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RESEARCH ARTICLE

First report on the phytochemical, nutritional and antioxidant potential of *Jacquemontia pentanthos,* a key plant species from Kerala, India

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Abstract

Jacquemontia pentanthos (Skyblue cluster vine) is an evergreen, twining vine that produces many small but showy flowers and is a member of the morning glory family. The Phytochemical profile and pharmacological aspects of the genus have been widely studied for *Jacquemontia tamnifolia and Jacquemontia paniculata*. However, *Jacquemontia pentanthos*, is the least studied species. This is the first study reporting the Phytochemical, proximate nutritional composition and *in vitro* antioxidant potential (via DPPH: 2,2-diphenyl-1-picrylhydrazyl, ABTS: 2,2-azino-bis and FRAP: Ferric reducing antioxidant power assays) of *Jacquemontia pentanthos* leaves. Proximate analysis quantified the primary metabolites in *Jacquemontia pentanthos* leaves, including protein (0.51 ± 0.03 mg BSA equivalent/g DW), dietary fibre (10.5 ± 0.3 mg/g dry weight), amino acids (19.85 ± 0.6 mg leucine equivalent/g DW), carbohydrates (0.38 ± 0.02 mg glucose equivalent/g DW), crude fat (0.14 ± 0.01% dry weight), moisture content (60.0 ± 1.2% fresh weight) and ash content (11.2 ± 0.5% dry weight). Secondary metabolite profiling of the extracts revealed high levels of flavonoids (87.049±0.011mg quercetin equivalents per gram mg QE/g), followed by alkaloids (2.717 ± 0.021mg atropine equivalents/g; mg AE/g), steroids (0.657 ± 0.031mg diosgenin equivalents/g; mg DE/g), phenolics (0.605 ± 0.008mg catechol equivalents/g; mg CE/g), terpenoids (0.459 ± 0.014 mg linalool equivalents/g; mg LE/g), saponins (0.663 ± 0.024 mg saponin equivalents/g; mg SE/g) and tannins (0.951 ± 0.017mg tannic acid equivalents/g; mg TAE/g). The antioxidant potential of the extract was evaluated using DPPH (IC_{50} = 0.450 ± 0.017mg/mL), ABTS (IC_{50} = 0.538 ± 0.027 mg/mL) and FRAP (137.72 ± 0.224 µg Trolox equivalent /g) assays. These results suggest that *Jacquemontia pentanthos* is a nutritionally rich species with promising antioxidant activity and potential for use in functional foods and phytopharmaceutical applications.

Keywords: antioxidant activity; *Jacquemontia pentanthos*; phytochemical analysis; proximate analysis

Introduction

Jacquemontia pentanthos (commonly known as Skyblue Clustervine) is a visually striking, lightweight evergreen climber belonging to the family Convolvulaceae. Its stems exhibit a velvety texture that occasionally transitions to smoothness. The leaves are ovate to broadly ovate, typically measuring 3-6 cm in length, with a heart-shaped base and tapering apex. The plant produces vibrant blue flowers, occasionally white, that are clustered densely and usually bloom one or two at a time. Each flower spans approximately 2-2.5 cm in diameter. After flowering, the plant forms spherical, brown capsules containing glabrous seeds, approximately 2.5 mm in size. Native to tropical America, *J. pentanthos* flourishes during the monsoon months (June-August) and has naturalized widely across tropical regions worldwide (1).

The genus *Jacquemontia* has garnered increasing interest in phytopharmacology in recent years. Multiple studies have highlighted the presence of diverse bioactive compounds across species in this genus, with demonstrated antioxidant, anti-inflammatory, antimicrobial, anticoagulant and cytotoxic activities (2-4). For instance, *Jacquemontia tamnifolia* has shown potent antioxidant, antinociceptive and anticoagulant properties, while *Jacquemontia caerulea* leaf extracts have exhibited promising anti-inflammatory effects in Wistar rat models (4). Similarly, *Jacquemontia pentanthos* has been reported to possess cytotoxic potential and a rich profile of secondary metabolites, along with recent investigations revealing its broad spectrum of bioactive constituents and biological activities (5).

Despite this, *Jacquemontia pentanthos* remains significantly underexplored compared to its congeneric species. There is currently limited comprehensive data on the nutritional, phytochemical and antioxidant potential of its leaves. The medicinal importance of traditional plants like *J. pentanthos* is primarily attributed to their complex phytochemical composition, which includes alkaloids, flavonoids, polyphenols, terpenoids and other compounds. This photochemical complexity not only underlies their pharmacological efficacy but also contributes to their ecological resilience under diverse environmental stresses.

Systematic evaluation of such underutilized plants is essential to discover novel bioactive molecules with therapeutic relevance. Furthermore, preservation and scientific documentation

of these species can provide valuable insights into the interplay between environmental adaptability, phytochemical composition and their implications for human health and nutrition.

Materials and Methods

Chemicals and reagents

DPPH (2, 2-diphenyl-1-picrylhydrazyl), Alpha Naphthol, Folin-Ciocalteu's reagent, Anthrone reagent, Benedict's reagent, quercetin, catechol, ascorbic acid, Bovine Serum Albumin, Ninhydrin, Mayer's reagent, Dragendorff's reagent, Diosgenin and Atropine were purchased from Sigma-Aldrich. The solvent and all reagents used in the analysis were of analytical grade. These reagents were selected based on their specificity and reliability in standard phytochemical and antioxidant assays. Each reagent targets a particular class of biomolecules-such as Folin-Ciocalteu's reagent for phenolics, DPPH for free radical scavenging activity and anthrone for carbohydrate estimation-ensuring accurate detection and quantification of phytoconstituents relevant to the study of *Jacquemontia pentanthos*. Their use is well -established in phytochemical research, contributing to reproducibility and comparability of results across similar studies.

Plant material and extraction

Plant collection and authentication

Specimens of *Jacquemontia pentanthos* were collected from Kulathupuzha, Kollam district, Kerala, India, in February 2024. Taxonomic identification was carried out by comparison with standard floras and confirmed by a qualified taxonomist. The plant was authenticated by Dr. A. Nazarudeen, Senior Scientist & Head of the Plant Systematics & Evolutionary Science Division, Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Kerala, India. A voucher specimen (No. 107817) was deposited in the JNTBGRI herbarium for future reference.

Sample preparation and extraction

Immediately after collection, the plant material was thoroughly washed with distilled water to remove any adhering soil or debris. The cleaned samples were oven-dried at 40 °C for 24 hr, then ground into a fine powder using a mechanical grinder. The powdered material was stored in a desiccator under dry conditions until further use.

Phytochemical extraction was conducted using the Soxhlet method, following protocols adapted from previous studies (6-8). A total of 10 g of powdered leaf material was subjected to continuous extraction with ethanol,hexane and ethyl acetate for 10 hours using a Soxhlet apparatus. The resulting extract was concentrated under reduced pressure using a rotary evaporator, yielding a crude residue. The extract was stored at 4°C in an airtight container for subsequent phytochemical and antioxidant analyses. The extraction yield was calculated to be 69 % (w/w), indicating efficient recovery of ethanol -soluble phytoconstituents. Soxhlet extraction was chosen for its proven ability to efficiently extract a broad spectrum of polar and semi-polar secondary metabolites, making it highly suitable for comprehensive phytochemical profiling.

Preliminary phytochemical screening

The crude extract of Jacquemontia pentanthos was subjected to preliminary phytochemical screening to identify the major classes of primary and secondary metabolites, including alkaloids, flavonoids, tannins, saponins, phenolics, proteins, carbohydrates, amino acids and terpenoids. Standard qualitative tests were employed based on colour change or precipitation reactions, following protocols described in recent phytochemical screening studies (2, 4, 5). These methods were selected for their reproducibility, reliability and frequent use in contemporary ethnobotanical research. Results were recorded qualitatively, with each phytochemical class marked as 'Present' or 'Absent' in the ethanol extract of Jacquemontia pentanthos leaves.

Proximate and nutritional analysis

A proximate analysis of *Jacquemontia pentanthos* was conducted to determine the levels of moisture, ash, crude protein, carbohydrates, crude fat, crude fiber, free amino acids and lipids. All measurements were conducted in triplicate and Results are expressed as mg equivalent or % on a dry weight (DW) or fresh weight (FW) basis, as appropriate. Standard methods were followed and where necessary, modifications were made to enhance extraction efficiency or practicality, as described below.

Moisture content: Moisture content was determined using the oven-drying method as described in AOAC (9). Approximately 10 g of fresh leaf sample was cut into 5 mm × 5 mm sections and dried in a hot air oven at 100°C for 24 hr until a constant weight was achieved. No modification was made to this method. The moisture percentage was calculated based on the weight difference before and after drying.

Total Carbohydrate (Anthrone Method): Total carbohydrate content was estimated by the Anthrone method according to Sadasivam (10). A 100 mg powdered sample was hydrolyzed with 5 ml of 2.5 N HCl by boiling in a water bath for 3 hr. After neutralization with solid sodium carbonate, the volume was made up to 100 ml and centrifuged. A 1 mL aliquot of the supernatant was reacted with 4 ml anthrone reagent, heated in a boiling water bath for 8 min, cooled and absorbance was measured at 630 nm. Glucose (0.2-1.0 mg/mL) was used as the standard.

Crude fat (Soxhlet method): Crude fat was extracted using hexane in a Soxhlet apparatus following Nielsen and AOCS guidelines (11, 12). A 5 g dried sample was extracted for 6 hr. In contrast to the conventional 8 hr extraction, the duration was reduced to 6 hr in this study to improve time efficiency without compromising fat yield or accuracy. The resulting extract was dried in a pre-weighed tube at 70 °C and crude fat content was calculated gravimetrically.

Free amino acids (Ninhydrin assay): Free amino acid content was estimated using the ninhydrin method described by Sadasivam (10). A 500 mg dried sample was extracted with 80 % ethanol and a 0.1 mL aliquot of the extract was reacted with 1 mL of ninhydrin reagent. The mixture was heated in a boiling water bath for 20 min, followed by the addition of 5 mL of diluent and absorbance was read at 570 nm after 15 min. Leucine (0.2-1.0 mg/mL) served as the standard. In this study, the method was modified by performing two successive extractions using a slightly increased volume of 80 % ethanol to ensure complete solubilization and recovery of free amino acids and the pooled extracts were used for analysis.

Crude protein (Lowry method): Crude protein was determined using the Lowry method and validated. A 500 mg dried sample was homogenized in phosphate buffer, centrifuged and 0.2 ml of the supernatant was mixed with 5 mL alkaline copper reagent

and incubated for 10 min. Then, 0.5 mL Folin-Ciocalteu reagent was added and absorbance was measured at 660 nm after 30 min. Bovine Serum Albumin (0.2-1.0 mg/mL) was used as the standard. To improve the solubilization and recovery of proteins, the volume of phosphate buffer used during homogenization was optimized slightly in this study.

Total ash: Ash content was determined by dry ashing in a muffle furnace at 525 °C (9). A 5 g dried sample was placed in a pre-weighed silica crucible, charred on a hot plate and then incinerated in the furnace until white ash remained. The percentage of ash was calculated from the weight of the residue. No modification was made to this procedure.

Crude fiber: Crude fiber was analyzed using acid-alkali digestion (9). A 2 g defatted sample was boiled with 200 mL of 1.25 % sulfuric acid for 30 min, filtered and washed. The residue was then boiled with 1.25 % sodium hydroxide for 30 min, filtered, washed again, dried at 110 °C and incinerated in a muffle furnace. The crude fiber content was calculated based on the loss in weight after ignition. The procedure was followed as per standard without any modification.

Lipid estimation: Lipids were estimated using the Bligh and Dyer method (13). A 5 g dried sample was homogenized with a chloroform: methanol mixture (1:1), followed by the addition of chloroform and water to induce phase separation. The lower chloroform layer was collected, evaporated in pre-weighed aluminium pans and the lipid content was calculated gravimetrically. A slight procedural modification was employed by adjusting the solvent volumes to optimize lipid extraction efficiency in plant matrices. As this method partially overlaps with crude fat estimation, the lipid content was analyzed but excluded from the final nutritional summary to avoid redundancy.

Quantitative analysis of phytochemicals

Quantitative estimation of phytochemicals was performed using standard colorimetric protocols, with slight modifications to accommodate the properties of the Jacquemontia pentanthos extract and available instrumentation. The methods used included the aluminium chloride ($AlCl_3$) method for flavonoids, the Folin-Ciocalteu method for phenolics, the bromocresol green (BCG) method for alkaloids and the Liebermann-Burchard method for steroids (14-17). Assays for tannins, terpenoids and saponins were also employed (18-20). All estimations were performed in triplicate and concentrations were determined using standard calibration curves. Results were expressed as milligrams of standard equivalents per gram of extract (mg/g).

Estimation of alkaloids

The method followed was based on the bromocresol green (BCG) dye binding assay (16). In the original method, acidified extracts are complexed with BCG and extracted using chloroform before spectrophotometric analysis. In our study, the ethanol extract was evaporated to dryness, reconstituted in 2 N HCl, filtered and washed three times with 10 mL of chloroform. After alkalinization with 0.1 N sodium hydroxide, 5 mL of BCG and 5 mL of phosphate buffer were added. The resulting solution was extracted with 1-4 mL of chloroform. To minimize emulsion formation, the number of washes and shaking time were optimized. Absorbance was recorded at 470 nm and results were calculated as mg atropine equivalents/g extract.

Estimation of flavonoid

Flavonoid content was measured using the aluminium chloride method, where flavonoids form a complex with $AlCl_3$ detectable at 510 nm (14). In the standard protocol, sodium nitrite and NaOH are added sequentially to the extract. In our modification, 1 mL of the extract was reacted with 4 mL distilled water, followed by 0.3 mL of 5 % sodium nitrite and 0.3 mL of 10 % aluminium chloride. After 6 min, 2 mL of 1 M sodium hydroxide was added and the total volume made up to 10 mL. The reaction was scaled to suit microcuvette-based spectrophotometry and quercetin was used as the calibration standard. Results were reported as mg quercetin equivalents/g extract.

Estimation of steroid

Steroids were quantified by the Liebermann-Burchard method, where sulphuric acid and ferric chloride form a coloured complex with steroids (17). In the original protocol, absorbance is measured at 780 nm after 30 min of incubation. Our procedure involved combining 1 mL of extract with 2 mL of 4 N sulfuric acid and ferric chloride, followed by 0.5 mL of 0.5% potassium hexacyanoferrate (III). The mixture was heated at 70°C for 30 min with intermittent shaking. Reagent ratios and incubation timing were optimized for consistent colour development. Diosgenin served as the standard.

Estimation of phenolic content

Phenolic compounds were determined by the Folin-Ciocalteu method, which relies on the reduction of the Folin reagent by phenolics to produce a blue chromophore (15). The extract was reacted with 0.4 ml of 1:10 diluted Folin-Ciocalteu reagent. After 5 min, 4 ml of sodium carbonate was added and the volume made up to 10 mL. The mixture was incubated at room temperature for 90 min. This extended incubation was implemented to ensure full chromophore development and absorbance was measured at 750 nm. Catechol was used for calibration and values were reported in mg catechol equivalents/g dry extract.

Estimation of tannin content

Tannins were quantified using the Folin-Denis colorimetric method (18). In the original method, tannins react with Folin-Denis reagent in alkaline medium, producing a blue complex. We followed the same procedure by mixing 1 mL of extract or standard tannic acid with distilled water to make up 7.5 mL. Then, 0.5 mL of Folin-Denis reagent and 1 mL of sodium carbonate solution were added and the final volume was adjusted to 10 mL. Absorbance was recorded at 700 nm after incubation. We optimized sample dilution and incubation to improve linearity and sensitivity. Results were expressed as mg tannic acid equivalents/g extract.

Estimation of terpenoid

Terpenoids were determined using the method of Ghorai et al (19), based on colour formation with concentrated sulphuric acid and methanol. One millilitre of extract was mixed with 3 ml of chloroform and allowed to stand for 5 min. Then, 200 µL of concentrated H_2SO_4 was added and the mixture incubated at room temperature in the dark for 1.5-2 hr. Finally, 3 ml of 95 % methanol was added and the solution was vortexed until the precipitate dissolved. We extended the colour development time to enhance absorbance stability and used vortexing to ensure clarity. Absorbance was measured at 538 nm and linalool was used as the standard.

Estimation of saponin

The method followed to estimate total saponin content, which involves acid hydrolysis and colorimetric detection (20). A 0.25 mL aliquot of aqueous extract was mixed with 1 mL of a 1:1 glacial acetic acid and sulphuric acid mixture. The reaction was vortexed and incubated at 60 °C in a water bath for 30 min. After cooling, absorbance was read at 527 nm. To accommodate small-volume cuvettes, we scaled down reagent volumes while maintaining stoichiometry. A commercial saponin standard was used and results were expressed as mg saponin equivalents/g extract.

Antioxidant assays

DPPH Radical Scavenging Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the ethanol, hexane and ethyl acetate extract of *Jacquemontia pentanthos* was assessed using a standard spectrophotometric method, with modifications based on recent protocols described (21-23). This method is widely recognized for its simplicity, sensitivity and reproducibility in evaluating antioxidant activity. A stock solution of ascorbic acid was prepared by dissolving 1 g in 100 mL of distilled water. From this stock, various concentrations ranging from 0.2 to 1.0 mL were transferred into separate test tubes. Likewise, the ethanol extract of *J. pentanthos* (1 g/100 ml) was serially diluted to obtain extract concentrations ranging from 0.2 to 1.0 mL. Each test tube was brought to a final volume of 5 ml using methanol. Subsequently, 1 ml of freshly prepared 0.1 mM DPPH solution was added to each tube. The reaction mixtures were incubated in the dark at room temperature for 1 hour to allow complete interaction between the antioxidants and DPPH radicals. After incubation, the absorbance was measured at 517 nm using a UV-Visible spectrophotometer. The percentage of DPPH radical scavenging activity was calculated using the formula:

$$\text{Scavenging Activity (\%)} = ((A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}) \times 100 \quad \text{(Eqn. 1)}$$

$A_{control}$ = Absorbance of control

A_{sample} = Absorbance of sample.

ABTS Radical Scavenging Assay

The ABTS radical cation ($ABTS^{+}\bullet$) scavenging activity of the ethanol, hexane and ethyl acetate extract of Jacquemontia pentanthos was evaluated following the method originally described, with slight modifications introduced to suit experimental conditions (24). In the original protocol, $ABTS^{+}\bullet$is produced by reacting ABTS (7 mM) with potassium persulfate (2.45 mM) and incubating the mixture in the dark for 12-16 hr. The resulting $ABTS^{+}\bullet$ solution is then diluted to achieve an absorbance of 0.70 ± 0.02 at 734 nm before being used for antioxidant activity measurement. In the modified procedure, $ABTS^{+}\bullet$ was generated by mixing 7 mM ABTS with 2.46 mM potassium persulfate and incubating the solution in the dark at room temperature for 12-16 hours. The resulting radical cation solution was diluted with ethanol (approximately 1:90, v/v) to obtain an absorbance of 0.70 ± 0.02 at 734 nm. For the assay, 100 µL of the diluted plant extract or Trolox standard solution was added to 2.4 mL of the $ABTS^{+}\bullet$ working solution. The reaction mixture was incubated at room temperature for 6 min, after which absorbance was measured at 734 nm using a UV-Visible spectrophotometer. In our modification, the final volume was adjusted to suit a standard 3 mL cuvette format and the incubation time was optimized to 6 minutes to improve consistency. Trolox (6 -hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), a water-soluble vitamin E analogue, was used as the standard antioxidant. A standard calibration curve was constructed using Trolox solutions ranging from 0 to 300 µM. The antioxidant capacity of the extract was calculated from the curve and expressed as Trolox Equivalent Antioxidant Capacity (TEAC) in mM TEAC.

Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing antioxidant power (FRAP) assay was carried out according to the standard method with minor modifications to suit the plant extract and laboratory conditions (25). In the original method, the FRAP reagent is composed of acetate buffer (300 mM, pH 3.6), TPTZ (2,4,6-tripyridyl-s-triazine) in HCl and ferric chloride. In our modification, the FRAP reagent was freshly prepared by mixing 25 mL of acetate buffer (300 mM, pH 3.6), 2.5 mL of TPTZ solution (10 mM in 40 mM HCl) and 2.5 mL of $FeCl_3 \cdot 6H_2O$ solution (20 mM). The reagent was pre-warmed to 37 °C to maintain reactivity. Next, 150 µL of the diluted Jacquemontia pentanthos extract or Trolox standard solution was added to 2.85 mL of the freshly prepared FRAP reagent. The mixture was incubated at 37 °C for 30 min, allowing full development of the blue ferrous-TPTZ complex. In our modified procedure, we optimized incubation timing and temperature for greater consistency in absorbance readings and scaled reaction volumes to accommodate spectrophotometric cuvettes. Absorbance was recorded at 593 nm using a UV-Visible spectrophotometer. A standard calibration curve was constructed using aqueous Trolox solutions ranging from 0 to 300 µM. The antioxidant capacity of the extract was calculated from the calibration curve and expressed as Trolox Equivalent Antioxidant Capacity (TEAC), reported in µg TE/g of dry extract.

Results and Discussion

Extraction yield and phytochemical screening

Solvent partitioning of the crude extract using hexane, ethyl acetate and ethanol yielded varying extract amounts (Table 1). The ethanol extraction of *Jacquemontia pentanthos* leaves yielded 68 % (w/w), indicating efficient recovery of ethanol-soluble phytoconstituents. The preliminary phytochemical screening of the three extracts revealing a broad spectrum of primary and secondary metabolites (Table 2). Qualitative analysis confirmed the presence of alkaloids, flavonoids, tannins, phenolics, saponins, terpenoids and steroids (Table 2). These classes of phytochemicals are widely recognized for their pharmacological properties, including antioxidant, antimicrobial, anti-inflammatory and cytoprotective activities (26-28). Their presence supports the traditional medicinal use of this plant and highlights its potential as a source of bioactive compounds.

Table 1. Extraction yield from *Jacquemontia pentanthos* with various solvents

Solvent	Extraction period (hr)	Yield (%)
Hexane	10	48.86
Ethyl Acetate	10	50.78
Ethanol	10	69.00

Table 2. Phytochemical screening of crude extracts of *Jacquemontia pentanthos*

Phytochemicals	Hexane	Ethanol	Ethyl acetate
Carbohydrate	+	+	+
Protein	+	+	+
Aminoacids	+	+	+
Terpenoides	+	+	+
Phenolics	+	+	+
Tannins	+	+	+
Saponins	+	+	+
Steroids	+	+	-
Alkaloids	+	+	+
Flavonoids	+	+	+
Starch	-	-	-
Fatty acid	+	+	-
Glycosides	-	-	-

Further quantitative analysis (Table 3) confirmed significant concentrations of phenolic compounds, flavonoids and alkaloids. These groups of compounds are well documented for their antioxidant, free radical scavenging and metal-chelating properties which may underlie the biological activities observed in *J. pentanthos* (29, 30).

Proximate and nutritional composition

The proximate analysis of *Jacquemontia pentanthos* leaves revealed its nutritional potential based on standard methods. Results are expressed as mg equivalent or % on a dry weight (DW) or fresh weight (FW) basis, as appropriate, are summarized in Table 4. The moisture content, determined by oven drying, was 62.0 ± 1.1 % (fresh weight), indicating high water content typical of leafy vegetables. This value is expected for fresh plant material but exceeds the WHO-recommended range of 8-12 % for dried medicinal plant samples intended for long-term storage (31, 32). This indicates the need for appropriate drying techniques to ensure shelf-life stability. For reference, the Recommended Dietary Allowance (RDA) for protein in adults is approximately 50 g/day, indicating that *J. pentanthos* may serve as a modest contributor rather than a primary source of nutrition.

Table 3. Quantification of total phenolics, flavonoids, steroids, tannins, terpenoids, saponins and alkaloid content

PLANT	*Jaquemontia pentanthos*		
Phytochemicals	**Hexane**	**Ethyl acetate**	**Ethanol**
Terpenoid	0.201 ± 0.005	0.417 ± 0.001	0.459 ± 0.014
Phenolic compounds	0.237 ± 0.002	0.576 ± 0.014	0.605 ± 0.008
Flavanoid	50.180±0.012	87.049±0.011	15.258±0.124
Steroid	0.251 ± 0.013	-	0.657 ± 0.031
Alkaloids	0.330 ± 0.006	0.579 ± 0.014	2.717 ± 0.021
Tannin	0.643 ± 0.003	0.951 ± 0.017	0.0062±0.0004
Saponin	0.553 ± 0.021	0.663 ± 0.024	0.304 ± 0.003

Table 4. Proximate composition of *Jacquemontia pentanthos*

Sl.no	Parameter	Unit	*Jacquemontia pentanthos*
1	Carbohydrate	mg glucose equivalent/g DW	0.38 ± 0.02
2	Ash	% dry weight	11.2 ± 0.5
3	Crude Fibre	mg/g dry weight	10.5 ± 0.3
4	Moisture	% fresh weight	60.0 ± 1.2
5	Protein	mg BSA equivalent/g DW	0.51 ± 0.03
6	Crude Fat	% dry weight	0.14 ± 0.01
7	Free Amino Acids	mg leucine equivalent/g DW	19.85 ± 0.6

Total ash content was 11.2 ± 0.5% dry weight, reflecting the mineral content and suggesting the presence of essential inorganic elements.

Crude protein content, estimated via the Lowry method using BSA as standard, was 0.51 ± 0.03 mg BSA equivalent/g DW. Free amino acids were19.85 ± 0.6 mg leucine equivalent/g DW, measured using the ninhydrin assay with leucine as standard. While the protein value is relatively low, it likely reflects extractable protein content rather than total protein in the leaf tissue. Total carbohydrates, measured by the Anthrone method, were 0.38 ± 0.02 mg glucose equivalent/g DW. The low yield of carbohydrates and proteins may be due to limited solubility in ethanol and the extraction-specific nature of the assay.

Crude fat content, extracted using the Soxhlet method and measured gravimetrically, was 0.14 ± 0.01% dry weight. As crude fat encompasses total lipid fractions, only the gravimetric crude fat value is presented here to avoid duplication.

Overall, *J. pentanthos* exhibits moderate moisture and mineral content, low crude fat and fiber and trace amounts of soluble proteins and carbohydrates. These values offer a preliminary insight into its nutritional profile, supporting its potential use as a functional food or phytopharmaceutical candidate. Further whole-leaf compositional analysis may better quantify total nutrient levels. These values are consistent with those of other leafy vegetables such as *Moringa oleifera* and *Amaranthus* species (32).

Quantitative phytochemical composition

Quantitative phytochemical analysis of *Jacquemontia pentanthos* leaf extracts revealed distinct variations in secondary metabolite content across the hexane, ethyl acetate and ethanol fractions. Tannins, expressed as tannic acid equivalents, were highest in the ethyl acetate extract (0.951 ± 0.017 mg TAE/g), moderate in hexane (0.643 ± 0.003 mg TAE/g) and present only in trace amounts in the ethanol extract (0.0062 ± 0.0004 mg TAE/g). The exceptionally low tannin level in the ethanol extract aligns with the absence of bitterness or astringency in traditional decoctions of *J. pentanthos* leaves used in southern Kerala, where the plant is occasionally consumed as a wild leafy vegetable or herbal tonic. The minimal tannin content may also lower potential anti-nutritional effects, improving its suitability for use in functional food and nutraceutical formulations.

Terpenoids, quantified using linalool as the standard, were found to be 0.201 ± 0.005 mg LE/g in the hexane extract, 0.417 ± 0.001 mg LE/g in ethyl acetate and 0.459 ± 0.014 mg LE/g in ethanol. Alkaloids, measured using atropine as the reference, were 0.330 ± 0.006, 0.579 ± 0.014 and 2.717 ± 0.021 mg AE/g in hexane, ethyl acetate and ethanol extracts, respectively showing a markedly higher concentration in the ethanol fraction. Steroids, expressed as diosgenin equivalents, were detected at 0.251 ± 0.013 mg DE/g in hexane and 0.657 ± 0.031 mg DE/g in ethanol, while absent in the ethyl acetate fraction.

Total flavonoid content, estimated using quercetin as the standard, varied widely among fractions 50.180 ± 0.012 mg QE/g in hexane, 87.049 ± 0.011 mg QE/g in ethyl acetate and 15.258 ± 0.124 mg QE/g in ethanol. The exceptionally high flavonoid content in the ethyl acetate extract likely contributes significantly to the plant's antioxidant potential. It is plausible that one or more major flavonoids or alkaloids serve as key radical scavengers, consistent with previous reports linking high flavonoid concentrations to enhanced antioxidant activity in medicinal plants (26, 28).

Phenolic compounds, determined using catechol as the standard, were 0.237 ± 0.002, 0.576 ± 0.014 and 0.605 ± 0.008 mg CE/g for the hexane, ethyl acetate and ethanol extracts, respectively. Saponin content was found to be 0.553 ± 0.021 mg SE/g in hexane, 0.663 ± 0.024 mg SE/g in ethyl acetate and 0.304 ± 0.003 mg SE/g in ethanol.

All assays were conducted in triplicate and results are presented as mean ± standard deviation (SD). Calibration curves for standard compounds (0.2-1.0 mg/mL) showed high linearity ($R^2 > 0.98$), confirming the accuracy, reproducibility and reliability of the quantification methods employed.

Antioxidant potential

The antioxidant potential of *Jacquemontia pentanthos* leaf extracts was evaluated using DPPH, ABTS and FRAP assays. All extracts exhibited concentration-dependent increase in radical scavenging activity. In the DPPH assay (Table 5, Fig. 1), The standard ascorbic acid showed an IC_{50} value of 0.316 ± 0.014 mg/mL, while the ethyl acetate, ethanol and hexane extracts exhibited IC_{50} values of 0.450 ± 0.017mg/mL, 0.558 ± 0.019 mg/mL and 0.762 ± 0.022 mg/mL, respectively. These findings suggest that the ethyl acetate extract possesses comparatively stronger antioxidant potential among the tested plant extracts, although its activity remains lower than that of the standard ascorbic acid. In the FRAP assay (Table 6, Fig.2), the ethanol extract exhibited the highest ferric reducing power, with a FRAP value of 0.134, which corresponded to 137.72±0.224µg Trolox equivalent. The ethyl acetate extract showed moderate reducing activity, with a FRAP value of 0.029 and a Trolox equivalent of 31.38±0.212µg. The hexane extract demonstrated the lowest FRAP activity, with a FRAP value of 0.015 corresponding to 17.61±0.114µg Trolox equivalent. These results indicate that the ethanol extract possesses the most significant reducing power, likely due to its higher concentration of phenolic and flavonoid

Table 6. FRAP Values and Trolox Equivalent Antioxidant Capacity of of *Jacquemontia pentanthos*

Sample	FRAP value	Trolox Concentration
Hexane extract	0.015	17.61306±0.114µg Trolox equivalent
Ethyl acetate extract	0.029	31.37633±0.212 µg Trolox equivalent
Ethanol extract	0.134	137.7202 ± 0.224 µg Trolox equivalent

Table 5. DPPH radical scavenging activity of ethanol, hexane and ethyl acetate extracts of *Jacquemontia pentanthos* at different concentrations compared to the standard (Ascorbic acid)

Concentration (mg/ml)	Average absorbance of standard	Average % radical scavenging activity of standard	Absorbance of ethanol extract (nm)	% Radical scavenging activity of ethanol extract	Average absorbance of hexane extract	Average % radical Scavenging activity of hexane extract	Average absorbance of EtOAc extract	Average % radical Scavenging activity of EtOAc extract
0.2	0.551	44.46	0.721	27.31	0.788	20.56	0.611	38.41
0.4	0.462	53.43	0.569	42.64	0.627	36.79	0.534	46.17
0.6	0.351	64.62	0.463	53.32	0.533	46.27	0.428	56.85
0.8	0.211	78.73	0.315	68.24	0.499	49.7	0.311	68.65
1	0.132	86.69	0.297	70.06	0.399	59.78	0.213	78.53

Concentration in mg mL^{-1}; absorbance measured at 517 nm (dimensionless); values are mean ± SD (n = 3). IC_{50} values were calculated from dose-response curves.

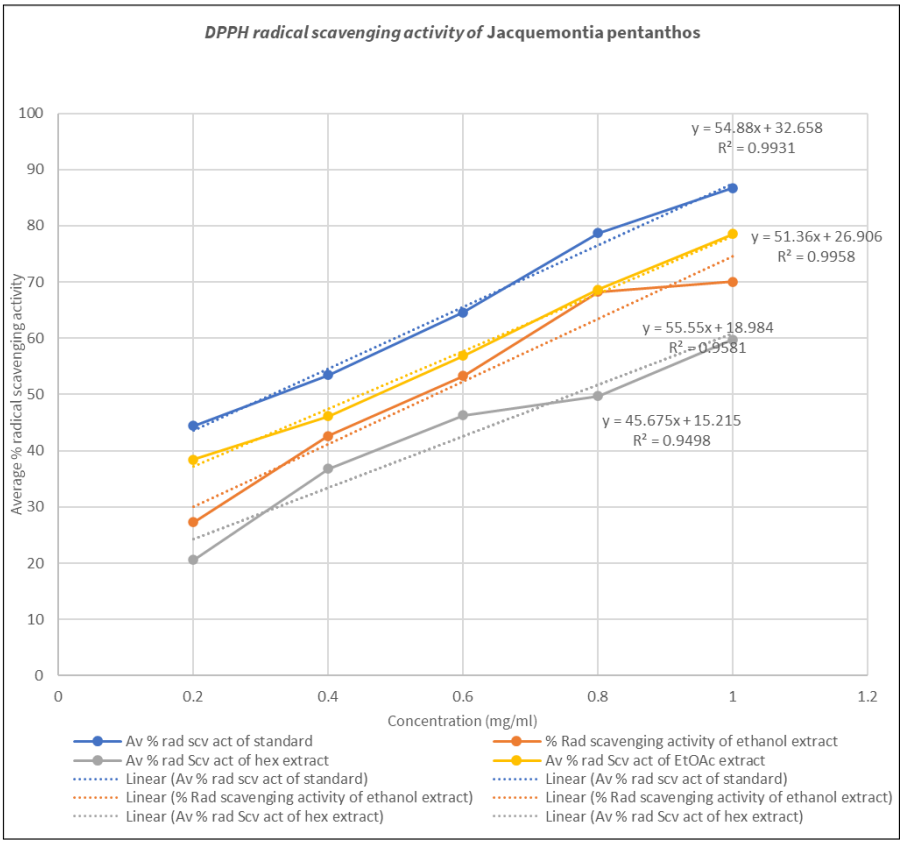

Fig. 1. DPPH radical scavenging activity of Standard, ethanol, hexane and ethyl acetate extracts of *Jacquemontia pentanthos.*

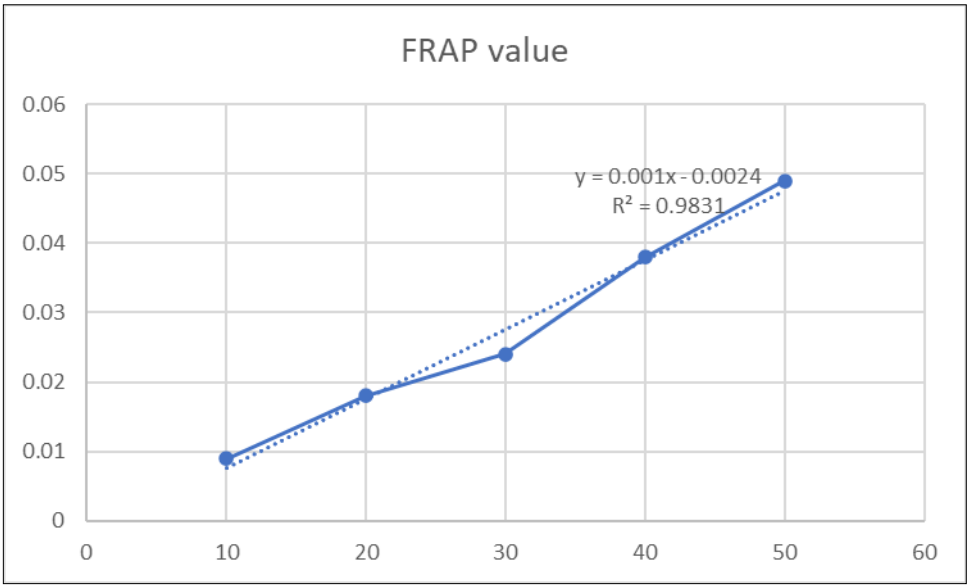

Fig. 2 Trolox standard curve for antioxidant estimation of *Jacquemontia pentanthos* in the FRAP assay.

compounds, which are known to contribute to antioxidant activity. Similarly, In the ABTS assay (Table 7, Fig 3) the ethyl acetate extract demonstrated the highest radical scavenging activity, followed by the hexane and ethanol extracts. Trolox, the standard, exhibited superior antioxidant potential at all concentrations. Trolox exhibited an IC_{50} of 0.431 ± 0.014 mg/mL, the ethyl acetate extract showed an IC_{50} of 0.538 ± 0.027 mg/mL, the hexane extract had an IC_{50} of 0.726 ± 0.031 mg/mL and the ethanol extract had an IC_{50} of 0.764 ± 0.029 mg/mL. These findings suggest that the ethyl acetate extract exhibits the most potent ABTS•⁺ radical scavenging activity among the extracts, approaching the efficacy of the Trolox standard.

Table 7. ABTS•⁺ radical scavenging activity of ethanol, hexane and ethyl acetate extracts of *Jacquemontia pentanthos* at different concentrations compared to the standard (Trolox)

Concentration (µg/ml)	Average absorbance of standard	Average % radical scavenging activity of standard	Absorbance of ethanol extract (nm)	%Rad scavenging activity of ethanol extract	Average absorbance of hexane extract	Average % radical Scavenging activity of hexane extract	Average absorbance of EtOAc extract	Average % radical Scavenging activity of EtOAc extract
0.2	0.598	34.36	0.744	18.33	0.711	21.95	0.601	34.03
0.4	0.451	50.49	0.651	28.54	0.632	30.63	0.533	41.49
0.6	0.361	60.37	0.598	34.35	0.514	43.58	0.412	54.77
0.8	0.254	72.12	0.422	53.67	0.422	53.68	0.374	58.95
1	0.174	80.9	0.311	65.86	0.314	65.53	0.214	76.51

Concentration in mg mL^{-1}; absorbance measured at 734 nm (dimensionless); values are mean ± SD (n = 3). IC_{50} values were calculated from dose-response curves.

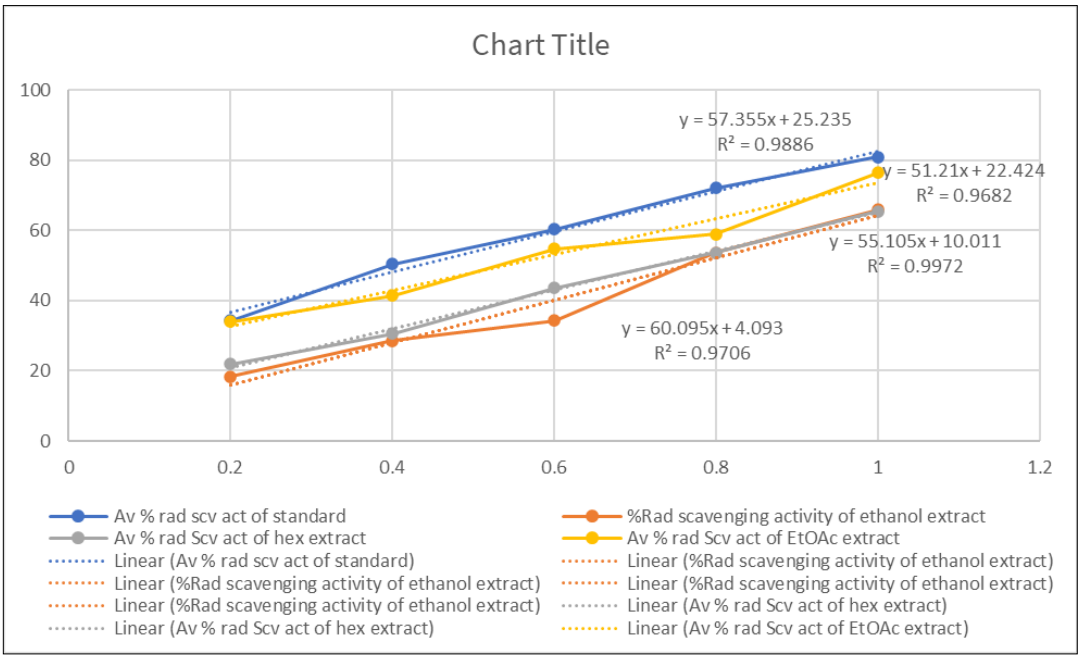

Fig. 3. ABTS•⁺ radical scavenging activity of standard, ethanol, hexane and ethyl acetate extracts of *Jacquemontia pentanthos.*

All antioxidant assays were performed in triplicate. IC_{50} values were derived from dose-response curves generated in Microsoft Excel by plotting percentage inhibition against concentration. The absorbance values of the control solutions (without extract) were 0.992and 0.911 at 517 nm and 734 nm for DPPH and ABTS assays, respectively. The consistency of replicates and statistically valid regression lines ($R^2 > 0.98$) enhance the credibility of the findings. Although no formal hypothesis testing (e.g., t-tests or ANOVA) was applied, the low standard deviations observed suggest good reproducibility of the results.

These results suggest that *J. pentanthos* is notably rich in antioxidant phytochemicals, especially flavonoids and phenolics and exhibits strong radical scavenging and ferric-reducing capabilities. This biochemical profile highlights its potential as a valuable source of natural antioxidants for nutraceutical and phytopharmaceutical applications.

Comparative evaluation within the genus Jacquemontia

In comparing the phytochemical composition and antioxidant potential of *Jacquemontia pentanthos* with other species in the genus, distinct differences are evident. The ethyl acetate extract of *J. pentanthos* exhibited a notably high flavonoid content of 87.049±0.011mg/g, which surpasses that reported in *J. tamnifolia* (10.6 mg/g extract), as documented (3). Similarly, phenolic content in *J. pentanthos* was measured at 0.605 ± 0.008mg/g, which is relatively lower than the values observed in *J. tamnifolia* (9.02 mg GAE/g) and *J. paniculata* (10.12 mg GAE/g), but within a comparable range to *J. caerulea* (8.5 mg GAE/g) (19-21). Despite lower phenolic values, *J. pentanthos* demonstrated stronger antioxidant efficacy, suggesting that flavonoids may play a dominant role in its radical scavenging capacity.

Alkaloid content in *J. pentanthos* (2.717 ± 0.021mg/g) was higher than the 1.1 % w/w reported in *J. tamnifolia* and both tannin and saponin contents were detectable, albeit in relatively lower concentrations. Remarkably, the antioxidant potential of *J. pentanthos* was evident in all three assays used in this study. The DPPH radical scavenging activity was particularly notable, with an IC_{50} value of 0.450 ± 0.017 mg/ml, which is significantly lower than that of *J. tamnifolia* (2.90 µg/mL) and even lower than most plant-based antioxidants documented in phytochemical literature (25). The ABTS assay further confirmed this trend, with an0.538 ± 0.027 mg/mL, while the FRAP assay showed a strong ferric reducing ability with137.72±0.224µg Trolox equivalent, exceeding the FRAP values reported for *J. paniculata* (104.5 µg TE) and *J. caerulea* (88.1 µg TE) (2, 4).With respect to nutritional constituents, *J. pentanthos* demonstrated moderate concentrations of protein (0.51 ± 0.03 mg BSA equivalent/g DW), carbohydrates (0.38 ± 0.02mg glucose equivalent/g DW) and amino acids (19.85 ± 0.6 mg leucine equivalent/g DW), although direct comparative values are not consistently available for other *Jacquemontia* species. The crude fat (0.14 ± 0.01% dry weight) and crude fibre (10.5 ± 0.3 mg/g dry weight) contents of *J. pentanthos* are comparable to those reported for *J. caerulea* (0.56% crude fat), further supporting its nutritional significance (2). These comparisons collectively highlight *Jacquemontia pentanthos* as a phytochemically rich and functionally promising species within its genus, with superior antioxidant potential and potential applications in nutraceutical or therapeutic formulations (3, 5).

Conclusion

This study provides a foundational investigation into the nutritional and antioxidant potential of *Jacquemontia pentanthos*, a relatively underexplored species with promising ethnobotanical relevance. The findings reveal that the plant is a rich source of essential macronutrients, including protein, carbohydrates and dietary fiber, along with moderate levels of crude fat and moisture content. The quantitative phytochemical screening confirmed the presence of bioactive compounds such as flavonoids, alkaloids, phenolics, saponins, terpenoids and tannins, while antioxidant assays (DPPH, ABTS and FRAP) demonstrated significant free radical scavenging capacity.

Despite these promising results, the pharmacological properties and specific mechanisms of action of the bioactive constituents remain largely unexplored. There is a clear research gap regarding the isolation, structural characterization and bioactivity validation of individual phytochemicals in *J. pentanthos*. Additionally, its toxicological safety profile and therapeutic efficacy *in vivo* require systematic evaluation to facilitate its potential development into functional foods or herbal formulations.

Future research should focus on detailed phytochemical profiling using advanced spectroscopic and chromatographic techniques to identify and characterize active compounds. Bioassay-guided fractionation and *in vivo* pharmacological studies are essential to validate the therapeutic claims and elucidate the mechanisms of action. Investigating the plant's anti-inflammatory, antimicrobial, anticancer, or metabolic regulatory activities could uncover novel applications. Moreover, integrating traditional knowledge with modern scientific validation can enhance the sustainable utilization of *Jacquemontia pentanthos* in nutraceutical and pharmaceutical industries.

Overall, this study not only highlights the nutritional and antioxidant promise of *Jacquemontia pentanthos* but also opens new avenues for its exploration as a plant-based resource for health and wellness.

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Authors' contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data. PC took part in drafting the article or revising it critically for important intellectual content. JL agreed to submit to the current journal, gave final approval of the version to be published and agree to be accountable for all aspects of the work. All the authors are eligible to be authors as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines. All the authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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