Jurnal Jamu Indonesia (2026) 11(6): 532–539
https://jamu-journal.ipb.ac.id/index.php/JJI/index

ISSN 2407-7178
eISSN 2407-7763

Research Article
DOI: https://doi.org/10.29244/jji.v11i6.599

Aqueous Extract of Gembili (*Dioscorea esculenta*) Tubers Exceeds Its Ethanol Fractions in Phenolic Content and Antioxidant Capacity

Ekstrak Air Umbi Gembili (*Dioscorea esculenta*) Melampaui Fraksi Etanolnya dalam Kadar Fenolik dan Kapasitas Antioksidan

Ika Puspitaningrum[1], **Lia Kusmita**[2]*

[1]*Department of Pharmacology and Clinical Pharmacy, Stifar Yayasan Pharmasi Semarang, Semarang 50193, Central Java, Indonesia*
[2]*Department of Pharmaceutical Biology, Stifar Yayasan Pharmasi Semarang, Semarang 50193, Central Java, Indonesia*

ARTICLE INFO

Article history:
Received on: 2026-10-11
Revised on: 2026-10-17
Accepted on: 2026-10-19
Published on: 2026-11-02

Keywords:
Ethanol precipitation
Folin–Ciocalteu
Inulin
Radical scavenging

ABSTRACT

Gembili (*Dioscorea esculenta*) tubers are used in Indonesia mainly as a source of inulin, recovered from hot-water extracts by ethanol precipitation, but how antioxidants partition during this step is unknown. A hot-water extract (AE) was compared with its ethanol-insoluble (EIF) and ethanol-soluble (ESF) fractions for total phenolic (TPC) and flavonoid (TFC) contents and for 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and ferric reducing antioxidant power (FRAP) activities. AE had the highest TPC (214.9 ± 6.1 mg gallic acid equivalents/g) and TFC (159.7 ± 6.5 mg quercetin equivalents/g), the lowest IC_{50} values (DPPH 30.0 ± 0.8; ABTS 28.5 ± 1.9 µg/mL), and the highest FRAP (269 ± 42 mg ascorbic acid equivalents/g). EIF exceeded ESF in all assays except FRAP ($p < 0.001$), and antioxidant capacity followed TPC across nine determinations (DPPH IC_{50}, $r = -0.94$). The inulin-rich precipitate therefore retains part of the antioxidant capacity of the tuber.

Kata kunci:

Folin–Ciocalteu
Inulin
Pengendapan etanol
Peredaman radikal

ABSTRAK

Umbi gembili (*Dioscorea esculenta*) di Indonesia terutama dimanfaatkan sebagai sumber inulin yang diperoleh dari ekstrak air panas melalui pengendapan etanol, tetapi sebaran antioksidan selama tahap ini belum diketahui. Ekstrak air panas (EA) dibandingkan dengan fraksi tidak larut etanol (FTLE) dan fraksi larut etanol (FLE) dalam kadar fenolik total (KFT) dan flavonoid total (KFlT) serta aktivitas 2,2-difenil-1-pikrilhidrazil (DPPH), asam 2,2′-azinobis(3-etilbenzotiazolin-6-sulfonat) (ABTS), dan daya reduksi besi (FRAP). EA memiliki KFT (214,9 ± 6,1 mg ekuivalen asam galat/g) dan KFlT (159,7 ± 6,5 mg ekuivalen kuersetin/g) tertinggi, IC_{50} terendah (DPPH 30,0 ± 0,8; ABTS 28,5 ± 1,9 µg/mL), dan FRAP tertinggi (269 ± 42 mg ekuivalen asam askorbat/g). FTLE melampaui FLE pada semua uji kecuali FRAP ($p < 0,001$), dan kapasitas antioksidan mengikuti KFT pada sembilan penetapan (IC_{50} DPPH, $r = -0,94$). Dengan demikian, endapan kaya inulin tetap membawa sebagian kapasitas antioksidan umbi.

**Corresponding author:*
Lia Kusmita (lia_kusmita@yahoo.com)

Citation: Puspitaningrum, I., & Kusmita, L. (2026). Aqueous extract of gembili (*Dioscorea esculenta*) tubers exceeds its ethanol fractions in phenolic content and antioxidant capacity. *Jurnal Jamu Indonesia, 11(6), 532–539*. https://doi.org/10.29244/jji.v11i6.599

© 2026 Authors. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (http://creativecommons.org/licenses/by-nc-sa/4.0/)

1. INTRODUCTION

Oxidative stress, an imbalance between the generation of reactive oxygen species and the capacity of cellular antioxidant systems, damages lipids, proteins, and DNA and contributes to many chronic diseases (Sies et al., 2017). Food plants that supply both nutrients and redox-active secondary metabolites are therefore of interest for functional foods and jamu, and the underused tubers of Indonesia form a large but poorly characterised part of this resource. Yams illustrate the point well. Their tubers are staple foods across the tropics, and tuber extracts of several *Dioscorea* species scavenge free radicals in proportion to their phenolic acid, flavonoid, and, in pigmented cultivars, anthocyanin contents (Adomėnienė & Venskutonis, 2022; Padhan et al., 2020).

Gembili, *Dioscorea esculenta* (Lour.) Burkill, is grown on a small scale in Java and is studied mainly for its water-soluble polysaccharides. The tuber contains about 15% inulin on a dry-weight basis, and hot-water extraction followed by ethanol precipitation is the usual route to an inulin-rich preparation with prebiotic activity (Hilman et al., 2021; Putri et al., 2021; Winarti et al., 2013; Zubaidah & Akhadiana, 2013). Its antioxidant profile has received far less attention. An ethanolic tuber extract contained 14.95 mg gallic acid equivalents (GAE)/g of total phenolics and 5.44 mg quercetin equivalents (QE)/g of total flavonoids and showed a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging IC_{50} of 76.4 µg/mL (Murtado et al., 2025). Crude non-starch polysaccharides of *D. esculenta* scavenged radicals less effectively than those of *D. opposita* and *D. alata* (Jiang et al., 2024), and long-term dietary intake of the tuber was associated with lower testicular inflammation in diabetic rats (Tatara & Sato, 2025).

What remains unknown is how antioxidant constituents distribute when the aqueous extract is split by ethanol precipitation, the very step used to produce gembili inulin. Phenolic compounds bind polysaccharides through hydrogen bonding and hydrophobic interactions and can precipitate with them, which would carry part of the antioxidant capacity into the polysaccharide-rich fraction, whereas small phenolics that stay soluble in aqueous ethanol would concentrate in the supernatant (Peng et al., 2023; Xue et al., 2024). Which of these outcomes dominates cannot be predicted from earlier work on whole-tuber extracts. Answering it also requires more than one assay, because the Folin–Ciocalteu reagent responds to any reductant, aluminium chloride to particular flavonoid structures, and the DPPH, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and ferric reducing antioxidant power (FRAP) assays to different radical or electron-transfer reactions (Munteanu & Apetrei, 2021; Platzer et al., 2021; Shraim et al., 2021).

This study aimed to compare the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity of a hot-water extract of gembili tubers (AE) with those of its ethanol-insoluble fraction (EIF) and ethanol-soluble fraction (ESF). Antioxidant capacity was measured with the DPPH and ABTS radical-scavenging assays and the FRAP assay, and the association between phenolic content and each antioxidant endpoint was tested across all determinations.

2. METHODS

2.1. Plant Material

Fresh tubers of *D. esculenta* were collected from Gundi hamlet, Ledokdawan Village, Geyer District, Grobogan Regency, Central Java, Indonesia, 8–9 months after planting (**Figure 1**). Mature, undamaged tubers were selected. The plant was identified and authenticated by a university pharmaceutical biology laboratory in Yogyakarta, Indonesia (certificate No. 17.12.4/UN1/FFA.2/BF/PT/2023).

2.2. Chemicals and Instruments

DPPH, ABTS, potassium persulfate, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), gallic acid, quercetin, iron(III) chloride hexahydrate, and the Folin–Ciocalteu phenol reagent were purchased from Sigma-Aldrich (St. Louis, MO, USA), and L-ascorbic acid, aluminium chloride, potassium acetate, sodium carbonate, acetic acid, sodium acetate, hydrochloric acid, methanol, and ethanol from Merck (Darmstadt, Germany). Absorbance was measured with a UV-1780 UV–Vis spectrophotometer (Shimadzu, Kyoto, Japan; slit width 2.0 nm). Extracts and fractions were dried in a freeze-dryer (Alpha 1-2 LDplus, Martin Christ Gefriertrocknungsanlagen GmbH, Germany) and concentrated in a rotary vacuum evaporator (Hei-VAP Core, Heidolph Instruments GmbH & Co. KG, Germany).

2.3. Extraction and Fractionation

The tubers were washed under running water, peeled, cut into small pieces, and blended with hot water (80–90 °C) at a tuber-to-water ratio of 1:4 (w/v). The mixture was filtered and the filtrate allowed to cool; this filtrate was designated the aqueous extract (AE), and a portion of it was freeze-dried (Winarti et al., 2013). For fractionation, 96% ethanol was added to the remaining filtrate at 40% of its volume, and the mixture was held at about −10 °C

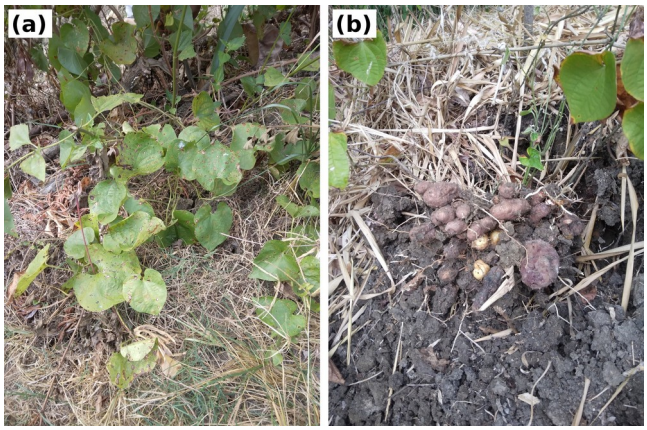

Figure 1. Gembili (*Dioscorea esculenta*) at the collection site in Grobogan Regency, Central Java, Indonesia. (a) Climbing vines with cordate leaves; (b) tubers at harvest, 8–9 months after planting.

for 18 h to complete precipitation, then thawed at 8 °C for 2 h (Zubaidah & Akhadiana, 2013). The precipitate, which contains the inulin-rich polysaccharides, was collected by vacuum filtration and freeze-dried to give the ethanol-insoluble fraction (EIF). The supernatant was concentrated in a rotary vacuum evaporator at 60 °C for 6–7 h and freeze-dried to give the ethanol-soluble fraction (ESF). The yield of each preparation was calculated as a percentage of the fresh tuber mass.

2.4. Sample Preparation

For all colorimetric assays, approximately 10 mg of each dried preparation was accurately weighed and dissolved in methanol to a final volume of 10 mL, yielding a stock solution with a concentration of approximately 1000 µg/mL. The resulting methanolic solutions were used for the total phenolic content (TPC), total flavonoid content (TFC), ABTS radical-scavenging, and ferric reducing antioxidant power (FRAP) assays.

2.5. Total Phenolic and Flavonoid Contents

TPC was determined with the Folin–Ciocalteu reagent (Singleton et al., 1999). A 0.3 mL aliquot of sample or gallic acid standard (20.4–102.0 µg/mL, five levels) was mixed with 1.0 mL of 10% (v/v) Folin–Ciocalteu reagent, vortexed for 3 min, and left for 8 min; 1.2 mL of 7% (w/v) Na_2CO_3 was then added, and the volume was made up to 10 mL with distilled water. After 2 h at room temperature, absorbance was read at 746 nm, the absorption maximum found by scanning a gallic acid reaction mixture from 400 to 800 nm. The AE solution was diluted twofold before the reaction. The calibration line was $A = 0.0052c + 0.134$ ($r = 0.991$), and TPC was expressed as mg GAE/g of dried preparation.

TFC was determined by the aluminium chloride method (Shraim et al., 2021). A 0.5 mL aliquot of sample or quercetin standard (20.6–103.0 µg/mL, five levels) was mixed in a 5 mL volumetric flask with 1.5 mL of analytical-grade ethanol, 0.1 mL of 10% (w/v) $AlCl_3$, and 0.1 mL of 1 M potassium acetate and made up to volume with distilled water; AE was diluted twofold before the reaction. After 1.5 h at room temperature, absorbance was read at 433 nm against a reagent blank containing ethanol, $AlCl_3$, and potassium acetate without sample. The calibration line was $A = 0.0065c + 0.0849$ ($r = 0.9997$), and TFC was expressed as mg QE/g of dried preparation.

2.6. DPPH Radical-Scavenging Assay

A 0.7 mM DPPH solution was prepared by dissolving 13.8 mg of DPPH in methanol to 50 mL. Its absorption maximum, 517 nm, was located by scanning a 1:1 mixture of the solution with methanol from 400 to 800 nm (Brand-Williams et al., 1995). Each preparation was dissolved in methanol and diluted serially (factor 0.8) to five concentrations: 19.7–52.5 µg/mL for AE, 39.3–108.0 µg/mL for EIF, 40.6–102.0 µg/mL for ESF, and 8.3–20.8 µg/mL for ascorbic acid, the reference antioxidant. Each range bracketed 50% inhibition for its preparation. A 0.2 mL aliquot of each solution was mixed with 4.0 mL of DPPH solution, vortexed for 1 min, and incubated in the dark at room temperature for 30 min before the absorbance was measured at 517 nm; the control contained methanol in place of sample. Inhibition was calculated with Equation (1), and IC_{50}, the sample concentration giving 50% inhibition, was obtained from the linear regression of inhibition on concentration for each replicate series. All concentrations refer to the sample solutions added to the reagent.

$$\text{Inhibition (\%)} = [(A_c - A_s) / A_c] \times 100 \quad (1)$$

where A_c is the absorbance of the control and A_s is the absorbance of the reaction mixture containing sample.

2.7. ABTS Radical-Scavenging Assay

The ABTS radical cation was generated by mixing 5 mL of 7 mM ABTS with 5 mL of 2.45 mM potassium persulfate and keeping the mixture in the dark at room temperature for 12–16 h (Re et al., 1999). Before use, it was diluted with 70% ethanol to an absorbance of 0.70 ± 0.02 at 735 nm, the absorption maximum found by scanning from 400 to 800 nm. A 0.2 mL aliquot of the sample was mixed with 4.0 mL of the ABTS working solution, vortexed for 1 min, and incubated in the dark for 30 min at room temperature. The absorbance was then measured at 734 nm against an appropriate blank. Inhibition and IC_{50} were calculated as described for DPPH.

2.8. Ferric Reducing Antioxidant Power Assay

The FRAP reagent was prepared from acetate buffer (pH 3.6; 46.3 mL of 0.2 M acetic acid and 3.7 mL of 0.2 M sodium acetate, adjusted with a calibrated pH meter), 10 mM TPTZ in 40 mM HCl, and an iron(III) chloride hexahydrate solution (0.1625 g in 50 mL of distilled water), mixed at 10:1:1 (v/v/v) immediately before use and warmed to 37 °C (Benzie & Strain, 1996). The absorption maximum of the reagent, 592 nm, was found by scanning from 400 to 800 nm. A 0.4 mL aliquot of sample solution (concentrations as in the DPPH assay) was mixed with 0.2 mL of FRAP reagent, vortexed for 1 min, and incubated in the dark for 30 min at room temperature. The absorbance was then measured at 592 nm. Because FRAP measures the formation of the coloured Fe^{2+}–TPTZ complex and has no 50% end point, absorbance was regressed on concentration for each replicate series, and the reducing capacity was expressed relative to ascorbic acid analysed in the same run, using the ratio of slopes in the manner of the TEAC calculation of Re et al. (1999), Equation (2).

$$\text{FRAP (mg AAE/g)} = (b_s / b_{AA}) \times 1000 \quad (2)$$

where b_s is the slope of absorbance against concentration for the sample (mL/µg), b_{AA} is the mean slope for ascorbic acid (0.0278 mL/µg, three series), and AAE denotes ascorbic acid equivalents.

2.9. Data Analysis

Each preparation was obtained from one extraction and fractionation batch. For every assay, three sample solutions were prepared from separately weighed portions of the dried material and analysed independently, so results are given as mean ± standard deviation (SD) of three analytical replicates ($n = 3$). In the DPPH, ABTS, and FRAP assays, each replicate consisted of a

five-point concentration series from which one IC_{50} or one slope was derived. Differences among preparations were tested by one-way analysis of variance (ANOVA) after confirming homogeneity of variance with Levene's test ($p \geq 0.31$ for all endpoints), followed by Tukey's honestly significant difference (HSD) test at $\alpha = 0.05$; ascorbic acid was included in the comparison of IC_{50} values. To relate radical-scavenging capacity to Folin–Ciocalteu response, IC_{50} values were also expressed as the GAE concentration at IC_{50} ($IC_{50} \times$ TPC). Pearson's correlation coefficients between TPC or TFC and each antioxidant endpoint were calculated across the nine determinations (three preparations × three replicates). Statistical analyses were performed in Python 3 with SciPy version 1.18 (SciPy Developers), and figures were drawn with Matplotlib version 3.11 (Matplotlib Development Team). Statistical re-analysis of the raw absorbance data and figure preparation were assisted by a generative AI tool, as stated in the Declaration of Generative AI Use; all values were checked against the original spreadsheets.

3. RESULTS AND DISCUSSION

3.1. Phenolic and Flavonoid Contents Concentrate in the Aqueous Extract and the Ethanol-Insoluble Fraction

The aqueous extract gave by far the strongest response in both colorimetric assays (**Table 1**). Its TPC was 2.7 and 4.5 times those of EIF and ESF, and its TFC 2.5 and 4.6 times, respectively (one-way ANOVA, TPC $F(2, 6) = 973.3$, TFC $F(2, 6) = 596.6$, both $p < 0.001$). The two fractions also differed from each other (Tukey's HSD, $p < 0.001$ for both TPC and TFC), with the ethanol-insoluble precipitate retaining 1.6–1.9 times more Folin–Ciocalteu-reactive and aluminium chloride-reactive material per gram than the ethanol-soluble fraction. The yields of AE, EIF, and ESF were 2.51%, 12.00%, and 2.75% (w/w of fresh tuber), respectively. TFC accounted for a similar share of TPC in all three preparations (0.73–0.82), and the two contents were almost perfectly correlated across the nine determinations ($r = 0.998$), so both assays ranked the material identically.

The phenolic content of AE is about fourteen times that reported for an ethanolic extract of gembili tuber (Murtado et al., 2025). Solvent and extraction conditions alone can change TPC and TFC substantially in the same plant material (Ghozaly et al., 2026), and hot water extracts many hydrophilic constituents that ethanol leaves behind. Part of that material is not phenolic. The Folin–Ciocalteu reagent is reduced by reducing sugars and other non-phenolic reductants as well as by phenols, and sugar-rich matrices are known to raise the apparent TPC (Lawag et al., 2023; Muñoz-Bernal et al., 2017; Pico et al., 2020). The values in **Table 1** are therefore best read as Folin–Ciocalteu reducing capacity expressed in gallic acid equivalents, which is what the assay measures (Pérez et al., 2023; Platzer et al., 2021). The same reasoning applies to TFC, because the response of the aluminium chloride complex varies with flavonoid structure and the quercetin equivalent is a calibration convention rather than a mass of flavonoids (Shraim et al., 2021).

The higher contents in EIF than in ESF are the more informative result, because EIF is the inulin-rich precipitate that would be collected in inulin production. Phenolic acids and flavonoids form non-covalent complexes with plant polysaccharides, and such complexes remain associated with the carbohydrate during its isolation (Peng et al., 2023; Xue et al., 2024). Antioxidant activity reported for crude polysaccharide preparations often originates in this co-extracted phenolic and protein material rather than in the carbohydrate itself (Fernandes & Coimbra, 2023), which fits the modest radical-scavenging capacity found for crude non-starch polysaccharides of *D. esculenta* (Jiang et al., 2024). Processing conditions are known to change the structure and associated components of yam polysaccharides (Bai et al., 2026), so the share of phenolics carried into the precipitate can be expected to depend on ethanol concentration and precipitation temperature.

3.2. The Aqueous Extract Is the Most Effective Radical Scavenger

Inhibition of both radicals rose linearly with concentration over the tested ranges (**Figure 2a**–b; coefficients of determination 0.86–0.99 for the gembili preparations), which allowed IC_{50} to be

Table 1. Total phenolic and flavonoid contents and antioxidant capacity of the aqueous extract and ethanol fractions of *Dioscorea esculenta* tubers

Preparation	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH IC_{50} (µg/mL)	ABTS IC_{50} (µg/mL)	FRAP (mg AAE/g)
AE	214.9 ± 6.1[a]	159.7 ± 6.5[a]	30.0 ± 0.8[c]	28.5 ± 1.9[c]	269 ± 42[a]
EIF	79.1 ± 5.5[b]	64.8 ± 4.1[b]	55.0 ± 0.2[b]	46.6 ± 1.8[b]	155 ± 13[b]
ESF	48.0 ± 2.3[c]	34.9 ± 2.1[c]	80.4 ± 1.1[a]	77.0 ± 1.1[a]	111 ± 4[b]
Ascorbic acid	–	–	10.4 ± 0.7[d]	9.2 ± 0.2[d]	1000 (reference)

Note. Values are mean ± SD of three analytical replicates (n = 3); all contents are expressed per gram of dried preparation. Different superscript letters in the same column indicate significant differences (one-way ANOVA followed by Tukey's HSD, $p < 0.05$). Ascorbic acid defines the FRAP scale (1000 mg AAE/g). AAE, ascorbic acid equivalents; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); AE, hot-water extract; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EIF, ethanol-insoluble fraction; ESF, ethanol-soluble fraction; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalents; IC_{50}, concentration giving 50% inhibition; QE, quercetin equivalents; TFC, total flavonoid content; TPC, total phenolic content.

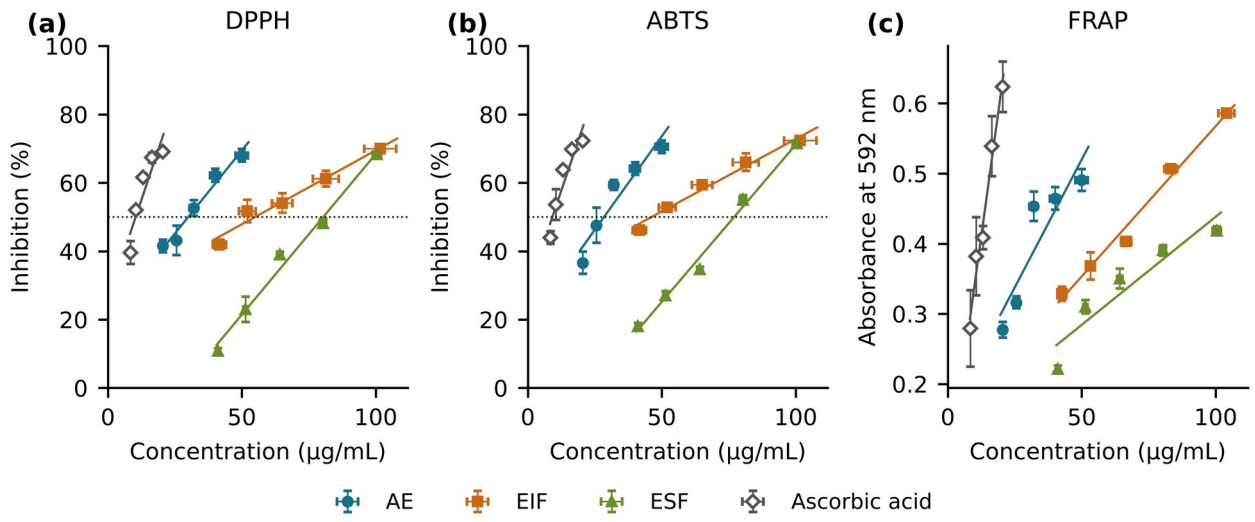

Figure 2. Concentration–response relationships of the aqueous extract (AE), ethanol-insoluble fraction (EIF), and ethanol-soluble fraction (ESF) of *Dioscorea esculenta* tubers and of ascorbic acid. (a) 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical inhibition; (b) 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical inhibition; (c) absorbance of the Fe^{2+}–2,4,6-tris(2-pyridyl)-*s*-triazine complex at 592 nm in the ferric reducing antioxidant power (FRAP) assay. Points are mean ± SD of three analytical replicates (n = 3) for both concentration and response; lines are least-squares fits to all replicate data; the dotted line marks 50% inhibition.

interpolated for every replicate. In both assays AE was the most active preparation and ESF the least, and all pairwise differences, including those with ascorbic acid, were significant (DPPH $F(3, 8) = 4624.2$; ABTS $F(3, 8) = 1241.2$; Tukey's HSD, $p < 0.001$ for every pair; **Table 1**). AE reached 50% inhibition at concentrations 1.8 and 2.7 times lower than EIF and ESF in the DPPH assay and 1.6 and 2.7 times lower in the ABTS assay, and it was about three times weaker than ascorbic acid in both.

The identical ranking in two assays that use different radicals and solvents argues that the differences reflect the preparations rather than the chemistry of one test (Munteanu & Apetrei, 2021). Compared on the same concentration basis, AE was about 2.5 times more active against DPPH than the ethanolic gembili extract (Murtado et al., 2025) and more active than tuber extracts of several wild and cultivated yams from Odisha, India, whose DPPH and ABTS IC_{50} values started at 77.9 and 101.2 µg/mL (Padhan et al., 2020). Aqueous preparations of other yams behave similarly: the cooking water of *D. alata* tubers carried phenolics whose content correlated with DPPH activity (Amarasekara & Wickramarachchi, 2021), and substituting purple yam flour into bread raised phenolic content and DPPH activity together (Tamaroh & Sudrajat, 2021). Such comparisons remain approximate because IC_{50} depends on radical concentration, solvent, and reaction time (Wołosiak et al., 2021).

Normalising the IC_{50} values to Folin–Ciocalteu response shows what drives the ranking. Expressed as GAE concentration at IC_{50}, AE required 6.5 ± 0.2 µg GAE/mL in the DPPH assay and 6.1 ± 0.3 µg GAE/mL in the ABTS assay, whereas EIF and ESF required 3.7–4.4 µg GAE/mL and did not differ from each other (DPPH $p = 0.085$; ABTS $p = 0.997$; both fractions versus AE, $p < 0.001$). Per unit of reducing capacity, therefore, the two fractions scavenged radicals at least as efficiently as the parent extract. AE owes its greater activity per gram to its larger amount of reductants rather than to more potent ones, and part of its Folin–Ciocalteu response probably comes from substances that reduce the reagent but react slowly with DPPH and ABTS radicals, such as sugars and proteins (Pico et al., 2020; Platzer et al., 2021).

3.3. Ferric Reducing Capacity Follows the Same Order

Absorbance of the Fe^{2+}–TPTZ complex increased with concentration for all preparations (**Figure 2c**). Expressed as ascorbic acid equivalents, AE had the highest reducing capacity, significantly above both fractions ($F(2, 6) = 31.5$, $p < 0.001$; AE versus EIF $p = 0.004$, AE versus ESF $p < 0.001$), while EIF and ESF did not differ ($p = 0.156$; **Table 1**). The larger SD for AE reflects curvature of its response at the upper concentrations (coefficients of determination 0.71–0.86).

FRAP reports electron transfer to Fe^{3+} at pH 3.6 and has no 50% end point, so it was expressed relative to ascorbic acid rather than as an inhibitory concentration (Benzie & Strain, 1996; Munteanu & Apetrei, 2021). With this endpoint, a higher value means stronger reducing power, and the ranking of the preparations matches the radical-scavenging assays. Single-electron-transfer assays tend to agree most closely with the Folin–Ciocalteu assay, which shares the same reaction principle (Rumpf et al., 2023), and across fifteen plant foods FRAP correlated with total polyphenols more strongly than ABTS or DPPH did (Kiss et al., 2025). The smaller and non-significant gap between EIF and ESF in this assay is consistent with their similar GAE-normalised radical-scavenging efficiency.

3.4. Antioxidant Capacity Tracks Phenolic and Flavonoid Contents

The relationship between phenolic content and antioxidant capacity is shown in **Figure 3**. All six associations were strong and

in the expected direction: preparations richer in Folin–Ciocalteu-reactive and aluminium chloride-reactive material needed lower concentrations to inhibit half of the DPPH and ABTS radicals and reduced more ferric iron. The points form three tight clusters, one for each preparation, so the coefficients mainly reflect the differences among AE, EIF, and ESF rather than variation among replicates. In the two radical assays the clusters follow a gently curved rather than a straight path, with EIF below and ESF above the fitted line. This is the pattern expected when IC_{50} is inversely proportional to phenolic content, and it agrees with the nearly constant GAE concentration at IC_{50} found for the two fractions in Section 3.2.

Because TPC and TFC were themselves almost collinear (Section 3.1), the upper and lower rows of **Figure 3** are nearly mirror images, and these data cannot apportion the activity between flavonoids and other phenolics. They do show that the antioxidant capacity of the three preparations rises and falls with their phenolic content. Comparable associations have been reported between phenolic metabolites and DPPH, ABTS, and FRAP values in yam peel (Yang et al., 2025) and across yam species (Adomėnienė & Venskutonis, 2022).

These results have a practical consequence for gembili processing. Ethanol precipitation, the standard step for preparing gembili inulin, does not strip the polysaccharide fraction of its antioxidants: EIF retained about 55–60% of the radical-scavenging potency of AE on a mass basis and was clearly more active than the ethanol-soluble supernatant. An inulin-rich gembili preparation can therefore be expected to carry phenolic co-precipitates, and the unfractionated hot-water extract delivers the highest antioxidant capacity per gram, which makes it the simpler candidate for jamu and functional food formulations. The evidence here is chemical and in vitro and was obtained from one harvest; identifying the phenolics by liquid chromatography–mass spectrometry, quantifying their mass balance between the fractions, and testing the preparations in cell-based antioxidant models are the logical next steps (Fernandes & Coimbra, 2023; Munteanu & Apetrei, 2021).

4. CONCLUSION

The hot-water extract of gembili tubers contained the most Folin–Ciocalteu-reactive phenolics (214.9 mg GAE/g) and aluminium chloride-reactive flavonoids (159.7 mg QE/g) and was the most effective antioxidant in all three assays, with IC_{50} values of 30.0 µg/mL (DPPH) and 28.5 µg/mL (ABTS) and a reducing capacity of 269 mg AAE/g. When the extract was split by ethanol precipitation, the inulin-rich insoluble fraction retained more phenolics and radical-scavenging capacity than the soluble fraction, and antioxidant capacity followed phenolic content across all preparations. Gembili hot-water extract and its inulin-

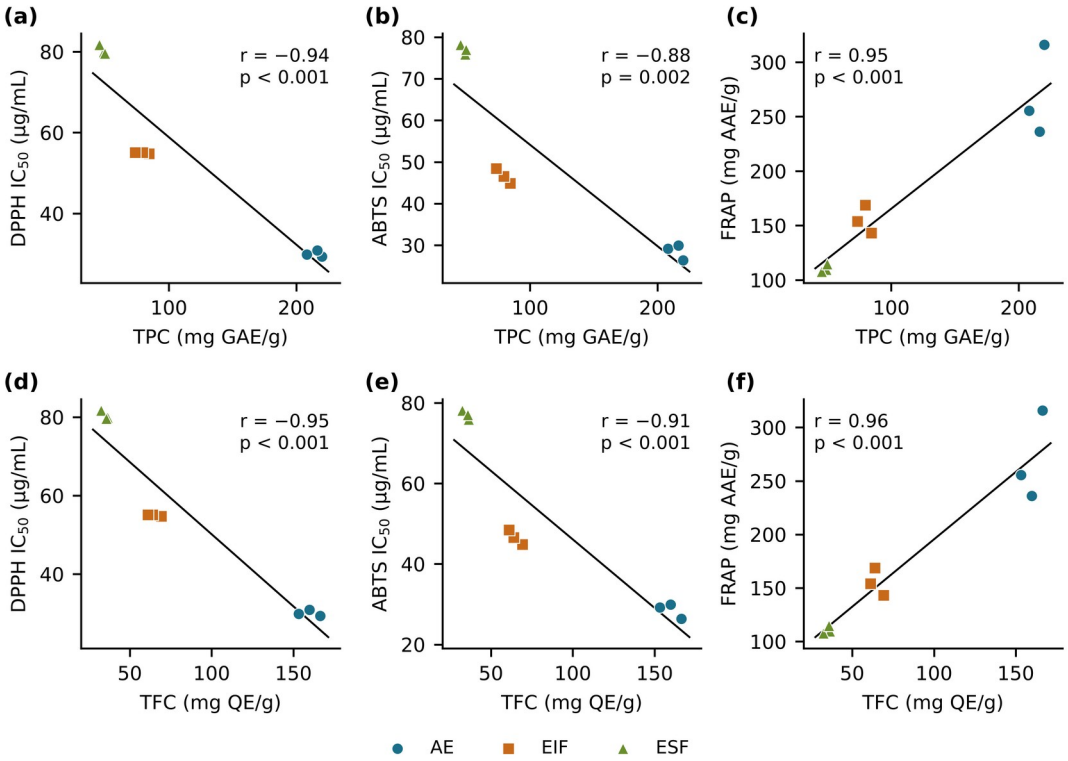

Figure 3. Relationships between phenolic content and antioxidant capacity of the aqueous extract (AE), ethanol-insoluble fraction (EIF), and ethanol-soluble fraction (ESF) of *Dioscorea esculenta* tubers. Total phenolic content (TPC) against (a) DPPH IC_{50}, (b) ABTS IC_{50}, and (c) FRAP; total flavonoid content (TFC) against (d) DPPH IC_{50}, (e) ABTS IC_{50}, and (f) FRAP. Each point is one determination (three per preparation, $n = 9$); lines are least-squares fits, and r and p are Pearson's correlation coefficients and their significance. GAE, gallic acid equivalents; QE, quercetin equivalents; AAE, ascorbic acid equivalents; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); FRAP, ferric reducing antioxidant power; IC_{50}, concentration giving 50% inhibition.

rich precipitate are therefore promising antioxidant-bearing ingredients for jamu and functional foods, and the phenolic constituents responsible should next be identified and their activity confirmed in biological models.

AUTHOR CONTRIBUTIONS

Conceptualization, I.P. and L.K.; methodology, I.P. and L.K.; validation, I.P. and L.K.; formal analysis, I.P. and L.K.; investigation, I.P. and L.K.; resources, I.P.; data curation, I.P. and L.K.; writing—original draft preparation, I.P.; writing—review and editing, I.P. and L.K.; visualization, I.P.; supervision, L.K.; project administration, I.P. and L.K. All authors have read and agreed to the published version of the manuscript.

INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable. The study used plant material and in vitro chemical assays only and involved neither human participants nor animals.

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

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

FUNDING

This research received no external funding.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ROLE OF FUNDERS

Not applicable.

DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) USE

During the preparation of this manuscript, the authors used Claude (Anthropic) for the purposes of text editing, statistical analysis of the raw data, and figure preparation. The authors have reviewed and edited the output and take full responsibility for the content of this publication.

REFERENCES

Adomėnienė, A., & Venskutonis, P. R. (2022). *Dioscorea* spp.: Comprehensive review of antioxidant properties and their relation to phytochemicals and health benefits. *Molecules*, *27*(8), 2530. https://doi.org/10.3390/molecules27082530

Amarasekara, R., & Wickramarachchi, S. R. (2021). Antioxidant activity of phenolic compounds in *Dioscorea alata* L. (raja ala) tuber cooking water. *Acta Chemica Iasi*, *29*(2), 183–200. https://doi.org/10.47743/achi-2021-2-0013

Bai, Y., Fan, B., Liu, J., Wang, F., & Zhang, M. (2026). Processing shapes architecture, cultivar dictates chemistry: A structural and functional paradigm for yam polysaccharides. *Foods*, *15*(5), 921. https://doi.org/10.3390/foods15050921

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry*, *239*(1), 70–76. https://doi.org/10.1006/abio.1996.0292

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology*, *28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Fernandes, P. A. R., & Coimbra, M. A. (2023). The antioxidant activity of polysaccharides: A structure–function relationship overview. *Carbohydrate Polymers*, *314*, 120965. https://doi.org/10.1016/j.carbpol.2023.120965

Ghozaly, M. R., Elya, B., Hanafi, M., & Setiawan, H. (2026). Ultrasound-assisted extraction enhances phenolic, flavonoid, and antioxidant profiles of *Spatholobus littoralis* roots. *Jurnal Jamu Indonesia*, *11*(3), 211–216. https://doi.org/10.29244/jji.v11i3.501

Hilman, A., Harmayani, E., & Cahyanto, M. N. (2021). The potential of gembili (*Dioscorea esculenta* L.) and dahlia (*Dahlia* spp. L.) from Indonesia as prebiotic compound. *IOP Conference Series: Earth and Environmental Science*, *782*(3), 032109. https://doi.org/10.1088/1755-1315/782/3/032109

Jiang, S., Xie, H., Zuo, Y., Sun, J., Wu, D., & Shu, X. (2024). Structural and functional properties of polysaccharides extracted from three *Dioscorea* species. *International Journal of Biological Macromolecules*, *281*(Pt 4), 136469. https://doi.org/10.1016/j.ijbiomac.2024.136469

Kiss, A., Papp, V. A., Pál, A., Prokisch, J., Mirani, S., Toth, B. E., & Alshaal, T. (2025). Comparative study on antioxidant capacity of diverse food matrices: Applicability, suitability and inter-correlation of multiple assays to assess polyphenol and antioxidant status. *Antioxidants*, *14*(3), 317. https://doi.org/10.3390/antiox14030317

Lawag, I. L., Nolden, E. S., Schaper, A. A. M., Lim, L. Y., & Locher, C. (2023). A modified Folin–Ciocalteu assay for the determination of total phenolics content in honey. *Applied Sciences*, *13*(4), 2135. https://doi.org/10.3390/app13042135

Munteanu, I. G., & Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: A review. *International Journal of Molecular Sciences*, *22*(7), 3380. https://doi.org/10.3390/ijms22073380

Muñoz-Bernal, Ó. A., Torres-Aguirre, G. A., Núñez-Gastélum, J. A., de la Rosa, L. A., Rodrigo-García, J., Ayala-Zavala, J. F., & Álvarez-Parrilla, E. (2017). Nuevo acercamiento a la interacción del reactivo de Folin–Ciocalteu con azúcares durante la cuantificación de polifenoles totales [A new approach to the interaction of the Folin–Ciocalteu reagent with sugars during the quantification of total polyphenols]. *TIP Revista Especializada en Ciencias Químico-Biológicas*, *20*(2), 23–28. https://doi.org/10.1016/j.recqb.2017.04.003

Murtado, A. D., Yani, A. V., & Idealistuti, I. (2025). Analysis of antioxidant activity and natural prebiotic in gembili tubers (*Dioscorea esculenta*). *Journal of Global Sustainable Agriculture*, *5*(3), 368–373. https://doi.org/10.32502/jgsa.v5i3.1367

Padhan, B., Nayak, J. K., & Panda, D. (2020). Natural antioxidant

potential of selected underutilized wild yams (*Dioscorea* spp.) for health benefit. *Journal of Food Science and Technology*, *57*(6), 2370–2376. https://doi.org/10.1007/s13197-020-04470-x

Peng, K., Li, Y., Sun, Y., Xu, W., Wang, H., Zhang, R., & Yi, Y. (2023). Lotus root polysaccharide–phenol complexes: Interaction, structure, antioxidant, and anti-inflammatory activities. *Foods*, *12*(3), 577. https://doi.org/10.3390/foods12030577

Pérez, M., Dominguez-López, I., & Lamuela-Raventós, R. M. (2023). The chemistry behind the Folin–Ciocalteu method for the estimation of (poly)phenol content in food: Total phenolic intake in a Mediterranean dietary pattern. *Journal of Agricultural and Food Chemistry*, *71*(46), 17543–17553. https://doi.org/10.1021/acs.jafc.3c04022

Pico, J., Pismag, R. Y., Laudouze, M., & Martinez, M. M. (2020). Systematic evaluation of the Folin–Ciocalteu and Fast Blue BB reactions during the analysis of total phenolics in legumes, nuts and plant seeds. *Food & Function*, *11*(11), 9868–9880. https://doi.org/10.1039/d0fo01857k

Platzer, M., Kiese, S., Herfellner, T., Schweiggert-Weisz, U., & Eisner, P. (2021). How does the phenol structure influence the results of the Folin–Ciocalteu assay? *Antioxidants*, *10*(5), 811. https://doi.org/10.3390/antiox10050811

Putri, W. D. R., Riyanto, E. I., Heliana, A., Wellang, A., & Zubaidah, E. (2021). Optimization of microwave-assisted extraction (MAE) time and material to solvent ratio of gembili (*Dioscorea esculenta*) water-soluble polysaccharides (WSP). *Industria: Jurnal Teknologi dan Manajemen Agroindustri*, *10*(1), 78–98. https://doi.org/10.21776/ub.industria.2021.010.01.9

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*(9–10), 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Rumpf, J., Burger, R., & Schulze, M. (2023). Statistical evaluation of DPPH, ABTS, FRAP, and Folin–Ciocalteu assays to assess the antioxidant capacity of lignins. *International Journal of Biological Macromolecules*, *233*, 123470. https://doi.org/10.1016/j.ijbiomac.2023.123470

Shraim, A. M., Ahmed, T. A., Rahman, M. M., & Hijji, Y. M. (2021). Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. *LWT*, *150*, 111932. https://doi.org/10.1016/j.lwt.2021.111932

Sies, H., Berndt, C., & Jones, D. P. (2017). Oxidative stress. *Annual Review of Biochemistry*, *86*, 715–748. https://doi.org/10.1146/annurev-biochem-061516-045037

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods in Enzymology*, *299*, 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1

Tamaroh, S., & Sudrajat, A. (2021). Antioxidative characteristics and sensory acceptability of bread substituted with purple yam (*Dioscorea alata* L.). *International Journal of Food Science*, *2021*, 5586316. https://doi.org/10.1155/2021/5586316

Tatara, K., & Sato, K. (2025). Chronic intake of *Dioscorea esculenta* suppresses testicular atrophy, enhances the ability of protection from inflammation in type 2 diabetes rats. *The Journal of Steroid Biochemistry and Molecular Biology*, *250*, 106730. https://doi.org/10.1016/j.jsbmb.2025.106730

Winarti, S., Harmayani, E., Marsono, Y., & Pranoto, Y. (2013). Pengaruh foaming pada pengeringan inulin umbi gembili (*Dioscorea esculenta*) terhadap karakteristik fisiko-kimia dan aktivitas prebiotik [Effect of foaming on the drying of gembili (*Dioscorea esculenta*) tuber inulin on its physicochemical characteristics and prebiotic activity]. *Agritech*, *33*(4), 424–432. https://journal.ugm.ac.id/agritech/article/view/9538

Wołosiak, R., Drużyńska, B., Derewiaka, D., Piecyk, M., Majewska, E., Ciecierska, M., Worobiej, E., & Pakosz, P. (2021). Verification of the conditions for determination of antioxidant activity by ABTS and DPPH assays—A practical approach. *Molecules*, *27*(1), 50. https://doi.org/10.3390/molecules27010050

Xue, H., Du, X., Fang, S., Gao, H., Xie, K., Wang, Y., & Tan, J. (2024). The interaction of polyphenols–polysaccharides and their applications: A review. *International Journal of Biological Macromolecules*, *278*(Pt 1), 134594. https://doi.org/10.1016/j.ijbiomac.2024.134594

Yang, L., Wang, W., Liu, Z., Zhai, Y., Wang, Z., Li, Y., Zhang, Z., Hou, B., Zhang, B., & Zhou, J. (2025). Widely targeted metabolomics reveals the bioactive metabolites and antioxidant activities of Chinese yam (*Dioscorea opposita* Thunb.) peel. *Biomedical Chromatography*, *39*(5), e70085. https://doi.org/10.1002/bmc.70085

Zubaidah, E., & Akhadiana, W. (2013). Comparative study of inulin extracts from dahlia, yam, and gembili tubers as prebiotic. *Food and Nutrition Sciences*, *4*(11A), 8–12. https://doi.org/10.4236/fns.2013.411A002

Publisher's Note & Disclaimer

All statements, opinions, and data in this publication were solely the responsibility of the individual authors or contributors and did not necessarily reflect the views of the publisher or editors. The publisher and editors did not guarantee the accuracy, completeness, or reliability of the content, and were not legally responsible for any errors, omissions, or consequences arising from its use. The publisher and editors also disclaimed any liability for injury, damage, or loss to persons or property resulting from the application of ideas, methods, or products mentioned herein. Readers were advised to independently verify all information before relying on it. The publisher accepted no responsibility for any consequences arising from the use of this publication's materials.