuivalen (GAE) per gram ekstrak, sedangkan kadar flavonoid dianalisis melalui uji aluminium klorida ($AlCl_3$) dan dinyatakan dalam mg ekuivalen kuersetin (QE) per gram ekstrak. Evaluasi aktivitas antioksidan dilakukan melalui metode penangkapan radikal ABTS dengan parameter nilai IC_{50}. Hasil menunjukkan kadar total fenol sebesar 92,89 ± 1,28 mg GAE/g ekstrak dan kadar flavonoid sebesar 95,12 ± 1,97 mg QE/g ekstrak. Hasil analisis aktivitas antioksidan dinyatakan berpotensi kuat dengan IC_{50} sebesar 46,03 ± 0,09 µg/mL. Aktivitas antioksidan meningkat seiring dengan peningkatan konsentrasi ekstrak, memperlihatkan korelasi linear kuat (R^2 = 0,9984). Hasil ini mengindikasikan bahwa ekstrak etil asetat daun Ampelocissus thyrsiflora berpotensi dikembangkan sebagai sumber antioksidan alami dalam bidang farmasi dan pangan fungsional.

Kata Kunci: *ABTS, aktivitas antioksidan, Ampelocissus thyrsiflora, ekstrak etil asetat*

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Highlight:

- Gagatan Harimau leaf extract demonstrated very strong antioxidant activity.
- The extract contained high total phenolic and flavonoid contents.
- Antioxidant activity increased strongly with increasing extract concentration.

INTRODUCTION

North Sumatra is known for its rich biodiversity, including plants from the Vitaceae family, such as *Ampelocissus thyrsiflora*, locally known as Gagatan Harimau. This plant has traditionally been used by the Karo community to treat various health conditions, including diarrhea, diabetic wounds, fatigue, and infections (Wasnis et al., 2022). This traditional use has prompted scientific interest in the bioactive potential of this plant.

Several studies indicate that *A. thyrsiflora* contains flavonoids, terpenoids, and phenylethanoid glycosides, which represent a class of bioactive compounds known to exhibit diverse pharmacological activities. Phenylethanoid glycosides are phenolic derivatives with significant potential as therapeutic agents for the prevention and management of diseases associated with oxidative stress and inflammation (Sun dan Shahrajabian, 2023). These compounds are hypothesized to act as antioxidants due to their ability to rapidly donate hydrogen atoms, thereby converting free radicals into more stable forms (Surbakti et al., 2024). A study by Wasnis et al. (2022) revealed that the phenylethanoid glycosides in *A. thyrsiflora* possess potential anti-inflammatory and antioxidant activities, operating through a hydrogen atom donation mechanism to

neutralize free radicals. Furthermore, Surbakti et al. (2023) reported that the leaf extract of *A. thyrsiflora* exhibits cytotoxic activity against *Artemia salina* larvae. The resulting IC_{50} value was categorized as active, thereby indicating the plant's potential anticancer properties.

Research by Nurani et al. (2024) further supports the antioxidant potential of this plant, with a total phenolic content of 298.128 mg GAE/g and an IC_{50} value of 28.04 mg/L, as determined using the *DPPH* method. Surbakti et al. (2024) further corroborated these findings by reporting an IC_{50} value of 34.79 µg/mL. However, most previous studies have predominantly used the *DPPH* method to evaluate antioxidant activity, while relatively few have investigated this activity using the ABTS method, which can provide a broader assessment of reactivity toward both hydrophilic and lipophilic radicals.

The selection of ABTS as the assay method was also influenced by the solvent used. In this study, *ethyl acetate*, a semipolar solvent, was employed. Ethyl acetate was selected as a suitable solvent for the ABTS assay because of its ability to extract both hydrophilic and lipophilic bioactive components. The ABTS method enables the measurement of antioxidant activity from lipophilic compounds, which are often more difficult to extract using polar solvents. The semipolar nature of ethyl acetate may therefore facilitate the detection of various bioactive compounds using the ABTS assay.

In addition, the limited comprehensive data regarding the relationship between the antioxidant activity of the *ethyl acetate* extract of Gagatan Harimau and its total phenolic and flavonoid contents warrant further investigation. Given the important roles of phenolic and flavonoid compounds in determining the antioxidant capacity of plants, quantitative analyses of these compounds are essential to gain a deeper understanding of their specific contributions to the observed bioactive activity.

Based on previous studies, this study focused on evaluating the free radical-scavenging activity of the *ethyl acetate* extract of Gagatan Harimau leaves using the ABTS assay, while simultaneously performing quantitative analyses of the total phenolic and flavonoid contents. The aim was to strengthen the scientific evidence supporting the potential use of this plant as a natural antioxidant agent in the pharmaceutical field.

METHODS

This study employed a descriptive design. The procedure commenced with sample collection and processing, followed by crude drug (*simplisia*) characterization and phytochemical screening to identify the chemical constituent groups present in the sample. Ethyl acetate was used for maceration after a defatting process using n-hexane. Furthermore, the Folin–Ciocalteu method was applied to analyze total phenolic content, while the $AlCl_3$ method was utilized to determine total flavonoid content. Free radical scavenging activity was evaluated using the ABTS assay. All experimental procedures were conducted at the Laboratory of the Pharmacy Research Center, Universitas Sumatera Utara.

Equipment used in this study included drop pipettes, graduated pipettes, funnels, porcelain dishes, graduated cylinders, and volumetric flasks. Additional tools comprised blue tips, aluminum foil, maceration bottles, cover slips, desiccators, a grinder, parchment paper, filter paper, Whatman filter paper, a drying cabinet, mortar and pestle, an analytical balance, glass slides, an oven, a ruler, a water bath, a pH meter, a rotary evaporator, a sonicator, and a UV-Vis spectrophotometer. Materials

used included distilled water, ethanol, ethyl acetate, n-hexane, and Gagatan Harimau leaves (*Ampelocissus thyrsiflora*). Chemical reagents consisted of aluminum chloride ($AlCl_3$), gallic acid, acetic acid, sodium acetate (CH_3COONa), sodium carbonate (Na_2CO_3), methanol, quercetin, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and Folin–Ciocalteu reagent.

Prior to size reduction, macroscopic and microscopic evaluations of the sample were performed. Total moisture and ash content were determined in accordance with the Indonesian Herbal Pharmacopoeia (*Farmakope Herbal Indonesia*). Following drying, the *simplisia* was defatted with n-hexane at a 1:3 (w/v) ratio for 24 hours. Subsequently, maceration was carried out using ethyl acetate at a 1:10 (w/v) ratio for 5 × 24 hours. The resulting filtrate was concentrated using a rotary evaporator to yield a dense extract.

The Folin–Ciocalteu assay was employed to quantify total phenolic content. The dissolved sample extract was reacted with Na_2CO_3, Folin–Ciocalteu reagent, and distilled water. The mixture was incubated for 56–58 minutes until a clear dark blue color developed, after which absorbance was measured at 765 nm using a UV-Vis spectrophotometer. Results were expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g).

The total flavonoid content was quantified spectrophotometrically using aluminum chloride ($AlCl_3$). The reaction mixture was incubated for 29 minutes to ensure complete reaction marked by a faint yellow color and absorbance was recorded at 415 nm. Flavonoid concentration was calculated and reported as milligrams of quercetin equivalent per gram of extract (mg QE/g).

Antioxidant capacity was evaluated via the ABTS assay. The $ABTS^+$ radical cation solution was prepared by mixing potassium persulfate with ABTS solution and incubating the mixture in the dark for 12–16 hours to generate a stable radical. After reacting the sample with the $ABTS^+$ solution, absorbance was measured at 734 nm using a UV-Vis spectrophotometer. The IC_{50} value served as the indicator of antioxidant activity, representing the extract concentration required to scavenge 50% of the $ABTS^+$ free radicals in the reaction system. Descriptive and quantitative data regarding total phenolic content, flavonoid content, and IC_{50} values were compiled in tabular format to illustrate the correlation between antioxidant capacity and bioactive compound concentrations (phenols and flavonoids).

RESULTS AND DISCUSSIONS

Crude drug characterization

To assess the physicochemical quality of the sample, characterization of the crude drug (*simplisia*) was performed prior to processing. Evaluated parameters included moisture content, water-soluble extractive content, ethanol-soluble extractive content, total ash content, and acid-insoluble ash content. Evaluating these factors is essential to ensure that the raw material meets the quality standards specified in the Indonesian Herbal Pharmacopoeia (*Farmakope Herbal Indonesia*). As a foundational evaluation for subsequent extraction procedures, Table 1 presents the characterization results, detailing the stability and purity of the herbal preparation.

According to the Indonesian Herbal Pharmacopoeia, the *simplisia* met all established characterization criteria. With a moisture content of 6.65%, the *simplisia* demonstrates stability against microbial growth and chemical degradation during storage, rendering it suitable for subsequent extraction steps. Based on total ash (5.87%)

and acid-insoluble ash contents (0.37%), inorganic contamination (such as dust or silica) remained within the safe permissible threshold of 2–5% (Kemenkes, 2017). The extractive values in ethanol (15.93%) and water (19.30%) indicate the potential presence of active constituents adaptable for herbal formulations. Overall, the low total ash and acid-insoluble ash values confirm the purity of the *simplisia*, with inorganic impurities remaining below regulatory limits. These findings demonstrate that the *simplisia* fulfills quality standards regarding stability, purity, and safety, affirming its eligibility as a raw material for herbal product development in compliance with the Indonesian Herbal Pharmacopoeia.

Table 1. Characterization of *Ampelocissus thyrsiflora* leaf *simplisia*

Parameter	Mean Value (%)
Moisture content	6,65
Water-soluble extractive content	19,30
Ethanol-soluble extractive content	15,93
Total ash content	5,87
Acid-insoluble ash content	0,37

Phytochemical screening of *simplisia* and ethyl acetate extract of *Ampelocissus thyrsiflora* leaves

The sample underwent phytochemical screening to identify the classes of secondary metabolites present. The screening was conducted to detect compounds such as alkaloids, tannins, flavonoids, saponins, steroids, terpenoids, and glycosides. Phytochemical screening is a qualitative technique that commonly involves color reactions. Table 2 summarizes the analytical findings and lists the various types of bioactive compounds identified in the sample.

Table 2. Secondary metabolite profiles of *simplisia* and ethyl acetate extract of *Ampelocissus thyrsiflora* leaves

Metabolite Group	Simplisia	Ethyl Acetate Extract
Alkaloids	+	-
Flavonoids	+	+
Glycosides	+	+
Tannins	+	+
Saponins	+	+
Steroids/Triterpenoids	+	+

Keterangan: (+) Ada; (-) Tidak ada

The presence of tannins, glycosides, and flavonoids was confirmed. These compounds exhibit diverse pharmacological properties, including antibacterial, anti-inflammatory, and antioxidant activities. The antioxidant capacity of the sample is specifically attributed to its flavonoid and tannin contents, which align with the observed radical scavenging capacity and total phenolic and flavonoid determinations (Surbakti et al., 2024). These results support the potential of the ethyl acetate extract as an active ingredient in pharmaceutical or nutraceutical formulations.

Determination of total phenolic content
Determination of maximum wavelength

Gallic acid was employed as the reference standard for quantifying total phenolic content due to its high stability and analytical precision. Gallic acid possesses three phenolic hydroxyl groups that readily undergo oxidation by the Folin Ciocalteu reagent under alkaline conditions (Ramadhan et al., 2021).

Reaction of a gallic acid solution with 10% Folin–Ciocalteu reagent yielded a dark green molybdenum complex. The addition of 10% Na_2CO_3 provided an alkaline medium that facilitated the dissociation of phenolic protons into phenolate ions, thereby intensifying the complex color (Ramadhan et al., 2021).

Spectrophotometric scanning of the standard gallic acid solution (20 µg/mL) across 200–800 nm demonstrated a maximum absorbance of 0.6914 at 731 nm.

Operating time determination for gallic acid

Operating time determination was conducted to determine the reaction time required for the complex formed between *gallic acid*, *Folin–Ciocalteu reagent*, and Na_2CO_3 to reach maximum stability. Absorbance was measured every minute for 60 minutes after the addition of *Folin–Ciocalteu reagent* and Na_2CO_3 to the *gallic acid* solution. A stable complex was formed between the 56th and 58th minutes, indicating that this time range was optimal for ensuring measurement precision.

Standard calibration curve of gallic acid

A gallic acid calibration curve was constructed using standard solutions at concentrations of 25, 50, 75, and 100 µg/mL. Absorbance was recorded at 731 nm following a 56-minute incubation period. The linear regression equation was determined as $y = 0.0078x + 0.02343$ with a coefficient of determination (R^2) of 0.99629, confirming a strong linear relationship between absorbance intensity and gallic acid concentration.

Table 3. Absorbance data of gallic acid standards at 731 nm

Concentration (µg/mL)	Absorbance (Replications)			Mean Absorbance
	I	II	III	
0	0.0000	0.0000	0.0000	0.0000
25	0.2434	0.2444	0.2424	0.2434
50	0.4250	0.4261	0.4232	0.4248
75	0.6026	0.6043	0.6025	0.6031
100	0.7949	0.7937	0.7948	0.7945

The data presented in Table 3 show that as the *gallic acid* concentration increased, the absorbance value also increased. This effect, which results in a more intense color and greater light absorption, is attributed to the formation of a greater number of molybdenum–tungsten complexes at higher concentrations (Hidayah and Budgeti, 2022). This finding indicates that the amount of phenolic compounds in the sample is directly correlated with absorbance.

Hasil penentuan kadar total fenol ekstrak etil asetat daun gagatan harimau

Penetapan kadar fenol dilakukan dengan mengolah data absorbansi rata-rata dari sampel, yang kemudian dimasukkan ke dalam model persamaan kalibrasi regresi. Hasil perhitungan dinyatakan dalam satuan *Gallic Acid Equivalent* (GAE), yang merepresentasikan kandungan senyawa fenolik setara dengan *asam galat*. Tabel 4 menampilkan nilai total kandungan fenol.

The total phenolic content was determined using a linear regression equation correlating the concentration of standard gallic acid solutions with absorbance values. Analysis revealed that the sample contained a total phenolic content of 92.8912 ± 1.28 mg GAE/g sample, indicating that each gram of sample contains phenolic constituents equivalent to 92.8912 mg of gallic acid. Quantifications were performed spectrophotometrically using the Folin–Ciocalteu assay. Based on the reduction reaction between phenolic compounds and the Folin–Ciocalteu reagent (a mixture of tungsten and molybdenum oxides), this technique offers high simplicity, precision, and sensitivity. The maximum absorbance of the blue complex resulting from metal reduction occurs at a wavelength of 765 nm (within the 750–770 nm range). The intensity of the resulting color directly correlates with phenolic concentration, as most phenolic compounds dissociate into phenolate ions at pH 10, rendering them more reactive toward the reagent (Nurkhasanah et al., 2023). The chemical architecture of phenolic constituents including the number and position of hydroxyl groups, the degree of glycosylation, and the presence of carboxyl groups modulates their antioxidant activity. Their contribution as potent antioxidants relies on their capacity to scavenge free radicals that pose potential damage to biological systems (Diniyah and Lee, 2020).

Table 4. Total phenolic content of *Ampelocissus thyrsiflora* leaf ethyl acetate extract

Replication	Mean Absorbance	Total Phenolic Content (mg GAE/g extract)	Mean Total Phenolic Content (mg GAE/g extract)
1	0.7632	94.8462	
2	0.7659	92.4197	92.8912 ± 1.28
3	0.7649	91.4078	

Determination of total flavonoid content

Determination of maximum wavelength

UV-Vis spectrophotometry was used to determine the maximum absorption wavelength following the addition of 10% $AlCl_3$, 1 M sodium acetate, and distilled water to a 60 µg/mL quercetin solution. Analysis yielded a maximum absorption peak at 430.5 nm with an absorbance of 0.4131, which falls within the characteristic quercetin absorption range of 400–450 nm (Daipadli et al., 2024).

Operating time determination for quercetin

Operating time determination was conducted to establish the optimal measurement timeframe, thereby minimizing procedural errors. This step is essential because the reaction product between $AlCl_3$ and quercetin is a complex compound that requires a specific duration to achieve maximum absorbance stability (Suharyanto and Prima, 2020). Absorbance was monitored at 1-minute intervals over a total period of 60 minutes at the maximum absorption wavelength of 430.5 nm. The results demonstrated that absorbance stability was attained between minutes 27 and 29, indicating that the quercetin–$AlCl_3$ complex had reached a stable state suitable for subsequent analytical measurements.

Standard calibration curve of quercetin

The quercetin calibration curve was constructed by measuring absorbance at concentrations of 0, 40, 60, 80, 100, and 120 µg/mL at a wavelength of 430.5 nm. The

flavonoid content of the sample was then calculated based on the absorbance values correlated with the calibration curve data.

As presented in Table 5, the increase in absorbance values correlates directly with the increase in quercetin solution concentration. The exceptionally strong linear relationship between concentration and absorbance is substantiated by the coefficient of determination (R^2 = 0.9983) and the linear regression equation y = 0.0072x – 0.003 derived from this study. The validity of the linear regression model applied is further confirmed by the correlation coefficient *(r)* value approaching 1. These findings align consistently with the Beer–Lambert Law, which asserts that under ideal conditions and at a constant wavelength, the absorbance of a solution is directly proportional to the concentration of the dissolved solute (Mukhriani et al., 2019).

Table 5. Absorbance data of quercetin standards at 430.5 nm

Concentration (µg/mL)	Absorbance (Replications)			Mean Absorbance
	I	II	III	
0	0.0000	0.0000	0.0000	0.0000
40	0.2883	0.2894	0.2898	0.2892
60	0.4072	0.4091	0.4094	0.4086
80	0.5867	0.5862	0.5862	0.5864
100	0.7005	0.7046	0.7048	0.7033
120	0.8651	0.8661	0.8665	0.8659

Total flavonoid content of ethyl acetate extract

The total flavonoid content of the sample was analyzed spectrophotometrically using UV-Vis, with quercetin serving as the reference standard. Across triplicate determinations, the recorded values were 93.6030 mg QE/g, 94.4174 mg QE/g, and 97.3472 mg QE/g. With a mean total flavonoid content of 95.1225 ± 1.969 mg QE/g extract, the sample was shown to contain a substantial concentration of flavonoids, which contributes to enhancing its pharmacological activities, such as antioxidant capacity. Table 6 details the complete analytical data for total flavonoid quantification.

Table 6. Total flavonoid content of *Ampelocissus thyrsiflora* leaf ethyl acetate extract

Replication	Mean Absorbance	Total Flavonoid Content (mg QE/g extract)	Mean Total Flavonoid Content (mg QE/g extract)
1	0.7024	97.9722	
2	0.6972	94.4174	95.1225 ± 1.969
3	0.6979	93.6030	

The quantitative analysis revealed a total flavonoid content of 95.1225 ± 1.969 mgQE/g extract, indicating that each gram of sample contains flavonoid constituents equivalent to 95.1225 mg of quercetin. The total flavonoid content was quantified using a linear regression equation describing the relationship between standard quercetin concentrations and absorbance levels. This methodology relies on the fundamental principle of spectrophotometry, wherein absorbance intensity increases proportionally with solute concentration. This principle aligns directly with the Beer–Lambert Law, which states that absorbance *(A)* is proportional to concentration *(c)* and optical path

length *(b)*, governed by the molar absorptivity *(ε),* expressed as A = εbc. In this analysis, constructing a calibration curve using quercetin as a reference standard enables precise determination of sample flavonoid content, provided measurements remain within the dynamic linear range. To ensure analytical accuracy, the sample absorbance values must fall strictly within the range of linearity, as high solute concentrations can induce deviations from this law due to optical and chemical interactions (Mukhriani et al., 2019).

Analysis of the antioxidant activity of gagatan harimau leaf samples using the ABTS method

Determination of the maximum absorption wavelength of ABTS

A UV-Vis spectrophotometer was used to determine the maximum absorption of the ABTS solution. The analysis showed a maximum absorbance of 0.3346 at a wavelength of 733 nm. This result is considered consistent with the literature, which states that ABTS absorption occurs within the wavelength range of 610–750 nm (Gandjar and Rohman, 2007).

Results of the ABTS free radical scavenging activity of the quercetin reference solution

In the ABTS antioxidant activity assay, quercetin was used as a positive control/reference standard. The antioxidant activity of quercetin was determined by measuring the absorbance of ABTS after the addition of the reference standard solution at concentrations of 1, 2, 3, 4, and 5 µg/mL, which were compared with the ABTS control (without the addition of the reference standard solution).

Plotting the concentration of the reference standard against the percentage of radical scavenging activity yielded a linear regression equation of y = −0.00950x + 0.88437, with a correlation coefficient of 0.99844. Based on these results, quercetin exhibited antioxidant activity with a value of 4.2316 ± 0.0310 µg/mL, which was classified as very strong antioxidant activity (<50 µg/mL).

The very strong antioxidant activity demonstrated by the reference standard indicates that quercetin is effective in neutralizing free radicals at low concentrations. This finding supports the potential of quercetin as a candidate for the development of health and cosmetic products that utilize active compounds with high antioxidant capacity. Further research into the molecular mechanisms underlying quercetin's activity may provide a better understanding of its potential role in protecting the body against free-radical-induced damage.

Results of the ABTS free radical scavenging activity of the ethyl acetate extract of gagatan harimau leaves

The ABTS method was used to evaluate the antioxidant activity of the sample at concentrations of 10, 20, 30, 40, and 50 µg/mL. The ability of the sample extract to scavenge free radicals was determined by measuring the decrease in absorbance after combining 0.1 mL of the sample with 2 mL of the ABTS solution. The antioxidant activity of the sample was indicated by a substantial decrease in absorbance. **Table 7** provides detailed information on the percentage of ABTS free radical inhibition at various concentrations.

The sample exhibited a high capacity to scavenge $ABTS^{+}$ radicals. The IC_{50} value of the sample, as determined from the linear regression equation y = −0.00950x + 0.88437 (coefficient of determination, R^2 = 0.99844), was 46.0304 ± 0.0946 µg/mL.

This value was classified as very strong antioxidant activity according to the categorization proposed by Molyneux (2003). According to Puspitasari and Sumantri (2019), the lower the IC_{50} value, the stronger the antioxidant capacity. The IC_{50} represents the concentration of a sample required to inhibit 50% of free radicals. The phenolic and flavonoid compounds present in the sample are believed to contribute to this activity through their ability to donate electrons or protons to neutralize free radicals (Sánchez-Moreno, 2002). The superior antioxidant potential of pure flavonoids is supported by the stronger activity of quercetin, which was used as the positive control and exhibited an IC_{50} of 4.23 ± 0.03 µg/mL (Rice-Evans et al., 1996).

Table 7. ABTS radical scavenging activity of quercetin standard and *Ampelocissus thyrsiflora* leaf extract

Sample	Concentration (µg/mL)	Absorbance (Replications)			Mean Inhibition (%)	IC50 (µg/mL)
		I	II	III		
Ethyl Acetate Extract	0	0.8893	0.8876	0.8866	0.00	46.0304 ± 0.0946 *(Very Strong)*
	10	0.7819	0.7818	0.7818	11.94	
	20	0.6957	0.6948	0.6976	21.60	
	30	0.6004	0.5993	0.5940	33.00	
	40	0.5146	0.5139	0.5165	41.74	
	50	0.4030	0.4022	0.4022	54.64	
Quercetin	0	0.8508	0.8575	0.8503	0.00	4.2316 ± 0.0310 *(Very Strong)*
	1	0.7560	0.7567	0.7588	11.22	
	2	0.6555	0.6518	0.6578	23.19	
	3	0.5387	0.5335	0.5395	37.01	
	4	0.4467	0.4485	0.4479	47.51	
	5	0.3572	0.3561	0.3552	58.24	

A very strong relationship (R^2 = 0.9984) was observed between sample concentration and the percentage of free radical inhibition, indicating that antioxidant activity increased linearly with increasing concentration. An R^2 value of 0.9984 indicates that approximately 99.84% of the variation in free radical inhibition could be explained by changes in extract concentration. In other words, there was a very strong and nearly linear relationship between sample concentration and its ability to inhibit free radicals. As the concentration increased, the antioxidant activity demonstrated by the sample also increased. The R^2 value approaching 1 indicates that the concentration–antioxidant activity model showed a very strong fit, supporting the reliability of the observed relationship in the antioxidant assay. These findings highlight the potential of the sample as a source of natural antioxidants for potential applications in the food, pharmaceutical, and cosmetic industries.

The results were also influenced by the use of the semipolar solvent ethyl acetate. Semipolar phenolic and flavonoid compounds can be extracted using this solvent and are believed to contribute substantially to the strong antioxidant activity of the sample. This finding is consistent with the reports of Kähkönen et al. (1999) and Harborne (1987), who indicated that semipolar solvents such as *ethyl acetate* can effectively extract flavonoid compounds compared with highly polar solvents such as *water* or *ethanol*. The effectiveness of *ethyl acetate* in extracting semipolar compounds, including phenolics and flavonoids, may therefore contribute to the high antioxidant activity observed in the sample. These compounds are known to scavenge free radicals

through electron- or hydrogen-donation mechanisms. In addition, the intermediate polarity of *ethyl acetate* enables the extraction of various bioactive compounds that may be less soluble in highly polar or nonpolar solvents. Therefore, the use of *ethyl acetate* as an extraction solvent may increase the concentration of antioxidant compounds in the sample, thereby contributing to its potential protective effects against oxidative stress. These findings further emphasize the importance of selecting an appropriate extraction solvent to optimize the recovery of bioactive compounds from natural materials.

CONCLUSIONS

The ethyl acetate extract of *Ampelocissus thyrsiflora* leaves exhibited very strong antioxidant activity, as evidenced by an IC_{50} value of 46.0304 ± 0.0946 µg/mL. This high activity strongly correlates with its total phenolic content (92.8912 ± 1.28 mg GAE/g) and total flavonoid content (95.1225 ± 1.969 mg QE/g). The pronounced antioxidant potential is attributed to the efficacy of ethyl acetate in extracting semipolar bioactive constituents, specifically phenolic and flavonoid compounds. Furthermore, the robust linear relationship between extract concentration and radical scavenging percentage confirms the extract's promise as a potent natural antioxidant source for prospective applications in the food, pharmaceutical, and cosmeceutical industries.

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