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Effect of Extraction Solvent and Plant Part on the Phytochemical Content and Bioactivity of *Jatropha tanjorensis*

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Abstract

Jatropha tanjorensis is eaten as a leafy vegetable and used in folk medicine in West Africa, yet how plant part and extraction solvent jointly shape its bioactivity has not been compared systematically. Leaves, stems and roots collected in Kaduna, Nigeria, were extracted by cold maceration with water, 70% ethanol and methanol, giving nine extracts. We measured extraction yield, screened for phytochemical classes, quantified total phenolic (TPC) and flavonoid (TFC) contents, and assessed antioxidant activity by DPPH, ABTS, FRAP and hydrogen peroxide assays. Antimicrobial activity was tested against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* by agar-well diffusion, and minimum inhibitory (MIC) and bactericidal/fungicidal (MBC/MFC) concentrations were determined for the four most active extracts selected from the screen. Data were analysed by two-way ANOVA, Pearson correlation, principal component analysis (PCA) and hierarchical clustering. Yield was highest for leaves and 70% ethanol (18.63 ± 0.50%). The 70% ethanol leaf extract had the highest TPC (68.4 ± 1.3 mg GAE/g) and TFC (36.8 ± 0.8 mg QE/g), the lowest DPPH IC_{50} (41.8 ± 1.3 µg/mL), the highest ABTS and FRAP values and the lowest H_2O_2 IC_{50} (48.7 ± 1.6 µg/mL). Plant part, solvent and their interaction significantly affected TPC ($p < 0.001$; n = 3 extraction replicates from one batch of plant material). TPC and TFC correlated strongly with antioxidant measures ($|r| = 0.94–1.00$). Among the four extracts tested, the 70% ethanol leaf extract was also the most active against all five

microorganisms (MIC 1.25–5.00 mg/mL). PC1 (96.0% of variance) ranked the ethanol and methanol leaf extracts highest and the aqueous root extract lowest. Leaves extracted with 70% ethanol gave the most bioactive profile; multivariate analyses are exploratory (n = 9).

Keywords: *Jatropha tanjorensis*; extraction solvent; plant part; phenolic content; antioxidant activity; antimicrobial activity; principal component analysis

1 Introduction

Plants remain a major source of bioactive compounds for food, medicine and drug discovery, and their secondary metabolites, including phenolic acids, flavonoids, tannins, alkaloids and terpenoids, underlie many of their antioxidant and antimicrobial properties [1]. Oxidative stress, in which reactive oxygen species overwhelm cellular defences, contributes to many chronic diseases, and dietary or medicinal plants rich in phenolics are widely studied as sources of natural antioxidants [2]. At the same time, antimicrobial resistance is a growing global health problem [3], which sustains interest in plant-derived antimicrobials [4].

Jatropha tanjorensis J.L. Ellis & Saroja (Euphorbiaceae) [5], known in Nigeria as “hospital too far” or “catholic vegetable”, is eaten as a leafy vegetable and used in folk medicine, notably as a blood tonic and for diabetes [6, 7] and malaria [8, 9]. Earlier work has reported alkaloids, flavonoids, tannins, saponins, glycosides and phenolics in its leaves [10–13], together with antioxidant [14, 15], antimicrobial [13, 16], antidiabetic [7] and antiplasmodial [17] activity in leaf extracts. Animal studies have also examined its effects on haematological indices, lipid profile, oxidative stress and inflammation [18–24]. The wider genus *Jatropha* is well known for its medicinal species and its chemistry [25].

Most of these studies examine a single plant part, usually the leaf, with one solvent. Yet the recovery of phenolics and other metabolites depends strongly on solvent polarity [26, 27], and phytochemical distribution can also vary with plant tissue [28]. As a result, the results of different studies cannot be compared directly, and it is unclear whether the leaf is really the most bioactive organ or whether a different solvent would change the ranking. Many studies rely on a single antioxidant assay, and comparatively few link chemical composition to activity with multivariate

statistics [29]. Because DPPH, ABTS, FRAP and peroxide assays probe different mechanisms, using several together gives a more reliable picture than any one alone [30, 31].

We therefore extracted the leaves, stems and roots of *J. tanjorensis* with water, 70% ethanol and methanol, in a full 3 × 3 design, and compared the nine extracts for yield, phytochemical classes, TPC, TFC, four antioxidant assays and activity against four bacteria and one yeast. We tested the hypothesis that phytochemical composition and biological activity differ significantly with plant part and solvent, and that phenolic and flavonoid content is associated with antioxidant activity. Two-way ANOVA, correlation analysis, principal component analysis (PCA) and hierarchical clustering were used to test these hypotheses.

2 Materials and methods

2.1 Plant material

Mature, healthy leaves, stems and roots of *J. tanjorensis* were collected at Barnawa, Kaduna South, Kaduna State, Nigeria, in May 2026. The plant was identified on 4 May 2026 by Dr. Abdullahi Shehu, and a voucher specimen (Ka/26/8766) was deposited in the Kaduna State University herbarium. No specific collection permit was required for this material. The plants were [wild-growing / cultivated: confirm] at the collection site, and their collection complied with institutional, local and national guidelines on the collection of plant material.

The plant parts were washed separately with distilled water and shade-dried at 25–28 °C for 7–10 days in a well-ventilated, dust-free laboratory, and were turned periodically. Dried samples were ground separately in a stainless-steel laboratory grinder and sieved through a 40-mesh sieve (about 425 µm). Powders were stored in airtight amber containers at room temperature until extraction.

2.2 Extraction

Each plant part was extracted separately with each of three solvents (distilled water, 70% ethanol and methanol), giving nine extracts: LW, LE, LM (leaf), SW, SE, SM (stem) and RW, RE, RM (root; W = water, E = 70% ethanol, M = methanol). Analytical-grade solvents were used: absolute ethanol (≥99.5%), methanol (≥99.8%) and distilled/deionised water; 70% ethanol was prepared by diluting absolute ethanol with distilled water (v/v). Extraction was by cold maceration: 100 g of powder was mixed with 1,000 mL of solvent (1:10 w/v) and macerated for 72 h at 25 ± 2 °C

with intermittent agitation every 6–8 h. Mixtures were filtered through Whatman No. 1 filter paper. Aqueous extracts were freeze-dried, and ethanolic and methanolic extracts were concentrated on a rotary evaporator at ≤40 °C under reduced pressure. Extraction yield was calculated as:

$$\text{Yield (\%)} = (\text{weight of dried extract} \div \text{weight of starting plant material}) \times 100$$

Dried extracts were stored in amber glass containers at −20 °C until analysis. Moisture content of the powders was determined gravimetrically by oven-drying triplicate 2 g samples at 105 °C to constant weight.

2.3 Qualitative phytochemical screening

All nine extracts were screened for alkaloids (Mayer's and Wagner's tests), flavonoids (Shinoda test), phenolics (ferric chloride test), tannins (ferric chloride/gelatin test), saponins (foam test), terpenoids (Salkowski test), steroids (Liebermann–Burchard test) and glycosides (Keller–Killiani test) [32]. Reactions were scored as absent (−), weak (+), moderate (++) or strong (+++).

2.4 Total phenolic and flavonoid content

Total phenolic content was measured by the Folin–Ciocalteu colorimetric assay [33, 34] with gallic acid as standard and expressed as mg gallic acid equivalents per gram of extract (mg GAE/g). Briefly, 0.5 mL of extract (1 mg/mL) was mixed with 2.5 mL of 10% (v/v) Folin–Ciocalteu reagent and 2.0 mL of 7.5% (w/v) sodium carbonate solution (final volume 5.0 mL), and absorbance was read at 765 nm after 30 min of incubation in the dark at room temperature. A gallic acid calibration curve was prepared over 10–200 µg/mL.

Total flavonoid content was measured by the aluminium chloride colorimetric method [35] with quercetin as standard and expressed as mg quercetin equivalents per gram of extract (mg QE/g). Briefly, 0.5 mL of extract (1 mg/mL) was mixed with 0.3 mL of 10% (w/v) aluminium chloride, 0.3 mL of 1 M potassium acetate and 4.0 mL of distilled water (final volume about 5.1 mL), and absorbance was read at 510 nm after 30 min at room temperature, against a quercetin calibration curve over 10–100 µg/mL.

2.5 Antioxidant assays

All assays were run on serial dilutions of each extract, with the reference compound given below.

DPPH. Radical scavenging activity was measured against a methanolic 2,2-diphenyl-1-picrylhydrazyl solution [36], with ascorbic acid as the reference. Extract or standard was mixed with DPPH solution, incubated in the dark at room temperature for 30 min, and absorbance was read at 517 nm. Percentage scavenging was calculated for each concentration and plotted, and the IC_{50} (µg/mL) was estimated from the concentration–response curve by linear regression. A lower IC_{50} indicates higher potency. Ascorbic acid (1.25–20 µg/mL) gave an IC_{50} of 7.1 µg/mL in this run.

ABTS. The ABTS radical cation was generated by reacting ABTS with potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12–16 h before dilution to a working absorbance. ABTS radical-cation decolorization was measured after Re et al. [37], with Trolox as the standard, absorbance read at 734 nm after 6 min, and results expressed as µmol Trolox equivalents per gram of extract (µmol TE/g), using a Trolox calibration curve over 0–500 µM.

FRAP. Ferric-reducing antioxidant power was measured after Benzie and Strain [38]. Freshly prepared FRAP reagent (acetate buffer, TPTZ and ferric chloride) was mixed with diluted extract, incubated at 37 °C for 4–10 min, and absorbance was read at 593 nm against a Trolox reference, with results expressed as µmol TE/g, using a Trolox calibration curve over 25–1000 µM.

Hydrogen peroxide scavenging. Scavenging of hydrogen peroxide was measured spectrophotometrically: extract was mixed with a hydrogen peroxide solution in phosphate buffer (pH 7.4) and absorbance read at 230 nm after 10 min, against ascorbic acid as the reference [39]. Results are expressed as IC_{50} (µg/mL), estimated from the concentration–response curve over 12.5–200 µg/mL. Ascorbic acid (5–100 µg/mL) gave an IC_{50} of 24.8 µg/mL in this run.

2.6 Antimicrobial assays

Test organisms. Four bacteria, two Gram-positive (*Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633) and two Gram-negative (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853), and one yeast (*Candida albicans* ATCC 10231) were used. Reference strains were obtained from the American Type Culture Collection (ATCC) and maintained on nutrient agar (bacteria) or Sabouraud dextrose agar (yeast) slants at 4 °C, with fresh subcultures prepared for each assay.

Agar-well diffusion. Extracts were dissolved in 5% DMSO and screened at 100 mg/mL by agar-well diffusion [40] on Mueller–Hinton agar (bacteria) or Sabouraud dextrose agar (*C. albicans*), inoculated with a suspension standardised to 0.5 McFarland turbidity. Wells 6 mm in diameter were cut in the inoculated agar, filled with 80 µL of extract, and plates were incubated at 37 °C for 24 h (bacteria) or 25–28 °C for 48 h (*C. albicans*). Gentamicin (10 µg/mL, bacteria) and nystatin (100 U/mL, *C. albicans*) were positive controls, and 5% dimethyl sulfoxide (DMSO) was the negative (vehicle) control. Growth controls (microorganism without extract) and sterility controls (uninoculated medium) were included. Zones are reported as total inhibition-zone diameter in mm including the 6-mm well, so a value of 6.0 mm indicates no inhibition.

MIC. For each plant part, the extract with the largest inhibition zones in the agar-well screen at 100 mg/mL (LE, SE and RE, all 70% ethanol), together with the methanol leaf extract (LM, the second most active extract overall), was selected. MICs were determined by broth microdilution in 96-well flat-bottom microtitre plates, using two-fold serial dilutions from 40 mg/mL down to 0.625 mg/mL in Mueller–Hinton broth (bacteria) or Sabouraud dextrose broth (*C. albicans*). Inocula were adjusted to 1×10^6 CFU/mL (bacteria) and 1×10^6 cells/mL (*C. albicans*). Each well contained a final volume of 200 µL of extract, inoculum and medium, with DMSO at ≤1% (v/v); vehicle, growth and sterility controls were included. Plates were incubated at 37 °C for 24 h (bacteria) or 25–28 °C for 48 h (*C. albicans*), and the MIC was taken as the lowest concentration showing no visible turbidity. All MICs were determined in three independent replicates. Because the four extracts were chosen after the inhibition-zone screen, MIC comparisons apply to these selected extracts only.

MBC/MFC. Aliquots from wells showing no visible growth were subcultured on extract-free agar, and the MBC or MFC was taken as the lowest concentration giving no visible colony growth after subculture. Extracts with an MBC/MIC (or MFC/MIC) ratio of ≤4 are described as bactericidal (or fungicidal) [41].

2.7 Statistical analysis

Each extraction was performed in triplicate from the same powdered plant material for each plant part (n = 3 extraction replicates from a single pooled batch), and spectrophotometric measurements were made in technical triplicate. Data are presented as mean ± standard deviation,

with each analytical determination performed in triplicate. Because the replicates share one batch of plant material, inference applies to this batch and does not cover plant-to-plant variability. Effects of plant part, solvent and their interaction were tested by two-way ANOVA, with effect sizes reported as partial η^2 and Tukey's multiple-comparison test after a significant result; $p < 0.05$ was considered significant, and exact p-values are given where $p \geq 0.001$. Analyses and graphs were made in GraphPad Prism, and PCA, hierarchical clustering and the heatmap were produced in R. Pearson correlation was performed on the nine extract means (LW, LE, LM, SW, SE, SM, RW, RE, RM), not on technical replicates, to avoid treating non-independent observations as independent. PCA and correlation were run on z-score-standardised extract means and are interpreted as exploratory because $n = 9$. The PCA used seven variables (extraction yield, TPC, TFC, DPPH IC_{50}, ABTS, FRAP and H_2O_2 IC_{50}); antimicrobial data were not included because MIC values were determined for only four extracts. Tukey comparisons were made among the nine extract means (part × solvent combinations). The heatmap was built from z-scored means, with hierarchical clustering by Euclidean distance and Ward's linkage.

3 Results

3.1 Moisture content and extraction yield

Moisture content was similar across plant parts, ranging from 7.85 ± 0.27% in stems to 8.76 ± 0.34% in roots, with leaves intermediate (8.42 ± 0.31%) (Table 1).

Table 1 Moisture content of *J. tanjorensis* plant parts (mean ± SD, n = 3)

Plant part	Moisture content (%)
Leaf	8.42 ± 0.31
Stem	7.85 ± 0.27
Root	8.76 ± 0.34

Extraction yield varied with both plant part and solvent (Table 2). Leaves gave the highest yield with every solvent, and 70% ethanol gave the highest yield for every plant part (leaf 18.63 ± 0.50%, stem 12.85 ± 0.38%, root 13.76 ± 0.35%). Water gave the lowest yields (8.91–14.82%). Plant part, solvent and their interaction all significantly affected yield (Table 6), and the 70% ethanol leaf extract differed from all other extracts (Tukey, $p < 0.05$; Fig. 1).

Table 2 Extraction yield (% w/w) of *J. tanjorensis* extracts (mean ± SD, n = 3). Different superscript letters within a table indicate significant differences among the nine extracts (Tukey HSD, $p < 0.05$).

Plant part	Water	70% ethanol	Methanol
Leaf	14.82 ± 0.28^{c}	18.63 ± 0.50^{a}	16.91 ± 0.34^{b}
Stem	9.74 ± 0.33^{f}	12.85 ± 0.38^{de}	11.93 ± 0.31^{e}
Root	8.91 ± 0.29^{f}	13.76 ± 0.35^{d}	12.48 ± 0.31^{e}

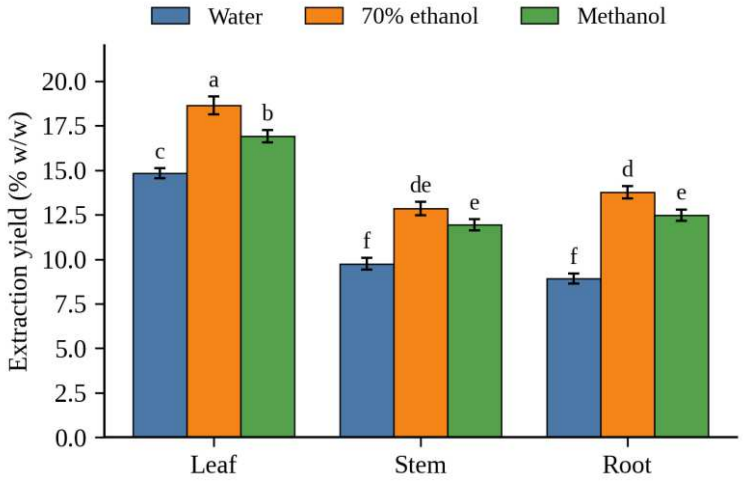


Fig. 1 Extraction yield of *J. tanjorensis* leaf, stem and root extracts prepared with water, 70% ethanol and methanol. Bars are means ± SD (n = 3); different letters indicate significant differences among the nine extracts (Tukey HSD, $p < 0.05$).

3.2 Qualitative phytochemical screening

All eight phytochemical classes were detected in all nine extracts (Table 3). Alkaloids, flavonoids and phenolics gave the strongest reactions (+++) in the 70% ethanol and methanol leaf extracts, and tannins in the 70% ethanol leaf extract only. Saponins (+++) in all root extracts, terpenoids (+++) in the 70% ethanol and methanol root extracts, and steroids (++) in all root extracts gave their strongest reactions in the roots. These tests indicate the presence of compound classes only and do not quantify them.

Table 3 Qualitative phytochemical composition of the nine *J. tanjorensis* extracts (+++ strong, ++ moderate, + weak, − absent)

Extract	Alkaloids	Flavonoids	Phenolics	Tannins	Saponins	Terpenoids	Steroids	Glycosides
Leaf–Water (LW)	++	++	++	++	++	+	+	++
Leaf–70% ethanol (LE)	+++	+++	+++	+++	++	++	+	++
Leaf–Methanol (LM)	+++	+++	+++	++	++	++	+	++
Stem–Water (SW)	++	+	++	+	++	++	+	+
Stem–70% ethanol (SE)	++	++	++	++	++	++	+	++
Stem–Methanol (SM)	++	++	++	++	++	++	+	++
Root–Water (RW)	+	+	+	+	+++	++	++	+
Root–70% ethanol (RE)	++	++	++	++	+++	+++	++	++

Extract	Alkaloids	Flavonoids	Phenolics	Tannins	Saponins	Terpenoids	Steroids	Glycosides
Root–Methanol (RM)	++	++	++	++	+++	+++	++	++

3.3 Total phenolic and flavonoid content

TPC ranged from 19.5 ± 0.6 mg GAE/g (aqueous root extract) to 68.4 ± 1.3 mg GAE/g (70% ethanol leaf extract) (Table 4). TFC followed the same pattern, from 8.9 ± 0.3 mg QE/g (aqueous root) to 36.8 ± 0.8 mg QE/g (70% ethanol leaf) (Table 5). Leaf extracts contained more phenolics and flavonoids than stem and root extracts with all three solvents, and 70% ethanol recovered the most of both.

Two-way ANOVA showed significant effects of plant part ($F_{2,18} = 2517.7$, $p < 0.001$, partial $\eta^2 = 0.996$), solvent ($F_{2,18} = 1169.7$, $p < 0.001$, partial $\eta^2 = 0.992$) and their interaction ($F_{4,18} = 33.3$, $p < 0.001$, partial $\eta^2 = 0.879$) on TPC, and similar effects on TFC (Table 6). The significant interaction means the advantage of a given solvent depended on the plant part being extracted. Tukey groupings are shown in Tables 4 and 5 and the values in Fig. 2.

Table 4 Total phenolic content (mg GAE/g extract; mean ± SD, n = 3). Different superscript letters within a table indicate significant differences among the nine extracts (Tukey HSD, $p < 0.05$).

Plant part	Water	70% ethanol	Methanol
Leaf	42.6 ± 0.9^{d}	68.4 ± 1.3^{a}	61.7 ± 0.8^{b}
Stem	28.7 ± 0.7^{g}	45.3 ± 1.1^{c}	41.2 ± 0.8^{d}
Root	19.5 ± 0.6^{h}	34.8 ± 0.8^{e}	31.6 ± 0.8^{f}

Table 5 Total flavonoid content (mg QE/g extract; mean ± SD, n = 3). Different superscript letters within a table indicate significant differences among the nine extracts (Tukey HSD, $p < 0.05$).

Plant part	Water	70% ethanol	Methanol
Leaf	21.4 ± 0.6^{d}	36.8 ± 0.8^{a}	33.5 ± 0.8^{b}
Stem	13.8 ± 0.4^{f}	23.7 ± 0.7^{c}	21.9 ± 0.6^{d}
Root	8.9 ± 0.3^{g}	15.6 ± 0.5^{e}	14.2 ± 0.5^{ef}

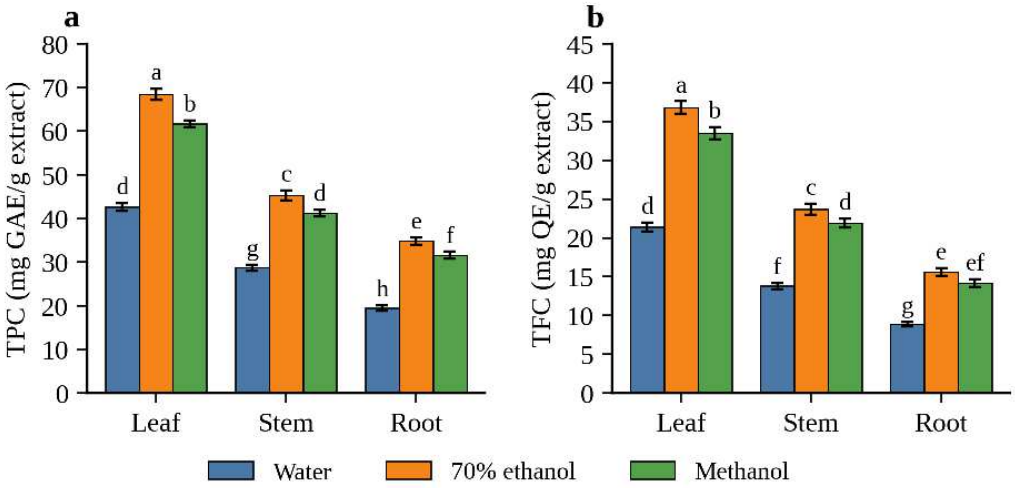


Fig. 2 Total phenolic content (a) and total flavonoid content (b) of the nine extracts. Bars are means ± SD (n = 3); different letters indicate significant differences (Tukey HSD, $p < 0.05$).

Table 6 Two-way ANOVA for extraction yield, phenolic and flavonoid contents and antioxidant assays: F values (p-values) [partial η^2]

Variable	Plant part F(2,18)	Solvent F(2,18)	Part × solvent F(4,18)
Extraction yield	659.4 ($p < 0.001$) [0.987]	294.4 ($p < 0.001$) [0.970]	6.6 ($p = 0.002$) [0.595]
TPC	2517.7 ($p < 0.001$) [0.996]	1169.7 ($p < 0.001$) [0.992]	33.3 ($p < 0.001$) [0.879]
TFC	2062.8 ($p < 0.001$) [0.995]	826.4 ($p < 0.001$) [0.989]	46.3 ($p < 0.001$) [0.908]
DPPH IC_{50}	1148.3 ($p < 0.001$) [0.992]	813.7 ($p < 0.001$) [0.989]	17.4 ($p < 0.001$) [0.795]
ABTS	1992.4 ($p < 0.001$) [0.995]	773.0 ($p < 0.001$) [0.988]	29.5 ($p < 0.001$) [0.863]
FRAP	2256.6 ($p < 0.001$) [0.996]	1181.4 ($p < 0.001$) [0.992]	58.3 ($p < 0.001$) [0.924]
H_2O_2 IC_{50}	1230.2 ($p < 0.001$) [0.993]	870.0 ($p < 0.001$) [0.990]	10.3 ($p < 0.001$) [0.695]

Replicates are extractions from a single batch of plant material, so effect sizes reflect very small within-extract variation and should be interpreted with that limitation in mind.

3.4 Antioxidant activity

The 70% ethanol leaf extract was the most potent extract in all four assays (Tables 7–10 and Figs 3 and 4; for H_2O_2 it was statistically tied with the methanol leaf extract). It had the lowest DPPH IC_{50} (41.8 ± 1.3 µg/mL), against 145.7 ± 4.1 µg/mL for the aqueous root extract, the least potent. It also gave the highest ABTS (61.8 ± 1.3 µmol TE/g) and FRAP (55.9 ± 1.2 µmol TE/g) values and the lowest hydrogen peroxide IC_{50} (48.7 ± 1.6 µg/mL, not significantly different from the methanol leaf extract at 53.9 ± 1.2 µg/mL; Table 10). Across all four assays, activity fell in the order leaf > stem > root and, within each part, 70% ethanol ≥ methanol > water. For comparison, ascorbic acid gave IC_{50} values of 7.1 µg/mL (DPPH) and 24.8 µg/mL (H_2O_2), and the Trolox calibration curves were linear

(R^2 = 0.998 for both ABTS and FRAP). Tukey groupings are given in Tables 7–10, and the values are plotted in Figs 3 and 4.

Table 7 DPPH radical-scavenging activity, IC_{50} (µg/mL; mean ± SD, n = 3). Lower values indicate higher potency. Different superscript letters within a table indicate significant differences among the nine extracts (Tukey HSD, $p < 0.05$).

Extract	IC_{50}
Leaf–Water	74.6 ± 1.7^{d}
Leaf–70% Ethanol	41.8 ± 1.3^{e}
Leaf–Methanol	46.3 ± 1.6^{e}
Stem–Water	112.5 ± 2.4^{b}
Stem–70% Ethanol	68.2 ± 1.7^{d}
Stem–Methanol	72.6 ± 2.1^{d}
Root–Water	145.7 ± 4.1^{a}
Root–70% Ethanol	91.4 ± 3.3^{c}
Root–Methanol	96.8 ± 3.1^{c}

Table 8 ABTS scavenging activity (µmol TE/g extract; mean ± SD, n = 3). Different superscript letters within a table indicate significant differences among the nine extracts (Tukey HSD, $p < 0.05$).

Extract	ABTS
Leaf–Water	38.4 ± 0.9^{cd}
Leaf–70% Ethanol	61.8 ± 1.3^{a}
Leaf–Methanol	56.7 ± 1.3^{b}
Stem–Water	24.3 ± 0.6^{f}
Stem–70% Ethanol	39.7 ± 1.1^{c}
Stem–Methanol	36.5 ± 1.1^{d}
Root–Water	16.8 ± 0.6^{g}
Root–70% Ethanol	28.9 ± 0.9^{e}
Root–Methanol	26.5 ± 0.8^{ef}

Table 9 Ferric-reducing antioxidant power (µmol TE/g extract; mean ± SD, n = 3). Different superscript letters within a table indicate significant differences among the nine extracts (Tukey HSD, $p < 0.05$).

Extract	FRAP
Leaf–Water	31.7 ± 0.8^{d}
Leaf–70% Ethanol	55.9 ± 1.2^{a}
Leaf–Methanol	51.4 ± 0.8^{b}
Stem–Water	20.8 ± 0.7^{f}
Stem–70% Ethanol	36.9 ± 0.9^{c}
Stem–Methanol	33.7 ± 0.8^{d}
Root–Water	14.7 ± 0.5^{g}
Root–70% Ethanol	25.8 ± 0.8^{e}
Root–Methanol	23.9 ± 0.7^{e}

Table 10 Hydrogen peroxide scavenging, IC_{50} (µg/mL; mean ± SD, n = 3). Lower values indicate higher potency. Ascorbic acid IC_{50} = 24.8 µg/mL in the same run. Different superscript letters within a table indicate significant differences among the nine extracts (Tukey HSD, $p < 0.05$).

Extract	IC_{50}
Leaf–Water	86.4 ± 1.6^{d}
Leaf–70% Ethanol	48.7 ± 1.6^{f}
Leaf–Methanol	53.9 ± 1.2^{f}
Stem–Water	124.6 ± 2.2^{b}
Stem–70% Ethanol	76.2 ± 2.3^{e}
Stem–Methanol	81.5 ± 2.3^{de}
Root–Water	158.3 ± 4.5^{a}
Root–70% Ethanol	103.7 ± 3.1^{c}
Root–Methanol	109.8 ± 3.2^{c}

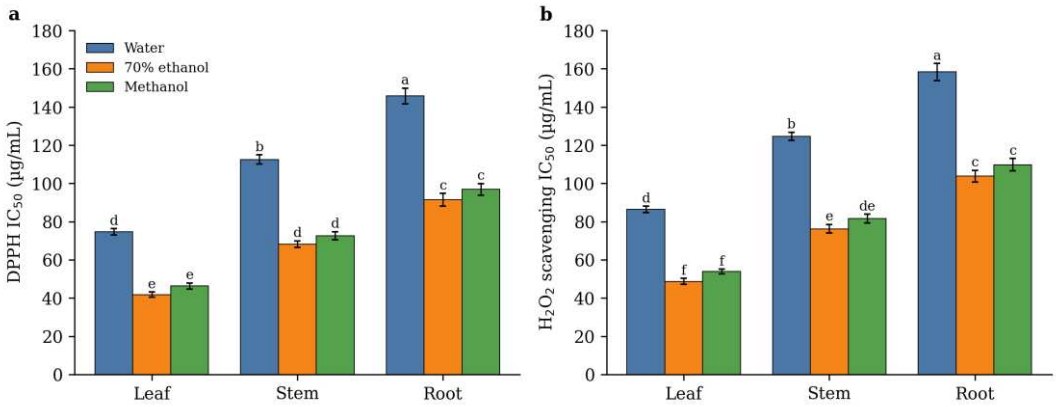


Fig. 3 DPPH radical-scavenging potency (a) and hydrogen peroxide scavenging potency (b), both expressed as IC_{50} (µg/mL; lower values mean higher potency), of the nine extracts. Bars are means ± SD (n = 3); different letters indicate significant differences (Tukey HSD, $p < 0.05$).

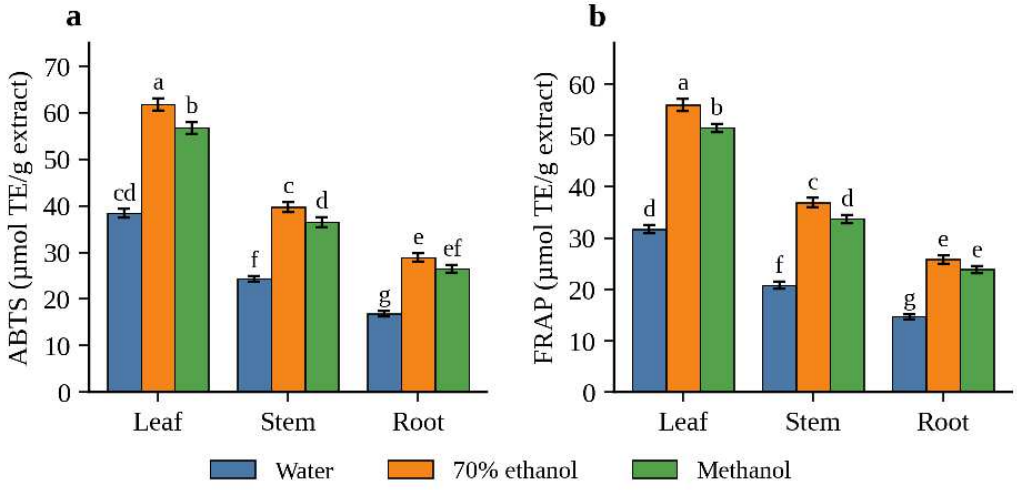


Fig. 4 ABTS radical-cation scavenging (a) and ferric-reducing antioxidant power (b) of the nine extracts. Bars are means ± SD (n = 3); different letters indicate significant differences (Tukey HSD, $p < 0.05$).

3.5 Antimicrobial activity

At 100 mg/mL, the 70% ethanol leaf extract gave the largest inhibition zones against all five microorganisms (11.8–15.7 mm), followed by the methanol leaf extract (10.9–14.2 mm) (Table 11). Aqueous extracts were weakest, and the aqueous root extract gave no inhibition against *P. aeruginosa* (6.0 mm, equal to the well diameter). For every extract, zones were largest against *S. aureus* and smallest against *P. aeruginosa*. Gentamicin gave zones of 22.4–27.3 mm against the bacteria, nystatin gave 21.8 mm against *C. albicans*, and the 5% DMSO vehicle control gave 6.0 mm, indicating no inhibition beyond the well.

Table 11 Zones of inhibition (mm, including the 6-mm well) at 100 mg/mL (mean ± SD, n = 3)

Extract	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*
Leaf–Water	8.2 ± 0.3	7.4 ± 0.2	6.8 ± 0.2	6.5 ± 0.2	7.1 ± 0.3
Leaf–70% Ethanol	15.7 ± 0.5	13.9 ± 0.4	12.4 ± 0.3	11.8 ± 0.4	13.2 ± 0.4
Leaf–Methanol	14.2 ± 0.4	12.8 ± 0.3	11.6 ± 0.4	10.9 ± 0.3	12.4 ± 0.4
Stem–Water	7.5 ± 0.2	7.0 ± 0.2	6.4 ± 0.2	6.2 ± 0.1	6.8 ± 0.2
Stem–70% Ethanol	10.4 ± 0.3	9.8 ± 0.3	8.9 ± 0.3	8.2 ± 0.2	9.5 ± 0.3
Stem–Methanol	9.6 ± 0.3	9.1 ± 0.3	8.3 ± 0.2	7.8 ± 0.2	8.9 ± 0.3
Root–Water	6.8 ± 0.2	6.5 ± 0.2	6.2 ± 0.1	6.0 ± 0.0	6.4 ± 0.2
Root–70% Ethanol	9.7 ± 0.3	8.9 ± 0.3	8.1 ± 0.3	7.5 ± 0.2	8.7 ± 0.3
Root–Methanol	9.1 ± 0.3	8.5 ± 0.2	7.7 ± 0.3	7.2 ± 0.2	8.2 ± 0.3
Gentamicin	24.6 ± 0.5	23.1 ± 0.4	27.3 ± 0.7	22.4 ± 0.6	–
Nystatin	–	–	–	–	21.8 ± 0.6
5% DMSO	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0

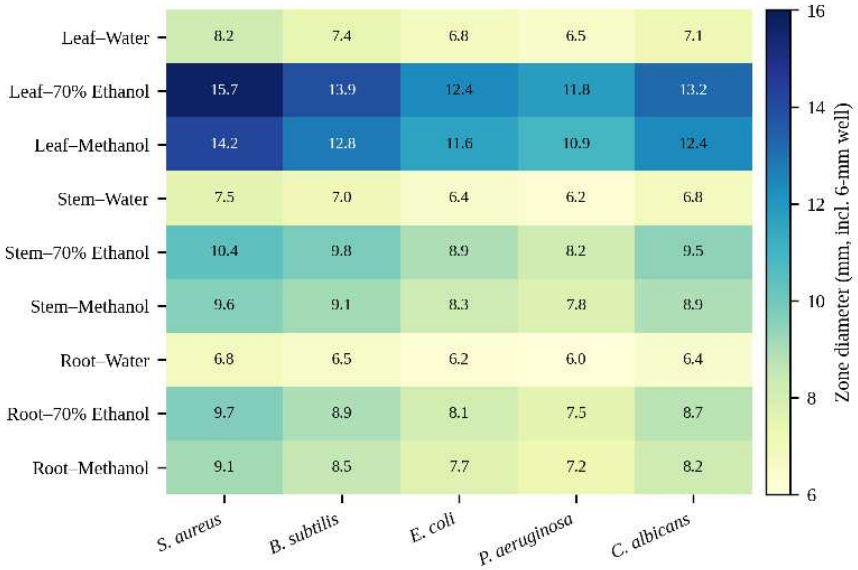


Fig. 5 Heatmap of inhibition-zone diameters (mm, including the 6-mm well) of the nine extracts at 100 mg/mL against four bacteria and one yeast. A value of 6 mm indicates no inhibition.

Four extracts (LE, LM, SE and RE) were taken forward for MIC determination under the selection criterion in Section 2.6 (Table 12; Fig. 6). The 70% ethanol leaf extract had the lowest MICs (1.25–5.00 mg/mL), and the 70% ethanol root extract the highest (5.00–20.00 mg/mL). MBC values (bacteria) and MFC values (*C. albicans*) followed the same ranking (Table 13 and Table 14; Fig. 6). Every MBC/MIC and MFC/MIC ratio was 2, so all four extracts were bactericidal and fungicidal by the ≤4 criterion.

Table 12 MIC of the four extracts selected for MIC determination (mg/mL)

Extract	***S. aureus***	***B. subtilis***	***E. coli***	***P. aeruginosa***	***C. albicans***
Leaf–70% Ethanol (LE)	1.25	2.50	2.50	5.00	2.50
Leaf–Methanol (LM)	2.50	2.50	5.00	5.00	2.50
Stem–70% Ethanol (SE)	5.00	5.00	10.00	10.00	5.00
Root–70% Ethanol (RE)	5.00	10.00	10.00	20.00	10.00

Table 13 Minimum bactericidal concentration (MBC) of the selected extracts (mg/mL)

Extract	***S. aureus***	***B. subtilis***	***E. coli***	***P. aeruginosa***
Leaf–70% Ethanol (LE)	2.50	5.00	5.00	10.00
Leaf–Methanol (LM)	5.00	5.00	10.00	10.00
Stem–70% Ethanol (SE)	10.00	10.00	20.00	20.00
Root–70% Ethanol (RE)	10.00	20.00	20.00	40.00

Table 14 Minimum fungicidal concentration (MFC) of the selected extracts against *C. albicans* (mg/mL)

Extract	***C. albicans***
Leaf–70% Ethanol (LE)	5.00
Leaf–Methanol (LM)	5.00
Stem–70% Ethanol (SE)	10.00
Root–70% Ethanol (RE)	20.00

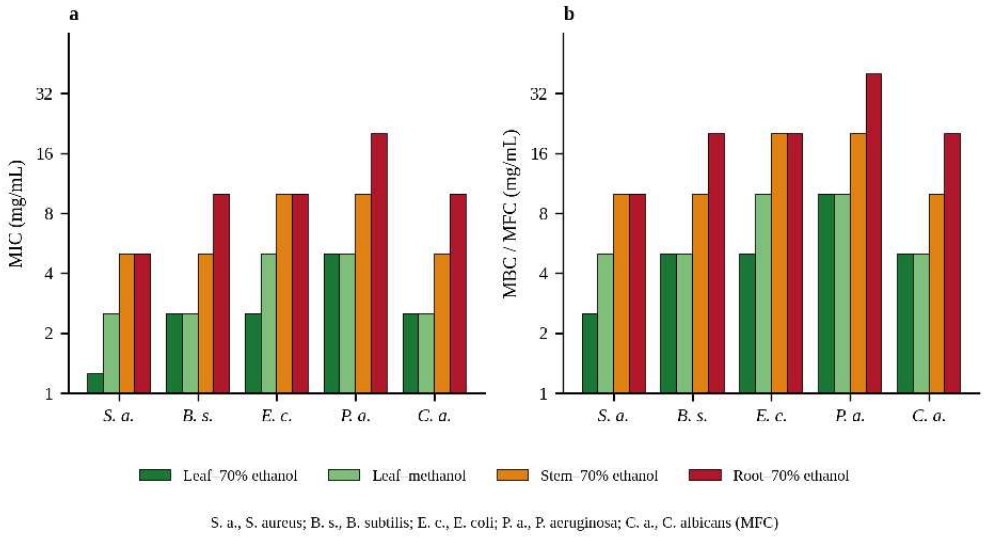


Fig. 6 Minimum inhibitory concentration (a) and minimum bactericidal or fungicidal concentration (b) of the four selected extracts (log_2 scale).

3.6 Relationships between phytochemical content and antioxidant activity

TPC and TFC were very strongly associated with antioxidant measures (Table 15; Figs 7 and 8). TPC correlated positively with ABTS (r = 0.998) and FRAP (r = 0.996) and negatively with DPPH IC_{50} (r = −0.951) and H_2O_2 IC_{50} (r = −0.957), so extracts richer in phenolics needed lower concentrations for 50% scavenging. TFC showed the same trend (ABTS r = 0.998; FRAP r = 0.998; DPPH IC_{50} r = −0.940; H_2O_2 IC_{50} r = −0.948), and TPC and TFC were themselves almost collinear (r = 0.996). The correlations were calculated on the nine extract means, which vary together along one gradient from the aqueous root to the 70% ethanol leaf extract. They therefore show association, not causation, and correlations on replicate-level data would be more informative.

Table 15 Pearson correlation coefficients between phenolic or flavonoid content and antioxidant measures (n = 9 extract means)

Variable pair	r	R^2	p
TPC vs ABTS	0.998	0.997	< 0.001
TPC vs FRAP	0.996	0.993	< 0.001
TPC vs DPPH IC_{50}	−0.951	0.905	< 0.001
TPC vs H_2O_2 IC_{50}	−0.957	0.916	< 0.001
TFC vs ABTS	0.998	0.996	< 0.001
TFC vs FRAP	0.998	0.995	< 0.001
TFC vs DPPH IC_{50}	−0.940	0.883	< 0.001
TFC vs H_2O_2 IC_{50}	−0.948	0.899	< 0.001

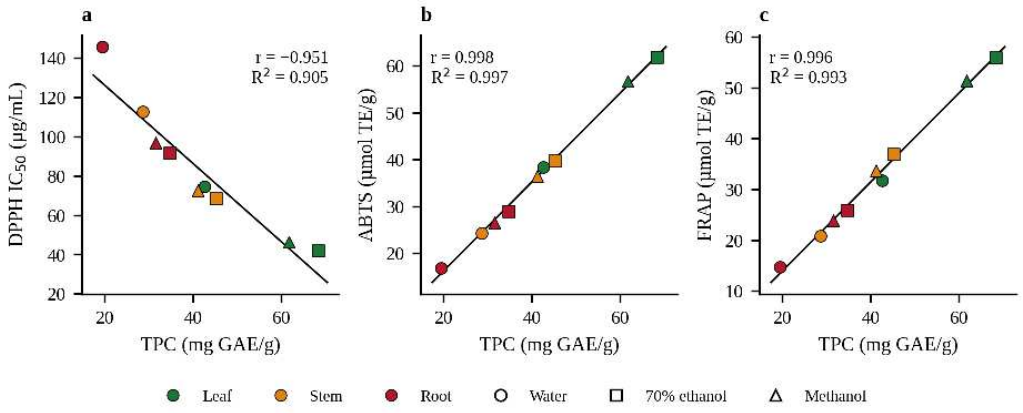


Fig. 7 Relationship between total phenolic content and DPPH IC_{50} (a), ABTS (b) and FRAP (c) across the nine extract means, with least-squares lines. Colour indicates plant part and symbol indicates solvent.

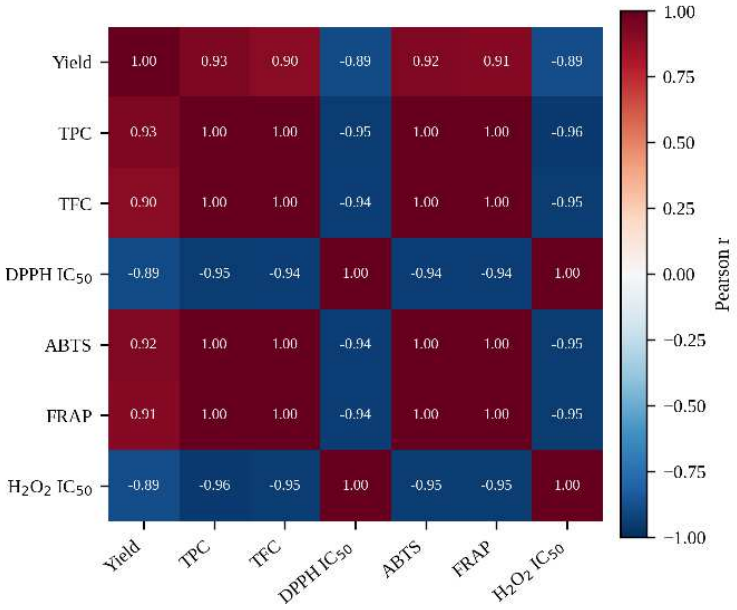


Fig. 8 Pearson correlation matrix of the seven variables used in the PCA (nine extract means).

3.7 Multivariate analysis

PCA of the standardised extract means (seven variables: extraction yield, TPC, TFC, DPPH IC_{50}, ABTS, FRAP and H_2O_2 IC_{50}) gave a first component that explained 96.0% of the variance (PC2 2.1%, PC3 1.8%, remaining components 0.1%; Table 16, Fig. 9a). PC1 was loaded positively by yield, TPC, TFC, ABTS and FRAP (loadings 0.94–1.00) and negatively by DPPH and H_2O_2 IC_{50} (−0.97 and −0.98; Table 17), so it is a single axis of overall potency. Extracts ranked along PC1 from the 70% ethanol leaf (score 4.19) and methanol leaf (3.32) extracts at the high end to the aqueous root (−3.99) and aqueous stem (−2.36) extracts at the low end, and the aqueous leaf, 70% ethanol stem and methanol stem extracts lay close to zero (Fig. 9b). PC2 accounted for only 2.1% of the variance and is not interpreted. Hierarchical clustering of the same standardised data (Fig. 10) first separated the 70%

ethanol and methanol leaf extracts (LE, LM) from the other seven extracts; at the three-cluster level the groups were LE and LM; LW, SE and SM; and SW, RW, RE and RM.

Table 16 Variance explained by the principal components (seven variables, nine extract means)

Component	Variance explained (%)	Cumulative (%)
PC1	96.0	96.0
PC2	2.1	98.1
PC3	1.8	99.9
PC4–PC7	0.1	100.0

Table 17 Loadings (correlations between variables and components) for the first three principal components

Variable	PC1	PC2	PC3
Yield	0.940	0.268	0.213
TPC	0.996	0.041	−0.071
TFC	0.989	0.007	−0.147
DPPH IC_{50}	−0.973	0.184	−0.142
ABTS	0.993	0.049	−0.101
FRAP	0.991	0.015	−0.126
H_2O_2 IC_{50}	−0.976	0.190	−0.106

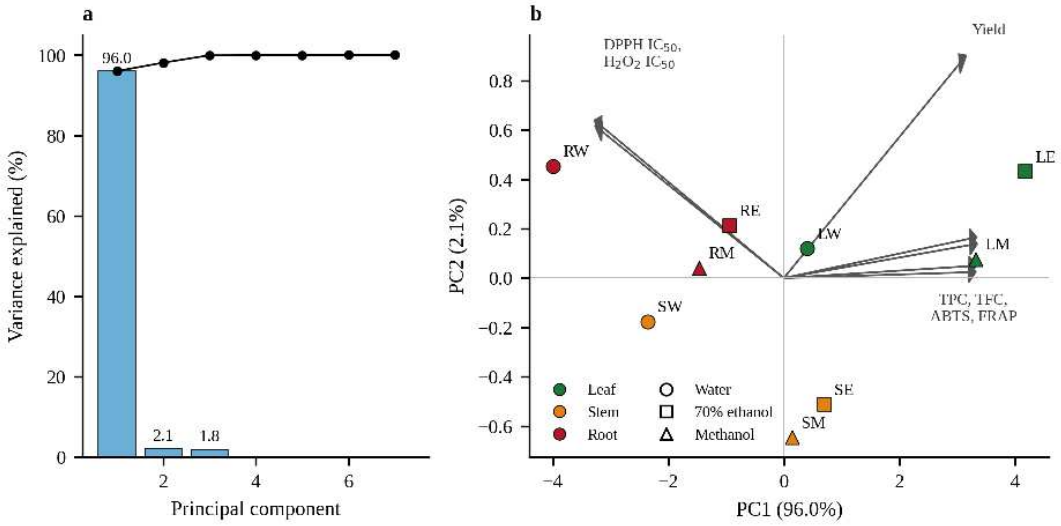


Fig. 9 PCA of the standardised extract means: variance explained by each component (a) and PC1–PC2 biplot with extract scores and variable loadings (b). LW, LE, LM, leaf; SW, SE, SM, stem; RW, RE, RM, root (W, water; E, 70% ethanol; M, methanol).

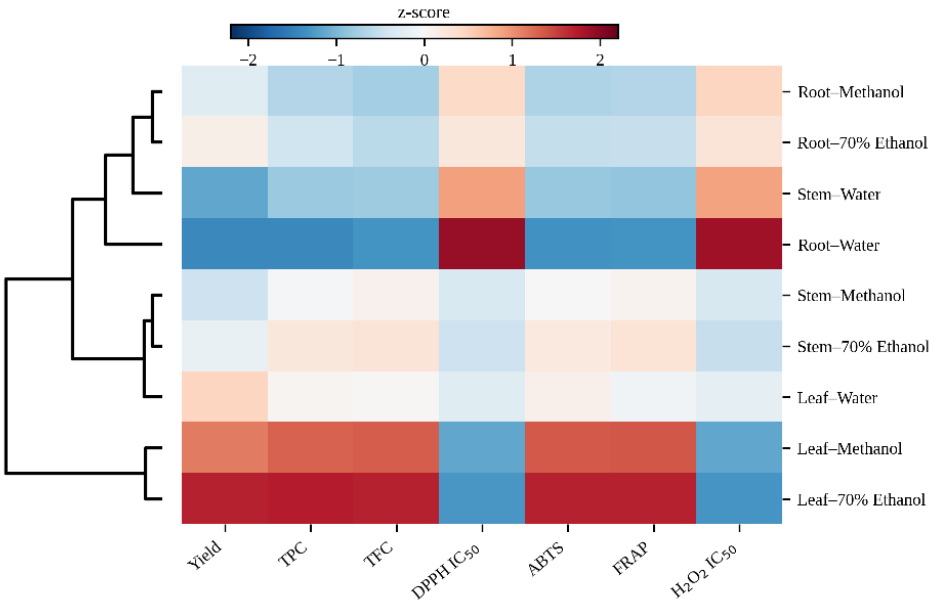


Fig. 10 Heatmap of z-scored variables with hierarchical clustering of the nine extracts (Euclidean distance, Ward's linkage).

4 Discussion

Plant part and solvent both matter, and they interact. Leaves gave the highest yield, phenolic and flavonoid contents, antioxidant activity and antimicrobial activity with every solvent, and 70% ethanol gave the highest values among the solvents tested for every plant part. Plant part explained the largest share of variation in every measure (leaf > stem > root), solvent effects were smaller but consistent (70% ethanol ≥ methanol > water), and the significant part × solvent interactions show that the size of the solvent effect depended on the organ. The choice of solvent should therefore not be fixed in advance across organs: for TPC, the gain from moving from water to 70% ethanol was 25.8 mg GAE/g in leaves but 15.3 mg GAE/g in roots. Aqueous ethanol often recovers more phenolics than either pure solvent [27], although the best solvent depends on the plant matrix, and 100% ethanol performed best for *Limnophila aromatica* [26]. This may reflect the complementary polarity of water and ethanol, which can extract phenolics that differ in physicochemical properties, although 70% ethanol is not superior across all plant matrices [26, 27]. The lower yields of water extracts may also reflect co-extraction or incomplete recovery of less polar phenolics, although this study did not test that directly.

Phenolics track antioxidant activity, but association is not proof of cause. TPC and TFC correlated strongly with ABTS, FRAP and DPPH IC_{50}, in line with the widely reported link between

plant phenolics and radical scavenging [42]. Phenolics can donate hydrogen atoms or electrons, and flavonoids in particular have structural features suited to radical scavenging and metal chelation [43]. However, the Folin–Ciocalteu assay also responds to non-phenolic reducing substances such as ascorbate and some amino acids [31, 34], and the correlations here rest on nine extract means. We therefore describe phenolics and flavonoids as associated with activity, not as its cause; identifying the active compounds would need fractionation and compound-level analysis such as LC–MS.

Antioxidant results agree across mechanisms. The same ranking (70% ethanol leaf best; aqueous root worst) appeared in the electron-transfer assay FRAP, in the mixed-mechanism radical assays DPPH and ABTS, and in hydrogen peroxide scavenging [30]; for H_2O_2 the 70% ethanol and methanol leaf extracts did not differ significantly (Table 10). Agreement across assays with different chemistry makes the ranking more credible than a single assay would. The 70% ethanol leaf DPPH IC_{50} of 41.8 µg/mL is a crude-extract value and should be read against the ascorbic acid value from the same run (7.1 µg/mL), not against pure compounds. Comparison with other reports of *J. tanjorensis* is limited by differences in solvents, assay conditions and units. Methanolic leaf extracts have been reported with DPPH IC_{50} values of 49.7 µg/mL [10] and 185.02 µg/mL [15], and with a TPC of 36.48 mg GAE/g [15], compared with 46.3 µg/mL and 61.7 mg GAE/g for the methanol leaf extract here; differences of this size are plausible given differences in collection site, extraction and assay conditions, but this study cannot resolve them.

Antimicrobial activity is modest but consistent. The 70% ethanol leaf extract gave the largest zones and lowest MIC against all five organisms among the extracts tested, and activity again ranked leaf > stem > root. Gram-positive bacteria and the yeast were more susceptible than Gram-negative bacteria, and *P. aeruginosa* was the least susceptible in every extract. This pattern is commonly attributed to the outer membrane of Gram-negative bacteria, which limits the entry of many plant compounds, and to the intrinsic resistance of *P. aeruginosa* [44, 45]. Flavonoids and tannins have been proposed to act on microbial membranes, enzymes and cell walls [4, 46, 47], and the leaf extracts, which were richest in these classes, were the most active. The present data do not test that explanation.

The strength of activity must be kept in perspective. Zones were measured at a high extract concentration (100 mg/mL) against gentamicin and nystatin controls at much lower concentrations,

and the best MIC (1.25 mg/mL) is high compared with conventional antibiotics. Because standardised clinical breakpoints are not established for crude plant extracts, the results are interpreted descriptively, and published criteria for grading crude-extract activity vary [48]. All MBC/MIC and MFC/MIC ratios were exactly 2. This is what a two-fold dilution scheme yields when the cidal endpoint lies one dilution above the inhibitory endpoint, so it supports a cidal action but also means the ratio does not discriminate between extracts. Zone-of-inhibition diameters also depend on diffusion of compounds through agar, which favours small and polar molecules, and should be read alongside MIC values [40]. Because MIC, MBC and MFC were determined only for four extracts chosen after the inhibition-zone screen, these comparisons apply to the selected extracts and not to all nine.

Multivariate view. PCA and clustering showed that the nine extracts lie along one dominant axis (96.0% of the variance) on which yield, phenolics, flavonoids and antioxidant capacity increase together, with the 70% ethanol and methanol leaf extracts at the top, which agrees with the univariate results. Because the measured variables are strongly collinear and rest on nine extract means, the PCA adds little beyond the pairwise results and should not be used to predict the behaviour of other samples [49]. Antimicrobial data were not included in the PCA because MIC values exist for only four extracts.

Limitations. All replicates were extractions of the same pooled powder, so biological variation among individual plants was not captured, and the very large F values and effect sizes should be read with that in mind. The plant material came from one location and one collection in May 2026, so seasonal, geographic and chemotypic variation was not covered. Phytochemical screening was qualitative, and individual compounds were not identified. The assays are in vitro, and no cytotoxicity or toxicity of the extracts was tested. Toxic compounds have been described in the related species *Jatropha curcas* [50], and the toxicological risks of herbal products are often underestimated [51]. Toxicity of *J. tanjorensis* leaf extracts has been examined in animals [11, 24, 52, 53], but activity in the present assays is not evidence of safety. Only a single concentration was used for the agar-diffusion screen, and only four of the nine extracts were taken forward to MIC/MBC/MFC.

Implications. For anyone preparing *J. tanjorensis* extracts for antioxidant or antimicrobial work, leaves extracted with 70% ethanol gave the most active material in this study, and stems and roots were consistently weaker. Future work should identify the active constituents by chromatography and mass spectrometry, test extracts from several plants, sites and seasons, and assess toxicity.

5 Conclusion

In a 3 × 3 comparison of plant part and solvent, leaves extracted with 70% ethanol gave the highest extraction yield, phenolic and flavonoid content, antioxidant activity in four assays, and, among the extracts tested for MIC, antimicrobial activity against two Gram-positive bacteria, two Gram-negative bacteria and *C. albicans*. Plant part, solvent and their interaction all affected phenolic content. Phenolic and flavonoid content were strongly associated with antioxidant activity, and PCA and clustering placed the 70% ethanol and methanol leaf extracts apart from the other seven extracts. The antimicrobial activity was modest at the concentrations tested, and the causal role of specific compounds, and the safety of the extracts, remain to be established.

Declarations

Ethics approval and consent to participate: Ethical approval was not required for the collection and laboratory analysis of plant material. The plant material (leaves, stems and roots of J. tanjorensis) was obtained from [wild-growing / cultivated: confirm] plants at Barnawa, Kaduna South, Kaduna State, Nigeria, and its collection complied with institutional, local and national guidelines and legislation. No permission or licence was required for the collection. All laboratory procedures involving microbial cultures followed applicable institutional biosafety procedures.

Consent for publication: Not applicable.

Plant material statement: Plant material was collected and used in accordance with institutional and national guidelines. A voucher specimen (Ka/26/8766) is held in the Kaduna State University herbarium.

Data availability: The data supporting the findings are available from the corresponding author on reasonable request.

Competing interests: The author declares no competing interests.

Funding: This research received no funding from any agency in the public, commercial, or not-for-profit sectors.

Author contributions: Oluwatoyin Ishola is the sole author and was responsible for all aspects of this study: conceptualization, methodology, investigation, formal analysis, and writing (original draft, review and editing).

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Use of AI tools: AI tools were used to assist with statistical analysis, figure preparation and manuscript drafting; all scientific content and conclusions were reviewed and approved by the author.

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