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Antioxidant Activities and Phytochemical Profiles of Pepino (*Solanum muricatum*) Fruit Ethanol Extract and Solvent Fractions

Aktivitas Antioksidan dan Profil Fitokimia Ekstrak Etanol serta Fraksi Pelarut Buah Pepino (*Solanum muricatum*)

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ABSTRACT

Pepino fruit (*Solanum muricatum* Aiton) contains bioactive compounds with potential antioxidant properties. This study evaluated the total phenolic and flavonoid contents, thin-layer chromatographic profiles, and antioxidant activities of a 70% ethanol extract and its *n*-hexane, ethyl acetate, and water fractions. The ethyl acetate fraction contained the highest total phenolic and flavonoid contents, at 13.07 ± 0.47 mg GAE/g and 4.33 ± 0.13 mg QE/g, respectively. It also exhibited the strongest antioxidant activity among the tested fractions, with IC_{50} values of 99.56 ± 6.00 µg/mL in the DPPH assay and 67.69 ± 0.86 µg/mL in the ABTS assay, and an EC_{50} of 59.95 ± 0.87 µg/mL in the FRAP assay. These findings identify the ethyl acetate fraction as the most promising fraction for further investigation as a natural source of antioxidant compounds.

ABSTRAK

Buah pepino (*Solanum muricatum* Aiton) mengandung senyawa bioaktif yang berpotensi sebagai antioksidan. Penelitian ini mengevaluasi kandungan total fenolik dan flavonoid, profil kromatografi lapis tipis, serta aktivitas antioksidan ekstrak etanol 70% dan fraksi *n*-heksana, etil asetat, serta air. Fraksi etil asetat memiliki kandungan total fenolik dan flavonoid tertinggi, masing-masing sebesar 13,07 ± 0,47 mg GAE/g dan 4,33 ± 0,13 mg QE/g. Fraksi tersebut juga menunjukkan aktivitas antioksidan terkuat di antara seluruh fraksi, dengan nilai IC_{50} sebesar 99,56 ± 6,00 µg/mL pada metode DPPH dan 67,69 ± 0,86 µg/mL pada metode ABTS, serta nilai EC_{50} sebesar 59,95 ± 0,87 µg/mL pada metode FRAP. Hasil ini menunjukkan bahwa fraksi etil asetat merupakan fraksi paling menjanjikan untuk diteliti lebih lanjut sebagai sumber alami senyawa antioksidan.

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1. INTRODUCTION

Aging is a natural biological process accompanied by progressive structural and functional changes in the skin, including reduced elasticity and increased susceptibility to oxidative damage (Csekes & Račková, 2021). Excessive production of reactive oxygen species can disrupt cellular homeostasis and accelerate skin-aging processes through damage to lipids, proteins, and nucleic acids. Natural polyphenols and flavonoids have therefore attracted considerable attention because of their ability to scavenge free radicals and contribute to antioxidant defense mechanisms (Csekes & Račková, 2021). The identification of safe and effective plant-derived antioxidants remains relevant for the development of functional, pharmaceutical, and cosmetic ingredients.

Pepino fruit (*Solanum muricatum* Aiton) contains several bioactive constituents, including phenolic compounds, flavonoids, β-carotene, and vitamin C, which may contribute to its antioxidant properties (Mahato et al., 2016). Previous studies have reported substantial phenolic and flavonoid contents in pepino fruit, although the measured levels vary according to cultivar, fruit maturity, extraction procedure, analytical method, and reporting unit (Di Scala et al., 2011; Maheshwari et al., 2014). Antioxidant activity has also been demonstrated in pepino fruit extracts, with differences attributed partly to the polarity of the extraction solvent and the distribution of bioactive constituents among solvent fractions (Sudha et al., 2012). In addition, 70% ethanol has been reported to be effective for extracting phenolic and flavonoid compounds from pepino fruit because its intermediate polarity facilitates the recovery of a broad range of phytochemicals (Firsty et al., 2023).

Despite these findings, comparative information on the phytochemical composition and antioxidant performance of pepino ethanol extract and its sequential *n*-hexane, ethyl acetate, and water fractions remains limited. Fractionation based on solvent polarity may concentrate different groups of antioxidant compounds and help identify the fraction with the greatest potential for further bioactive-compound isolation. Therefore, this study aimed to determine the total phenolic and flavonoid contents, characterize the thin-layer chromatographic profiles, and evaluate the antioxidant activities of the ethanol extract and solvent fractions of *S. muricatum* fruit using DPPH, FRAP, and ABTS assays. The findings are expected to provide a scientific basis for the further investigation of pepino fruit as a natural source of antioxidant ingredients.

2. METHODS

2.1. Materials and Instruments

Pepino fruit (*S. muricatum*) was obtained from Melody Orchard, Wonolelo Village, Sawangan District, Magelang Regency, Central Java, Indonesia. Botanical authentication was performed by the Herbal Laboratory of Materia Medica Batu, Malang, East Java, Indonesia, under reference number 000.9.3/3290/102.20/2025.

The materials used included distilled water, technical-grade ethanol, *n*-hexane, and ethyl acetate (Brataco, Jakarta, Indonesia); analytical-grade ethanol, methanol, chloroform, acetone, acetic acid, ammonia, ferric chloride, aluminum chloride, sodium acetate, Folin–Ciocalteu reagent, potassium dihydrogen phosphate, and other analytical-grade reagents (Merck, Darmstadt, Germany); and gallic acid, quercetin, DPPH, ABTS, and trichloroacetic acid (Sigma-Aldrich, St. Louis, MO, USA). Vitamin C and quercetin were used as positive controls in the FRAP and ABTS assays, respectively.

The main instruments included a drying oven (Getra, China), blender (Philips, Amsterdam, the Netherlands), rotary evaporator (Biobase, Shandong, China), water bath (Biobase, Shandong, China), analytical balance (Ohaus, Parsippany, NJ, USA), and UV–visible spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan).

2.2. Sample Preparation and Extraction

Pepino fruit was cleaned, cut into small pieces, dried, and ground into powder. A total of 150 g of fruit powder was macerated with 70% ethanol at a material-to-solvent ratio of 1:10 (w/v) for 24 h at room temperature. The mixture was filtered, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 50 °C. The liquid extract was further concentrated in a water bath until a viscous ethanol extract was obtained. The extract was stored at 4 °C until further analysis (Sudha et al., 2012, with modifications).

2.3. Fractionation

A total of 40 g of the concentrated ethanol extract was suspended in 100 mL of distilled water and sequentially partitioned with *n*-hexane and ethyl acetate. First, 100 mL of *n*-hexane was added to the aqueous suspension in a separatory funnel, shaken, and allowed to stand until two distinct layers formed. The *n*-hexane layer was collected, and the partitioning process was repeated with fresh solvent until the organic layer became visually clear. The remaining aqueous layer was subsequently partitioned with ethyl acetate using the same procedure. The *n*-hexane, ethyl acetate, and water fractions were concentrated separately using a rotary evaporator at 40 °C (Marfan et al., 2024, with modifications).

2.4. Determination of Extraction and Fractionation Yields

The extraction yield was calculated relative to the initial weight of dried fruit powder, whereas the yield of each solvent fraction was calculated relative to the 40 g of ethanol extract used for partitioning:

$$\text{Yield (\%)} = \frac{\text{weight of recovered extract or fraction}}{\text{weight of starting material or extract}} \times 100$$

2.5. Thin-Layer Chromatography

Thin-layer chromatography was performed using silica gel F_{254} plates. Before use, the plates were activated in an oven at 100 °C for 10 min. The ethanol extract, solvent fractions, and reference standards were prepared at a concentration of 10 mg/mL in methanol and applied to the plates.

For phenolic profiling, the mobile phase consisted of ethyl acetate–water–formic acid–toluene (20:2:2:1, v/v/v/v), and gallic acid was used as the reference standard. The developed plate was observed under ultraviolet light and subsequently sprayed with ferric chloride reagent.

For flavonoid profiling, the mobile phase consisted of *n*-butanol–acetic acid–water (4:1:5, v/v/v), and quercetin was used as the reference standard. The chromatograms were observed under UV light at 254 and 366 nm and after exposure to ammonia vapor. Retention factor values were calculated as follows:

$$R_f = \frac{\text{distance traveled by the compound}}{\text{distance traveled by the solvent front}}$$

The procedure was adapted from Pratama et al. (2018) and Moldovan et al. (2020), with modifications.

2.6. Determination of Total Phenolic Content

Total phenolic content was determined using the Folin–Ciocalteu method. Gallic acid standard solutions were prepared at concentrations of 25, 50, 75, and 100 µg/mL, whereas the ethanol extract and solvent fractions were prepared at 8,000 µg/mL. A 1 mL aliquot of each standard or sample solution was mixed with 5 mL of 7.5% Folin–Ciocalteu reagent. After standing for 8 min, 4 mL of 1% sodium hydroxide solution was added. The mixture was incubated for 60 min, and the absorbance was measured at 760.5 nm using a UV–visible spectrophotometer. Measurements were performed in triplicate. Total phenolic content was calculated from the gallic acid calibration curve and expressed as milligrams of gallic acid equivalents per gram of sample (mg GAE/g) (Kementerian Kesehatan Republik Indonesia, 2017, with modifications).

2.7. Determination of Total Flavonoid Content

Total flavonoid content was determined using an aluminum chloride colorimetric method. Quercetin standard solutions were prepared at concentrations of 25, 50, 75, and 100 µg/mL, whereas the ethanol extract and solvent fractions were prepared at 8,000 µg/mL. A 0.5 mL aliquot of each standard or sample solution was mixed with 1.5 mL of analytical-grade ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M sodium acetate, and 2.8 mL of distilled water. The mixture was incubated for 28 min, and the absorbance was measured at 425 nm. Measurements were performed in triplicate. Total flavonoid content was calculated from the quercetin calibration curve and expressed as milligrams of quercetin equivalents per gram of sample (mg QE/g) (Kementerian Kesehatan Republik Indonesia, 2017, with modifications).

2.8. DPPH Radical-Scavenging Assay

DPPH radical-scavenging activity was evaluated using quercetin as the positive control. Quercetin solutions were prepared at concentrations of 1, 2, 3, 4, and 5 µg/mL. The ethanol extract and the *n*-hexane, ethyl acetate, and water fractions were prepared at concentrations of 200, 400, 600, and 800 µg/mL. A 2 mL aliquot of each quercetin or sample solution was mixed with 2 mL of 0.01% DPPH solution in methanol and incubated for 25 min in the dark. Absorbance was measured at 515.4 nm using a UV–visible spectrophotometer. Measurements were performed in triplicate (Sugihartini et al., 2024, with modifications).

The percentage inhibition was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where A_{control} is the absorbance of the negative control and A_{sample} is the absorbance of the tested solution. The IC_{50} value was estimated from the linear regression equation constructed from the concentration-response data obtained for each sample. The regression equation was generated using the measured percentage inhibition values within the tested concentration range.

2.9. FRAP Assay

Ferric-reducing antioxidant power was evaluated using vitamin C as the positive control. Vitamin C solutions were prepared at concentrations of 2, 4, 6, 8, and 10 µg/mL. The ethanol extract and the *n*-hexane, ethyl acetate, and water fractions were prepared at concentrations of 60, 70, 80, 90, and 100 µg/mL. A 1 mL aliquot of each test solution was mixed with 1 mL of 0.2 M phosphate buffer at pH 6.6 and 1 mL of 1% potassium ferricyanide solution. The mixture was incubated at 50 °C for 20 min, after which 1 mL of 10% trichloroacetic acid was added and the mixture was vortexed. A 1 mL aliquot of the resulting solution was mixed with 1 mL of distilled water and 0.5 mL of 0.1% ferric chloride solution. Analytical-grade ethanol was then added to obtain a final volume of 5 mL. After standing for 10 min, absorbance was measured at 706 nm. Measurements were performed in triplicate (Purnamasari et al., 2024, with modifications). The EC_{50} value was estimated from the linear regression equation constructed from the concentration-response data for each sample and was defined as the concentration required to produce 50% of the maximum reducing response.

2.10. ABTS Radical-Cation Scavenging Assay

ABTS radical-cation scavenging activity was assessed using quercetin as the positive control. Quercetin was prepared at concentrations of 5, 10, 15, 20, and 25 µg/mL. The ethanol extract and the *n*-hexane, ethyl acetate, and water fractions were prepared at concentrations of 100, 200, 300, 400, and 500 µg/mL. A 1 mL aliquot of each test solution was mixed with 2 mL of ABTS working solution and incubated for 46 min. Absorbance was measured at 739.5 nm using a UV–visible spectrophotometer. Measurements were performed in triplicate (Afifah et al., 2023, with modifications). The percentage inhibition was calculated using the same equation applied in the DPPH assay. The IC_{50} value was estimated from the linear regression equation constructed from the concentration-response data obtained for each sample.

2.11. Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation. Statistical analyses were

conducted using IBM SPSS Statistics version 24. Data normality was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated using Levene's test. Differences among the ethanol extract, solvent fractions, and positive control were analyzed using one-way analysis of variance. When the homogeneity-of-variance assumption was satisfied, pairwise comparisons were performed using Tukey's honestly significant difference test. When the assumption was violated, Welch's analysis of variance followed by the Games–Howell post hoc test was applied. Differences were considered statistically significant at $p < 0.05$. Different letters in the figures indicate statistically significant differences among samples.

3. RESULTS AND DISCUSSION

3.1. Extraction Yield

Maceration of *S. muricatum* fruit using 70% ethanol produced a concentrated extract with a yield of 62.08%, which was subsequently partitioned using *n*-hexane, ethyl acetate, and water to separate constituents according to their relative polarity. As presented in **Table 1**, the water fraction produced the highest yield at 52.70% (21.09 g), followed by the *n*-hexane fraction at 6.30% (2.52 g) and the ethyl acetate fraction at 5.05% (2.02 g). The predominance of the water fraction suggests that a substantial proportion of the extractable constituents remained in the polar phase, consistent with the abundance of water-soluble components commonly found in fruit matrices and with previous reports that polar fractions may recover greater amounts of dissolved material than semi-polar or non-polar fractions (Marfan et al., 2024). However, the highest fraction yield did not correspond to the highest phytochemical content or antioxidant activity, as the lower-yield ethyl acetate fraction contained the highest total phenolic and flavonoid contents and showed the strongest antioxidant performance. This finding indicates that fraction yield reflects the total mass of recovered constituents rather than the concentration of specific bioactive compounds.

Table 1. Fractionation yields of the ethanol extract of *S. muricatum* fruit

Fraction	Initial extract weight (g)	Fraction weight (g)	Yield (%)
n-Hexane	40.00	2.52	6.30
Ethyl acetate	40.00	2.02	5.05
Water	40.00	21.09	52.70

3.2. Quantification of Total Phenolic Content

Total phenolic content differed significantly among the ethanol extract and solvent fractions, as shown in **Figure 3**. The ethyl acetate fraction exhibited the highest phenolic content (13.07 ± 0.47 mg GAE/g), followed by the n-hexane fraction (10.49 ± 0.55 mg GAE/g), the water fraction (7.90 ± 0.18 mg GAE/g), and the ethanol extract (5.87 ± 0.48 mg GAE/g), with all samples differing significantly ($p < 0.05$). The preferential accumulation of phenolic compounds in the ethyl acetate fraction suggests that the major phenolic constituents of *S. muricatum* fruit are predominantly semi-polar and were efficiently enriched during solvent partitioning. Similar observations have been reported in other medicinal plants, where ethyl acetate effectively concentrates phenolic constituents because of its intermediate polarity (Kumalasari et al., 2021; Putri et al., 2023; Rondonuwu et al., 2017). As phenolic compounds are recognized as important hydrogen- and electron-donating antioxidants, the higher phenolic content of the ethyl acetate fraction may contribute to its superior antioxidant performance, which is discussed in the subsequent antioxidant assays.

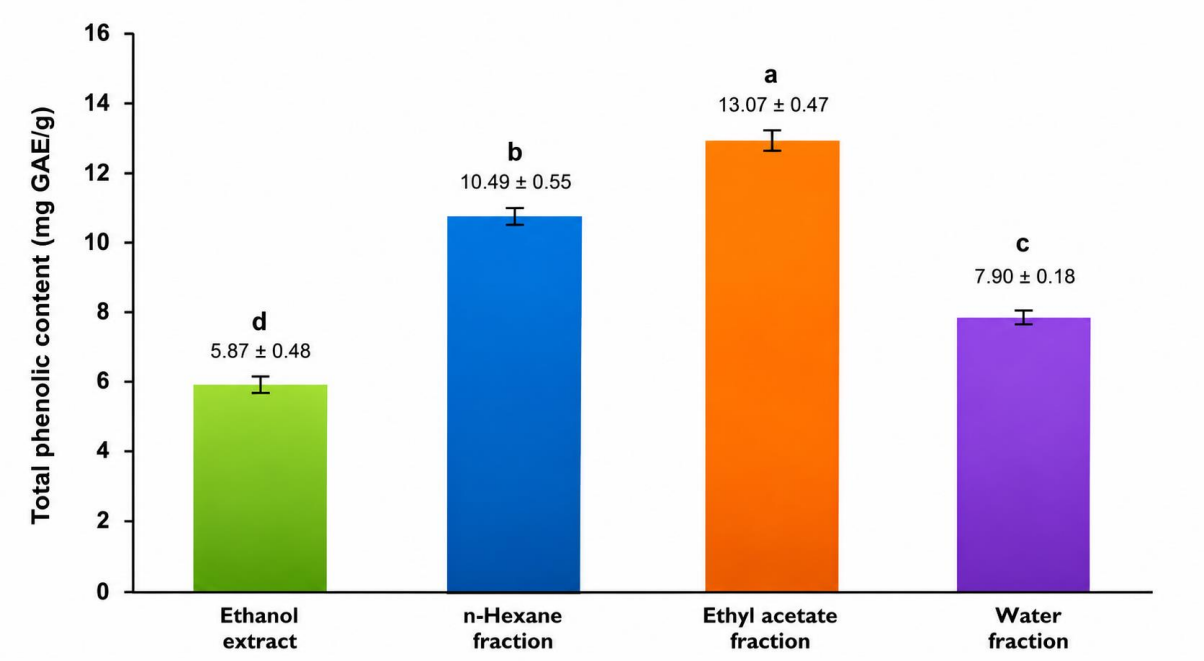


Figure 3. Total phenolic content of the ethanol extract and solvent fractions of *Solanum muricatum* fruit. Data are presented as mean ± standard deviation (n = 3). Different letters indicate significant differences ($p < 0.05$). GAE, gallic acid equivalent.

3.3. Quantification of Total Flavonoid Content

Total flavonoid content also differed significantly among the ethanol extract and solvent fractions, as shown in **Figure 4**. The ethyl acetate fraction contained the highest flavonoid level at 4.33 ± 0.13 mg QE/g, followed by the n-hexane fraction at 3.42 ± 0.18 mg QE/g, the ethanol extract at 3.26 ± 0.16 mg QE/g, and the water fraction at 2.70 ± 0.07 mg QE/g. The ethyl acetate fraction differed significantly from the other samples, while the ethanol extract and n-hexane fraction were not significantly different from each other ($p < 0.05$). The higher flavonoid level in the ethyl acetate fraction indicates that semi-polar flavonoid constituents were preferentially transferred into this solvent during partitioning. Similar enrichment patterns have been reported in other plant extracts, where ethyl acetate fractions contained higher flavonoid concentrations than crude or aqueous fractions (Furi et al., 2024; Saputri & Sa'ad, 2023). Because flavonoids can donate electrons or hydrogen atoms and stabilize radical intermediates through their conjugated structures, their accumulation in the ethyl acetate fraction may contribute to the stronger antioxidant activity observed in the subsequent assays.

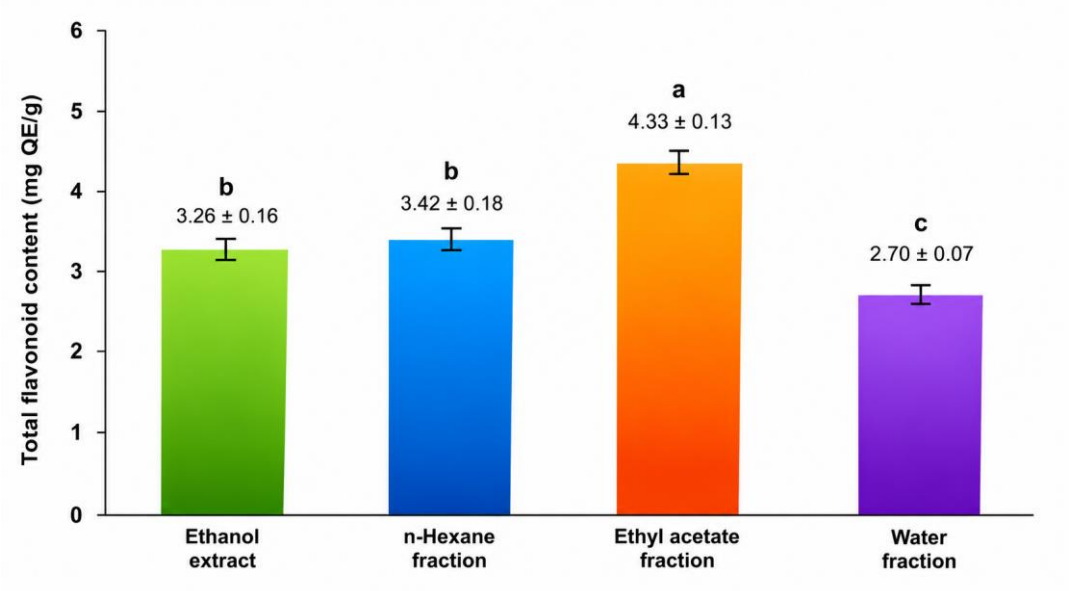


Figure 4. Total flavonoid content of the ethanol extract and solvent fractions of *Solanum muricatum* fruit. Data are presented as mean ± standard deviation (n = 3). Different letters indicate significant differences ($p < 0.05$). QE, quercetin equivalent.

3.4. TLC Fingerprint Analysis

The TLC fingerprints of the ethanol extract and solvent fractions of *Solanum muricatum* fruit are presented in **Figure 5**. The phenolic profile was developed on silica gel GF_{254} using ethyl acetate–water–formic acid–toluene (20:2:2:1, v/v/v/v) as the mobile phase. The ethyl acetate fraction exhibited the most complex chromatographic profile, producing four spots at *Rf* values of 0.50, 0.70, 0.80, and 0.90. The ethanol extract produced two spots at *Rf* 0.50 and 0.90, while the *n*-hexane fraction produced two spots at *Rf* 0.50 and 0.85. Following correction of the tabulated data, the water fraction was recorded as producing two spots at *Rf* 0.50 and 0.90. The gallic acid standard produced a single spot at *Rf* 0.70. The spot observed in the ethyl acetate fraction at *Rf* 0.70 exhibited chromatographic mobility comparable to that of gallic acid. However, similarity in *Rf* value alone is insufficient to confirm compound identity.

The flavonoid profile was developed on silica gel GF_{254} using *n*-butanol–acetic acid–water (4:1:5, v/v/v) as the mobile phase and was visualized under UV light at 254 nm and after exposure to ammonia vapor. The ethyl acetate fraction again exhibited the most complex profile, producing four spots at *Rf* values of 0.50, 0.70, 0.80, and 0.90. The *n*-hexane fraction produced three spots at *Rf* 0.50, 0.70, and 0.80, whereas the ethanol extract and water fraction each produced one spot at *Rf* 0.50. The quercetin standard produced a single spot at *Rf* 0.90, corresponding to one of the spots detected in the ethyl acetate fraction. These chromatographic patterns are consistent with the higher total phenolic and flavonoid contents of the ethyl acetate fraction. Nevertheless, confirmation of individual compounds requires co-chromatography with authenticated standards or further analysis using HPTLC, HPLC, or LC–MS.

3.5. Antioxidant Activity Using the DPPH Method

The free radical-scavenging activities of the ethanol extract and solvent fractions of *Solanum muricatum* fruit are presented in **Figure 6**. Significant differences in antioxidant capacity were observed among the tested samples ($p < 0.05$). The ethyl acetate fraction exhibited the strongest DPPH radical-scavenging activity, with an IC_{50} value of 99.56 ± 6.00 µg/mL, followed by the *n*-hexane fraction (245.98 ± 10.40 µg/mL), the water fraction (376.36 ± 10.13 µg/mL), and the ethanol extract (398.84 ± 6.56 µg/mL). As expected, quercetin showed the greatest activity with an IC_{50} value of 1.01 ± 0.086 µg/mL. Lower IC_{50} values indicate greater radical-scavenging activity, demonstrating that fractionation substantially enhanced the antioxidant potency of the ethyl acetate fraction.

The superior activity of the ethyl acetate fraction is consistent with its higher total phenolic and flavonoid contents and its more complex TLC fingerprint, indicating enrichment of semi-polar

antioxidant constituents during solvent partitioning. Phenolic compounds readily donate hydrogen atoms or electrons to stabilize DPPH radicals, while flavonoids further enhance radical-scavenging activity through their hydroxyl groups and conjugated aromatic structures. Similar enrichment of antioxidant activity in ethyl acetate fractions has been reported for numerous medicinal plants, where solvent partitioning concentrates phenolic antioxidants with intermediate polarity (Kumalasari et al., 2021; Putri et al., 2023; Furi et al., 2024). Although quercetin remained considerably more potent than all extract fractions because it represents a purified reference compound, the ethyl acetate fraction demonstrated the highest antioxidant potential among the tested samples, supporting its selection for further investigation as a source of natural antioxidant constituents.

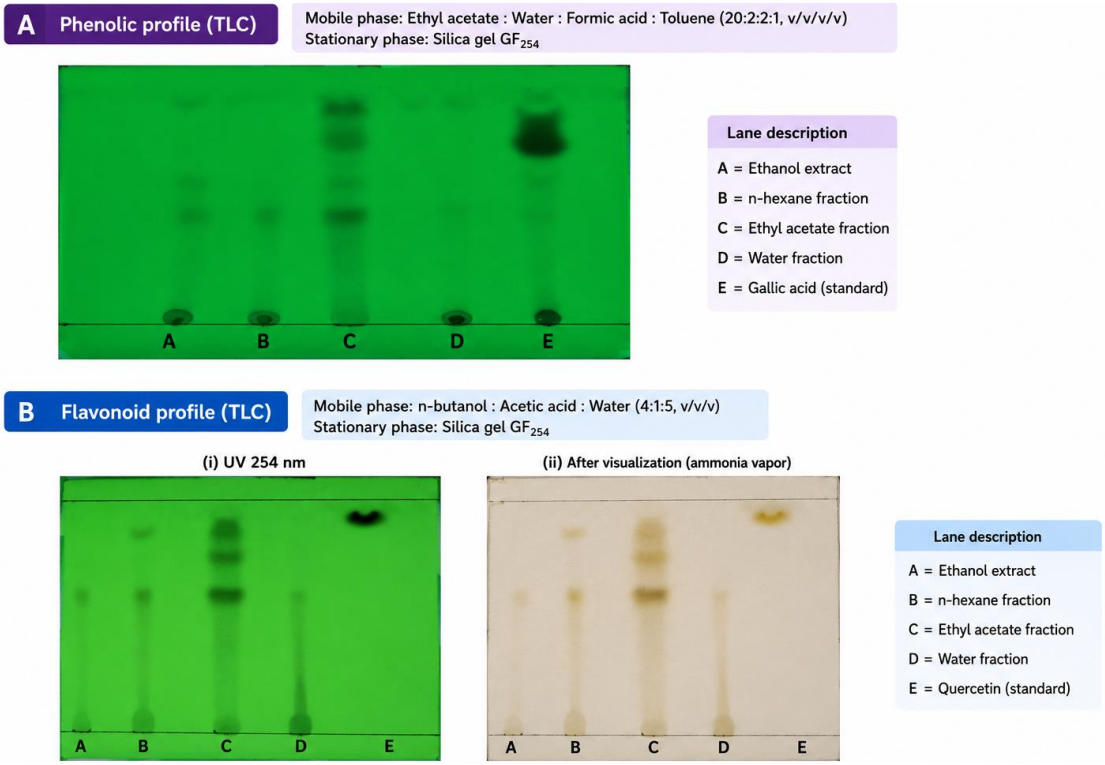


Figure 5. TLC fingerprints of phenolic and flavonoid constituents in the ethanol extract and solvent fractions of *Solanum muricatum* fruit. (A) Phenolic profile developed on silica gel GF_{254} using ethyl acetate–water–formic acid–toluene (20:2:2:1, v/v/v/v). (B) Flavonoid profile developed on silica gel GF_{254} using *n*-butanol–acetic acid–water (4:1:5, v/v/v) and visualized under UV light at 254 nm and after exposure to ammonia vapor. Lanes: A, ethanol extract; B, *n*-hexane fraction; C, ethyl acetate fraction; D, water fraction; and E, gallic acid standard in panel A or quercetin standard in panel B.

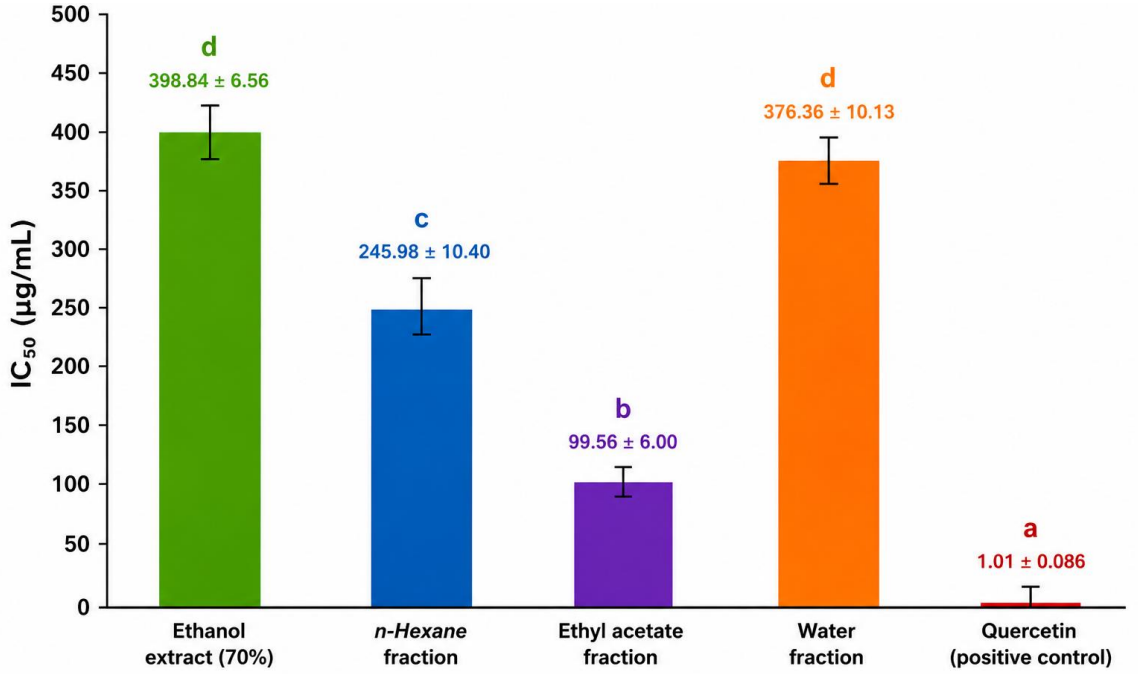


Figure 6. DPPH radical-scavenging activity of the ethanol extract and solvent fractions of *Solanum muricatum* fruit. Data are expressed as IC_{50} values. A lower IC_{50} value indicates stronger antioxidant activity. Quercetin was used as the positive control.

3.6. Antioxidant Activity Using the FRAP Method

The ferric-reducing capacities of the ethanol extract and solvent fractions of *Solanum muricatum* fruit are presented in **Figure 7**. The differences among the pepino samples were relatively small. The ethyl acetate fraction exhibited the lowest mean EC_{50} value at 59.95 ± 0.87 µg/mL, followed by the *n*-hexane fraction at 60.52 ± 0.41 µg/mL, the water fraction at 61.99 ± 0.17 µg/mL, and the ethanol extract at 62.84 ± 0.19 µg/mL. Vitamin C demonstrated substantially stronger reducing activity, with an EC_{50} value of 6.35 ± 0.70 µg/mL. Lower EC_{50} values indicate greater ferric-reducing capacity.

Although the ethyl acetate fraction had the lowest mean EC_{50} value, it was not significantly different from the *n*-hexane fraction. The water fraction was not significantly different from the ethanol extract or the n-hexane fraction but differed significantly from the ethyl acetate fraction. The slightly greater reducing capacity of the ethyl acetate fraction was consistent with its higher total phenolic and flavonoid contents and its more complex TLC profile. Phenolic constituents can reduce Fe^{3+} to Fe^{2+} through electron donation, and comparable relationships between phenolic content and FRAP activity have been reported in plant extracts and fractions (Kumar et al., 2014; Minarti et al., 2024). However, because the differences among the pepino samples were narrow, the FRAP results should be interpreted together with the DPPH and ABTS assays rather than as standalone evidence of markedly different antioxidant potency.

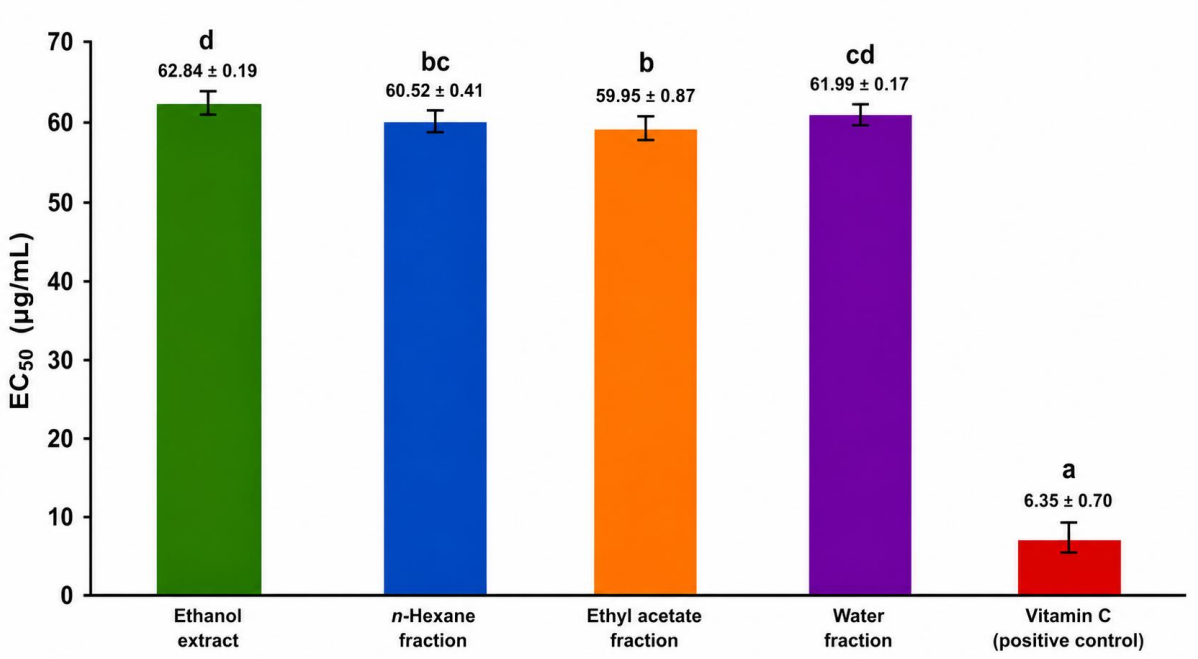


Figure 7. Ferric-reducing antioxidant power of the ethanol extract and solvent fractions of *Solanum muricatum* fruit. Data are presented as mean ± standard deviation ($n = 3$). Different letters indicate significant differences ($p < 0.05$). Lower EC_{50} values indicate stronger ferric-reducing capacity.

3.7. Antioxidant Activity Using the ABTS Method

The ABTS radical-cation scavenging activities of the ethanol extract and solvent fractions of *Solanum muricatum* fruit are presented in **Figure 8**. The ethyl acetate fraction exhibited the strongest activity among the pepino samples, with an IC_{50} value of 67.69 ± 0.86 µg/mL, followed by the *n*-hexane fraction at 193.23 ± 4.44 µg/mL, the water fraction at 229.34 ± 9.38 µg/mL, and the ethanol extract at 234.37 ± 9.49 µg/mL. Quercetin showed the greatest overall activity, with an IC_{50} value of 3.97 ± 0.08 µg/mL. Lower IC_{50} values indicate stronger ABTS radical-cation scavenging activity.

The ethyl acetate fraction differed significantly from all other pepino samples. The water fraction was not significantly different from the ethanol extract, whereas the n-hexane fraction differed significantly from both. Its stronger ABTS activity is consistent with the higher total phenolic and flavonoid contents and the more complex TLC profile observed for this fraction. Semi-polar phenolic and flavonoid constituents may contribute to ABTS radical-cation neutralization through electron- and hydrogen-donating mechanisms. Differences between the present findings and previous reports may be attributable to variations in fruit maturity, geographical origin, extraction procedure, solvent composition, phytochemical profile, and assay conditions. Because ABTS can detect both hydrophilic and lipophilic antioxidants, the assay complements the DPPH and FRAP results and provides a broader assessment of the antioxidant potential of the pepino fractions.

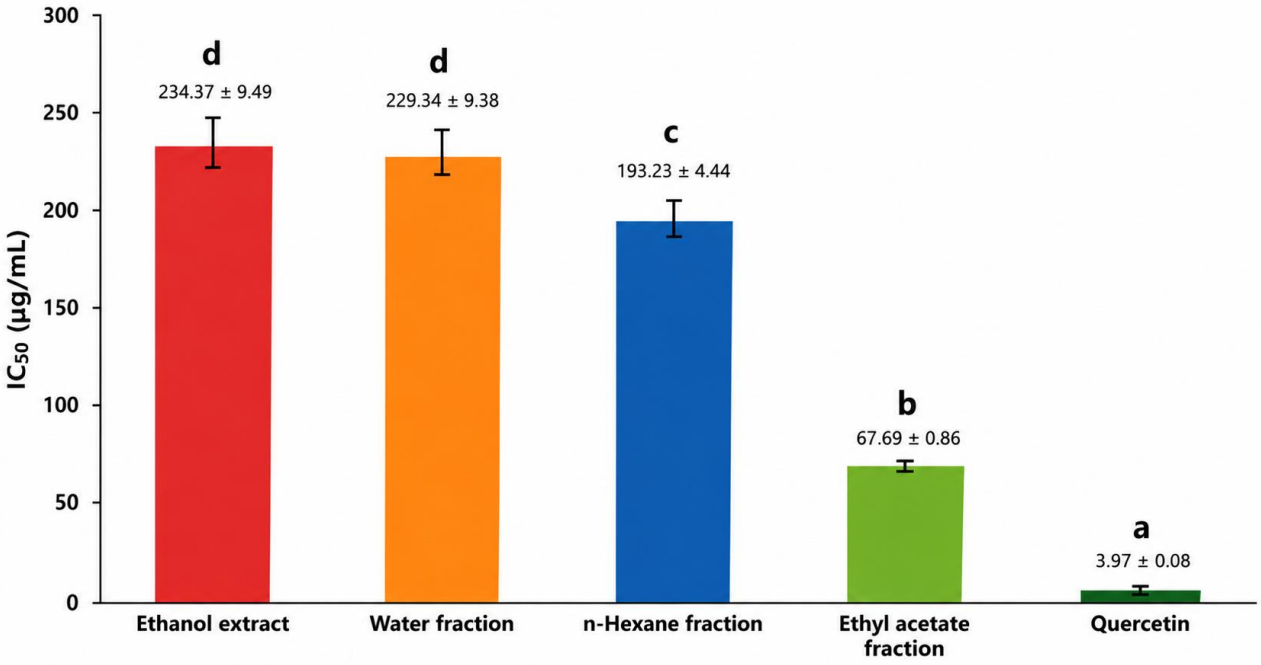


Figure 8. ABTS radical-cation scavenging activity of the ethanol extract and solvent fractions of *Solanum muricatum* fruit. Data are presented as mean ± standard deviation ($n = 3$). Different letters indicate significant differences ($p < 0.05$). Lower IC_{50} values indicate stronger antioxidant activity. Quercetin was used as the positive control.

3.8. Overall Antioxidant Performance of Pepino Fruit Fractions

Across the three antioxidant assays, the ethyl acetate fraction consistently exhibited the strongest activity among the pepino-derived samples. It showed the lowest IC_{50} values in the DPPH and ABTS assays and the lowest mean EC_{50} value in the FRAP assay. This superior performance was supported by its higher total phenolic and flavonoid contents and its more complex TLC fingerprint, indicating that solvent partitioning effectively enriched semi-polar antioxidant constituents.

Slight differences in sample ranking were observed among the DPPH, FRAP, and ABTS assays because each method evaluates a different antioxidant mechanism. DPPH and ABTS primarily assess radical-scavenging activity, whereas FRAP measures electron-donating capacity through the reduction of Fe^{3+} to Fe^{2+}. The clearer separation among samples in the DPPH and ABTS assays, compared with the relatively narrow EC_{50} range observed in the FRAP assay, suggests that differences in radical-scavenging capacity were more pronounced than differences in ferric-reducing power.

The ranking of antioxidant activity was generally consistent across the three assays, with the ethyl acetate fraction showing the strongest activity among the pepino-derived samples, followed by the n-hexane fraction, water fraction, and ethanol extract. However, the magnitude of the differences among samples varied across the assays.

Although the ethyl acetate fraction was less active than the purified reference antioxidants—quercetin and vitamin C—this difference is expected because plant fractions contain complex mixtures of active and inactive constituents, whereas the reference compounds are purified antioxidants. Collectively, the integration of phytochemical characterization and complementary antioxidant assays provides a robust evaluation of the antioxidant potential of *S. muricatum* fruit fractions. The ethyl acetate fraction therefore represents the most promising fraction for future isolation of bioactive compounds and further investigation as a natural source of antioxidant ingredients.

4. CONCLUSION

The ethyl acetate fraction of *S. muricatum* fruit exhibited the strongest antioxidant activity across the DPPH, FRAP, and ABTS assays and contained the highest total phenolic and flavonoid levels. Its richer TLC profile further supported the greater diversity of phenolic and flavonoid constituents in this fraction. These findings identify the ethyl acetate fraction as the most promising fraction for further isolation and characterization of antioxidant compounds. Future studies should identify the major active constituents, evaluate their safety, and investigate relevant skin-aging-related mechanisms before development as a natural antioxidant ingredient.

AUTHOR CONTRIBUTIONS

Conceptualization, G.R.F.; methodology, G.R.F., O.P., and P.S.W.; software, P.S.W.; validation, G.R.F.; formal analysis, G.R.F.; investigation, P.S.W.; resources, G.R.F.; data curation, G.R.F. and P.S.W.; writing—original draft preparation, G.R.F.; writing—review and editing, O.P., and P.S.W.; visualization, G.R.F.; supervision, G.R.F., O.P., and P.S.W.; project administration, P.S.W.; funding acquisition, G.R.F. All authors have read and agreed to the published version of the manuscript.

INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable. This study did not involve human participants or experimental animals.

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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The funder had no role in the design of the study; in the collection, analysis, or interpretation of the data; in the writing of the manuscript; or in the decision to publish the results.

DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE USE

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist in improving the clarity, structure, language, and readability of the text. Following the use of this tool, the authors thoroughly reviewed, edited, and verified the entire manuscript to ensure that it accurately represented their data, ideas, and interpretations. The authors take full responsibility for the integrity, accuracy, and originality of the published work.

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