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Identifying food-based medicinal indigenous leafy vegetables and evaluating their bioactive compounds in the Ga-Molepo community of Limpopo Province

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Introduction: Indigenous leafy vegetables (ILVs) remain central to South African rural diets and ethnomedicine, yet their bioactive composition is insufficiently documented. This study identified ILVs used for nutritional and medicinal purposes in Ga-Molepo and assessed their phytochemical and antioxidant profiles.

Methods: A systematic ethnobotanical survey (n = 216) identified commonly used ILVs. Five species—*Bidens pilosa*, *Cleome gynandra*, *Crotalaria juncea*, *Tribulus terrestris*, *Amaranthus cruentus*—were analyzed for total carotenoids, phenolics, antinutritional factors, antioxidant activity (DPPH, ABTS, FRAP), and metabolite profiles using UHPLC-QTOF-MS.

Results: ILVs exhibited significantly higher carotenoid and phenolic contents than spinach. *B. pilosa* had the highest antioxidant capacity, while *C. juncea* contained the most carotenoids. Over 200 metabolites were detected, mainly flavonoids, with distinct species-specific clustering in PCA and OPLS-DA.

Discussion: Biochemical and metabolomic evidence strongly supports the traditional medicinal uses of ILVs. Species differ substantially in metabolite composition, suggesting varied therapeutic potential.

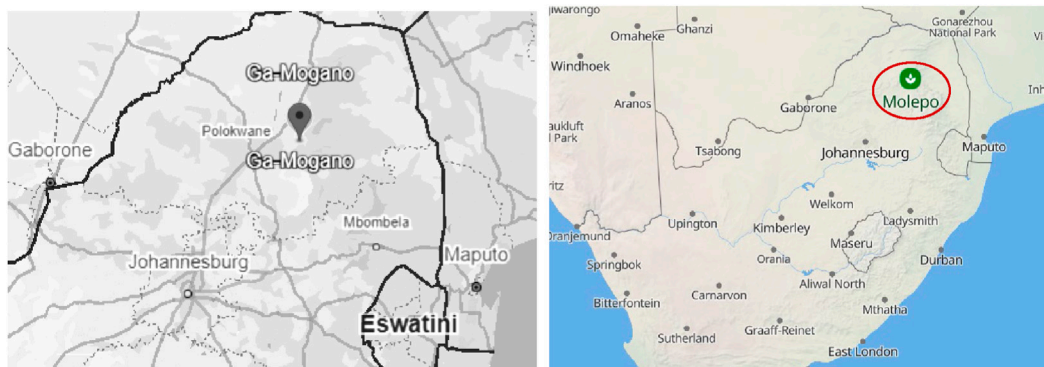
Conclusion: ILVs are nutritious, rich in bioactive compounds, and relevant for non-communicable disease prevention. Further studies should include quantitative metabolomics, micronutrient profiling, and clinical validation.

KEYWORDS

antinutritive factors, antioxidant activity, bioactive compounds, chemometrics, ethnobotany, indigenous leafy vegetables, metabolite profiling, UHPLC-QTOF

1 Introduction

Non-communicable diseases (NCDs) continue to impose a major public health burden in South Africa, contributing significantly to national mortality. Statistics South Africa reports that cardiovascular diseases, diabetes mellitus, chronic respiratory disorders, and cancers collectively account for a substantial proportion of annual deaths in the country (Stats SA, 2021; Stats SA, 2024). While global figures estimate that NCDs cause



GRAPHICAL ABSTRACT
Map of Ga Molepo, Ga-Mogano (google earth).

approximately 41 million deaths each year, national statistics provide more relevant insight into the disproportionate impact on rural and resource-limited communities. These areas often face restricted healthcare access, limited availability of chronic medications, and growing concerns regarding the long-term safety, affordability, and efficacy of synthetic drugs used to manage NCDs.

Within this context, indigenous leafy vegetables (ILVs) remain central to traditional food systems and ethnomedicinal practices in South Africa. ILVs—such as *Bidens pilosa*, *Cleome gynandra*, *Crotalaria juncea*, and *Amaranthus* species—are widely consumed and valued for their therapeutic properties, including anti-inflammatory, antioxidant, antidiabetic, and digestive benefits (Moyo et al., 2020; Mushapi et al., 2022). Their importance as food-based medicinal resources has been further emphasized by recent reviews. Netshiluvhi and Eloff (2025), for example, highlight the significant health-promoting effects of South African indigenous fruits and vegetables and argue for their inclusion as functional foods that may complement or enhance the management of chronic diseases. ILVs were specifically prioritised in the present study because they are more consistently available and more frequently consumed than most indigenous fruits, many of which are seasonal, region-specific, or underutilized.

ILVs are rich in bioactive compounds—particularly phenolic acids, flavonoids, and carotenoids—that play protective roles against oxidative stress and inflammation, both key drivers of NCD development (Mungofa et al., 2022; Aryal et al., 2019). Their accessibility, cultural embeddedness, and adaptability to local environments position them as promising dietary interventions in communities facing increasing NCD prevalence. Despite their demonstrated nutritional and medicinal relevance, ILVs used in the Ga-Molepo area have not been scientifically documented in sufficient detail. Existing studies have explored ILVs in other regions of South Africa (Managa et al., 2020a; Managa et al., 2020b; Sarkhel and Roy, 2022), but little research integrates ethnobotanical knowledge with phytochemical and metabolomic analyses at the community level.

This study therefore addresses a critical gap by combining ethnobotanical survey methods with laboratory-based biochemical and metabolomic profiling to characterize ILVs widely used in Ga-Molepo. This integrated approach provides

both cultural and scientific perspectives necessary for understanding the role of ILVs as food-based medicines and their potential contribution to managing NCD-related health challenges in rural South African setting.

Accordingly, the objective of this study was twofold: (a) to identify and document indigenous leafy vegetables (ILVs) consumed for nutritional and medicinal purposes in the Ga-Molepo area; and (b) to evaluate their bioactive compounds in line with ethnomedicinal uses in comparison with a reference vegetable (*Spinacia oleracea*, spinach).

2 Materials and methods

2.1 Study area

The ethnobotanical survey was conducted in Ga-Molepo, Ga-Mogano ($-24^{\circ}3'6''\text{S}$; $29^{\circ}44'48''\text{E}$), located in the Polokwane area of the Capricorn District Municipality, Limpopo Province, South Africa. The region has a hot semi-arid (steppe) climate and receives an average annual rainfall of 478 mm.

Laboratory analyses were performed at the University of Limpopo, Green Biotechnologies Research Centre of Excellence, within the Green Innovation Laboratory.

2.1.1 The survey

Participants for the ethnobotanical survey were selected using a systematic sampling method. A random starting point was chosen, after which four households were skipped; the fifth household was included in the sample. This interval ensured an adequate representation of the population, following the method described by Alemu et al. (2024).

The ethnobotanical survey included structured and semi-structured interviews guided by a questionnaire developed to document local knowledge of ILVs. High-level themes and example questions included:

1. Household ILV Usage
 - Which indigenous leafy vegetables are commonly consumed in your household?

○ How frequently are these vegetables eaten?

2. Medicinal Applications

- Which ILVs do you use for treating or preventing illnesses?
- What specific symptoms or ailments do these ILVs address?

3. Preparation Methods

- How are these ILVs prepared for consumption or medicinal use?

4. Knowledge Transmission

- From whom did you learn about the uses of these vegetables?
- Do you teach these uses to younger family members?

5. Availability and Seasonality

- Where are these ILVs harvested?
- Are there seasonal limitations affecting access?

These themes ensure replicability and transparency of the survey by emphasising the ethnobotanical, culinary, and medicinal dimensions of ILV use.

Ethical approval was obtained from the University of Limpopo TREC committee (TREC/112/2024: PG), and permission was granted by the Mogano tribal authority. Questionnaires were prepared in English and translated into Sepedi. Participation was voluntary, consent forms were signed before interviews, and no incentives were provided.

2.2 Number of participants in the study

In 2022, Ga-Molepo (Ga-Mogano) had an estimated 469 households. The sample size was determined using the formula by Althubaiti (2023):

Sample size:

$$n = N / (1 + N(e)^2)$$

$$n = 469 / 1 + 469(0.05)^2$$

$$n = 469 / 2.1725$$

$$n = 215.88 \text{ participants}$$

$$n = 216 \text{ participants}$$

Thus, a total of 216 participants were included in the study.

2.3 Plant collection

Following the survey, five indigenous leafy vegetables (ILVs) were selected based on their frequency index values. The frequency index (FI) was calculated as:

$$FI = \frac{FC}{N} \times 100$$

where FC = number of informants citing the species; N = total number of informants; Tangjang et al., 2011.

The selected ILVs were *Bidens pilosa* L., *Cleome gynandra* L., *Crotalaria juncea* L., *Tribulus terrestris* L., and *Amaranthus cruentus* L. Specimens collected from natural vegetation and home gardens

were taken to the University of Limpopo Herbarium for taxonomic confirmation. Each specimen was assigned a unique voucher number. Reference vegetable: spinach was purchased from a local market.

2.4 Preparation of plant extracts and justification for biochemical and phytochemical analyses

Plant materials were shade-dried at approximately 25 °C until constant mass, ground into powder, and stored at room temperature. Extracts of the ILVs and spinach were prepared by mixing 1 g of dry material with 10 mL of 80% aqueous methanol in sealed centrifuge tubes. Samples were vortexed, protected from light, and sonicated at room temperature for 60 min. They were then centrifuged for 10 min to separate the supernatant used for subsequent assays.

The decision to focus on total carotenoids, total phenolics, antioxidant activity, and antinutritive compounds was informed by their relevance to the ethnomedicinal uses reported by participants. Carotenoids and phenolics are widely recognised as key phytochemicals that exhibit antioxidant, anti-inflammatory, and protective effects against NCD-related oxidative stress. Antioxidant assays (DPPH, ABTS, FRAP) were selected to capture complementary mechanisms of antioxidant activity. Meanwhile, tannins and phytates were included because antinutritive factors influence the bioavailability of beneficial nutrients and are important for assessing ILVs intended for regular consumption. Other phytochemical classes and micronutrients were beyond the scope of the present study but are recommended for future work.

2.5 Total carotenoids assay

Following Kaseke et al. (2020), 0.2 g of each ILV sample was extracted with 2 mL hexane, followed by 0.5 mL of 0.5% NaCl. After vortexing and centrifugation at 1,500 rpm for 10 min, absorbance of the supernatant was measured at 460 nm. Total carotenoids were expressed as mg β-carotene per 100 g sample antioxidant assays.

2.6 Total phenolic contents assay

Total phenolic content (TPC) was determined using a modified Folin-Ciocalteu method (Apea-Bah et al., 2020). A standard curve (0–800 mg/mL gallic acid) was prepared. Each microplate well received 18.2 μL of extract or standard, 36.4 μL of 10% Folin-Ciocalteu reagent, and 145.4 μL of 700 mM sodium carbonate. After incubation in the dark for 2 h at ~25 °C, absorbance was measured at 750 nm. TPC was expressed as mg gallic acid equivalents (GAE) per 100 g dry weight.

Extracts (1 mL) from each ILV (in triplicate) were analyzed using UHPLC-QToF LC-MS (LC-MS-9030, Shimadzu). Chromatographic separation was performed on a Shim-pack Velox C₁₈ column (100 × 2.1 mm, 2.7 μm) at 55 °C with a 3 μL injection volume. Solvent A was Milli-Q water with 0.1% formic acid; solvent B was UHPLC-grade methanol. Negative electrospray ionization and data-dependent acquisition followed Makhumbila et al. (2022).

Data were exported as *mzML* files and processed using Sirius 6 and MetaboAnalyst, applying UPLC-QTOF parameters with the centWave algorithm. The analysis used a 5 ppm *m/z* deviation limit, signal-to-noise threshold of 7, prefilters of 3 peaks and intensity ≥ 700 , retention-time correction via obiwarp (profStep 0.5), and peak alignment requiring ≥ 0.5 sample fraction and grouping width of 0.025 *m/z*.

2.7 Antioxidant activity

The DPPH radical scavenging capacity was determined following Apea-Bah et al. (2020). A 60 $\mu\text{mol/L}$ DPPH solution was prepared by dissolving 1.2 mg of DPPH in 30 mL methanol. In each microplate well, 5 μL of sample extract or Trolox standard was mixed with 195 μL of DPPH solution. After 60 min of incubation in the dark, absorbance was measured at 515 nm. Results were expressed as μmol Trolox equivalents per gram dry weight ($\mu\text{mol TE/g}$).

The Ferric Reducing Antioxidant Power (FRAP) assay followed Bhengu et al. (2024). FRAP reagent consisted of acetate buffer (0.3 M, pH 3.6), ferric chloride (20 mM), and TPTZ (10 mM in hydrochloric acid). A homogenate was prepared using dried plant material mixed in a 4:1 methanol:water solution. The reaction was initiated by adding 50 μL of homogenate to 950 μL of pre-warmed FRAP reagent (37 °C). Antioxidant capacity was expressed as $\mu\text{mol TE/g}$ dry weight.

The ABTS radical cation decolorization assay was performed according to Apea-Bah et al. (2020). Stock solutions of potassium persulfate (6 mM) and ABTS were mixed and incubated in the dark for 12–16 h. The mixture was diluted 30-fold with 200 mM PBS to create the working solution. In each microplate well, 190 μL of ABTS solution was combined with 10 μL of diluted extract. After 60 min of incubation, absorbance was measured at 750 nm, and results were expressed as $\mu\text{mol TE/g}$ dry weight.

2.8 Antinutritive compounds

Tannin content was quantified using the vanillin-HCl method described by Li et al. (2023). Briefly, 0.2 g of the sample was extracted in 10 mL of 1% HCl via vortexing for 20 min, followed by centrifugation at $2,500 \times g$ for 5 min. A 50 μL aliquot of the supernatant was then reacted with vanillin-HCl reagent in a microplate and incubated at 30 °C for 20 min. Absorbance was measured at 500 nm, with results reported as mg/100 g. Catechin was used as the standard.

Phytate levels were determined using the method of Priya et al. (2025). Each 0.5 g ILV sample was extracted in 100 mL of 2.4% HCl. Wade's reagent (containing ferric chloride monohydrate and sulfosalicylic acid) was added to aliquots of the extract, followed by centrifugation. The supernatant was measured at 450–550 nm, and results were expressed as mg/100 g dry weight. Calcium phytate was used as the standard.

2.9 Statistical analysis

Survey data were analyzed descriptively using IBM SPSS Statistics version 29 (International Business Machines Statistical Package for the Social Sciences IBM SPSS, 2023; Mabala, 2018). Laboratory data were

subjected to analysis using Statistix 10.0. Means were separated using Least Significant Difference (LSD) test. Prior to multivariate analysis, the data were normally distributed and mean-centred. Principal Component Analysis (PCA) and heatmaps were generated using MetaboAnalyst 6.0. Specifically, HPLC data were processed through the Statistical Analysis module, where a custom colour scale was applied to the resulting heatmaps to improve visual clarity.

3 Results and discussion

3.1 Ethnobotanical survey

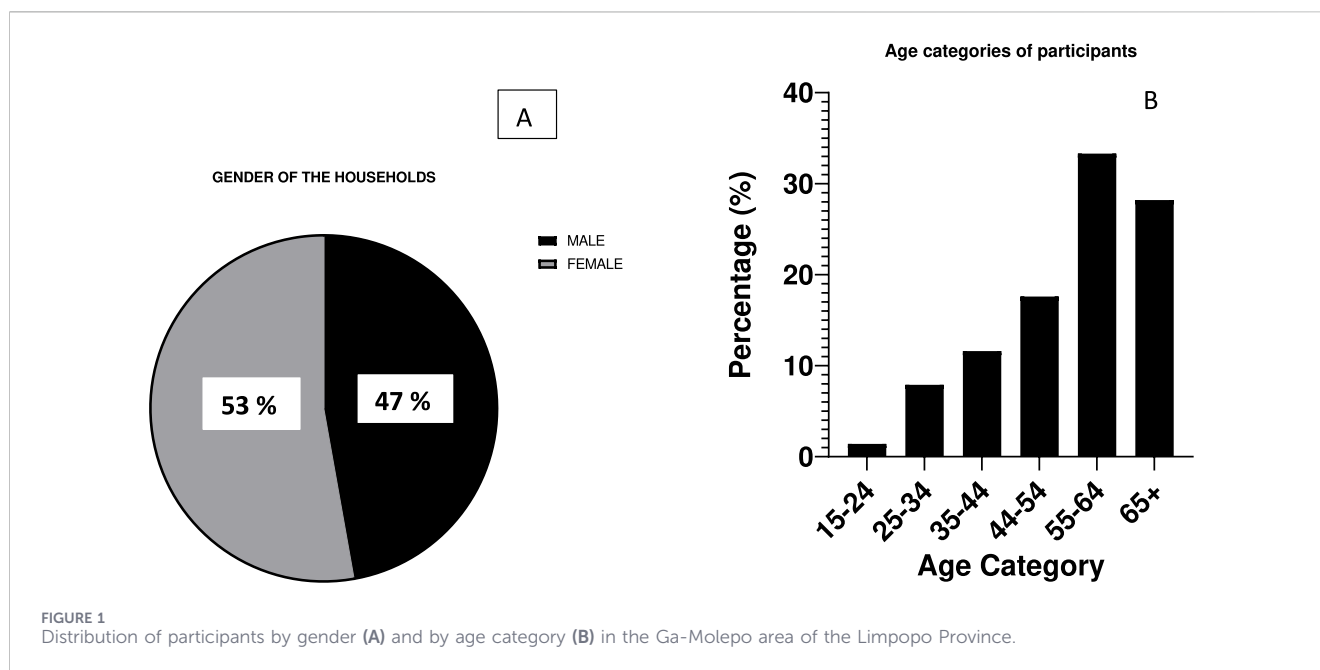
3.1.1 Demographic characteristics

A total of 216 participants were included in the study, of whom 53% were female and 47% male, as illustrated in Figure 1A. This aligns with Statistics South Africa (2021), which notes that most households in Limpopo Province are female-headed, with women often serving as custodians of indigenous knowledge and playing active roles in farming. The 2024 General Household Survey similarly reports that 47.0% of Limpopo households are female-headed, with the proportion increasing to 47.6% in rural areas. Figure 1B shows the age distribution of participants, indicating that the majority were between 55 and 64 years of age, while the fewest were aged 15–24 years. This trend suggests that older generations hold most of the indigenous knowledge and actively preserve it, whereas younger individuals tend to be less familiar with these systems and sometimes associate indigenous leafy vegetables (ILVs) with poverty and low socio-economic status (Qwabe and Pittawaty, 2023).

Participants' educational levels were relatively high (Figure 2A), with over 80% having attained at least primary education and 40% having completed secondary school. Education is known to shape socioeconomic status, health and nutrition awareness, comprehension of research questions, and willingness to participate (Ndhlovu et al., 2024).

The Ga-Molepo community maintains a rich repository of indigenous knowledge and continues to cultivate and consume ILVs for both nutritional and medicinal purposes. ILVs contribute to the treatment of ailments and supply essential nutrients due to their rich profiles of bioactive compounds. As shown in Figure 2B, 76.4% of participants consumed ILVs to treat or prevent illnesses, whereas 23.6% consumed them solely for nutritional purposes.

Frequency Index (FI) values for the documented ILVs ranged from 2.12 to 19.90. *Amaranthus cruentus* had the highest FI (19.90), followed by *Bidens pilosa* (12.51), *Cleome gynandra* (9.26), *Tribulus terrestris* (8.33), and *Crotalaria juncea* (6.48). *Pentarrhinum inspidum* had the lowest FI (2.12). The Amaranthaceae and Cucurbitaceae families were the most represented, each comprising two ILVs (Table 1). Many of these ILVs are traditionally used to treat inflammatory and skin-related conditions, consistent with existing literature indicating that ILVs contain phenolic acid, flavonoids, capable of neutralizing ROS free radicals through antioxidant activity and reducing inflammation (Mushaphi et al., 2022). Table 2 provides further details on the selected ILVs, including their scientific and common names, families, medicinal uses, and herbarium voucher numbers.



3.2 Phytonutrient composition and antioxidant activity

3.2.1 Total carotenoid content (TCC)

Significant variations ($p \leq 0.05$) in total carotenoid content (TCC) were observed (Figure 3A). The *C. juncea* had the highest TCC (30.99 mg/100 g DW), followed by *B. pilosa* (25.69 mg/100 g DW), *A. cruentus* (14.48 mg/100 g DW), *T. terrestris* (11.81 mg/100 g DW), and *C. gynandra* (4.11 mg/100 g DW). *S. oleracea* recorded the lowest TCC at 1.19 mg/100 g (DW). The TCC values in this study exceed those reported by Gowelee et al. (2019), who found values of 1.01–4.84 mg/100 g (DW). The TCC achieved in all species in from *B. pilosa*, *C. gynandra*, *C. juncea*, *T. terrestris* and *A. cruentus* ranging from 1.19 mg/100 g to 30.99 mg/100 g were lower than contents found in different African leafy vegetables including African nightshade, nightshade, green amaranth, red amaranth, collard leaf, cowpea leaf, pumpkin leaf and spider plant (Lugumira et al., 2025). However, contents of TCC found in *C. juncea* were higher than accession AT5 of the *A. tricolor* species (Sarker et al., 2022). Mallor et al. (2023) reported higher TCC from the chard and lettuce relative to the obtained results in this study. However, our results on TCC were relative to those reported on baby spinach and pea shoots microgreen (Bhengui et al., 2024; Selelepoo et al., 2025). However, the observed variation with other reports could be linked to different techniques used. Carotenoid stability is further influenced by structural factors, storage conditions, and processing methods (Van Jaarsveld et al., 2014; Liu et al., 2023).

3.2.2 Total phenolic content (TPC)

The TPC describes a broad spectrum of polyphenols contents available in the ILV, including phenolic acids, flavonoids, which are related to ILV antioxidant activity (Aryal et al., 2019). Significant

differences ($p \leq 0.05$) in total phenolic content (TPC) were observed among the ILVs (Figure 3B). *Bidens pilosa* had the highest TPC (792.97 mg GAE/100 g DW), followed by *C. gynandra* (694.85 mg GAE/100 g DW), *C. juncea* (692.59 mg GAE/100 g), *A. cruentus* (635.06 mg GAE/100 g DW), and *T. terrestris* (660.28 mg GAE/100 g DW). Although not significantly different from one another, these ILVs exhibited TPC values markedly higher than that of *S. oleracea* (423.78 mg GAE/100 g DW), representing a two-to three-fold reduction relative to ILVs. The TPC found in all the studied samples (*B. pilosa*, *C. gynandra*, *C. juncea*, *T. terrestris* and *A. cruentus*) was higher than the one reported on *Nasturtium officinale*, *Diplazium esculentum* and *Chenopodium album*, and lower than that found in *Urtica dioica* leafy vegetables (Sharma et al., 2022). Furthermore, these TPC contents in *B. pilosa*, *C. gynandra*, *C. juncea*, *T. terrestris* and *A. cruentus* were comparable relative to contents found in other African leafy vegetables including African nightshade, nightshade, green amaranth, red amaranth, collard leaf, cowpea leaf, pumpkin leaf and spider plant (Lugumira et al., 2025). Nguyen et al. (2023) reported extracts of *B. pilosa* to demonstrate peak efficacy in DPPH radical scavenging, α -amylase and α -glucosidase inhibition, and the prevention of albumin denaturation, thus suggesting its potential medicinal role. The TPC values recorded in this study exceed those reported by Moyo et al. (2020), who documented TPC values of 2.68–5.16 mg GAE/g fresh weight in similar vegetables. The TPC of *C. gynandra* determined here was notably higher than values reported in earlier studies. Conversely, Onyango et al. (2021) found higher TPC values (0.98–3.89 g/100 g); however, *B. pilosa* consistently ranked highest across studies. Previous research attributes several bioactive properties to *B. pilosa*, including antioxidant, anti-inflammatory, antidiabetic, anticancer, hepatoprotective, and immunomodulatory activities (Mtenga and Ripanda, 2022).

Phenolic compounds constitute a major class of secondary metabolites whose concentrations vary across and within ILV

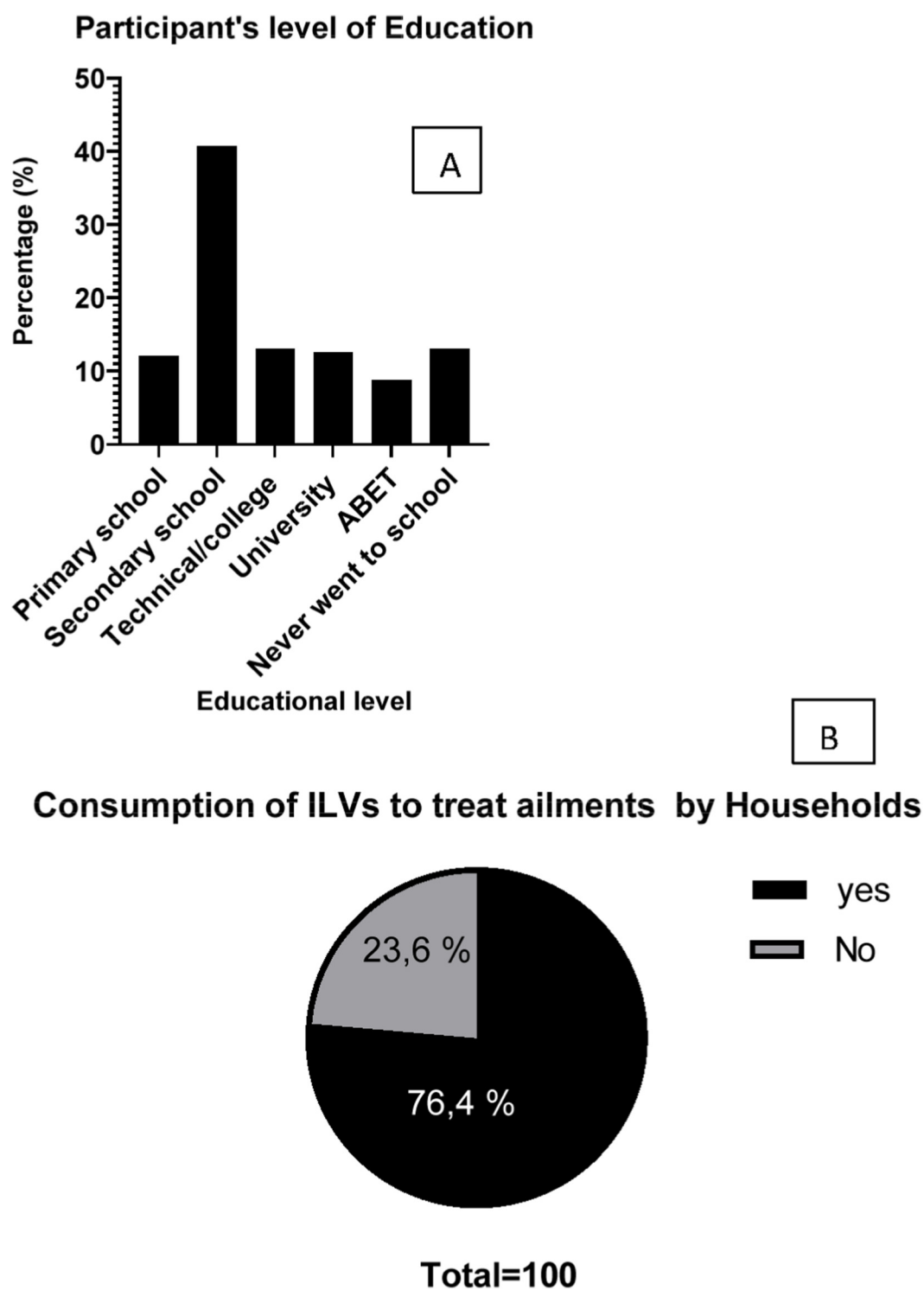


FIGURE 2
Percentage of participants by educational background (A) and proportion reporting consumption of indigenous leafy vegetables (ILVs) for treating ailments (B).

species (Robards et al., 1999; Ahlawat et al., 2024). These compounds demonstrate anticancer and anti-inflammatory activities and contribute to protection against chronic diseases such as diabetes mellitus and osteoporosis (Cuevas-Cianca et al., 2023; Oresanya

et al., 2025). They also influence nitric oxide bioavailability (Choudhary et al., 2015; Festa et al., 2024). Therefore, the inclusion of ILVs rich in phenolic in the diet may help reduce disease prevalence.

TABLE 1 Ethnobotanical information on indigenous leafy vegetables (ILVs) used as food-based medicine in the Ga-Molepo area of Limpopo Province, South Africa.

Common name	Local (Pedi) name	Scientific name	Family	Medicinal uses	Frequency index (FI)
Amaranth	Thepe	<i>Amaranthus hybridus</i> L.	Amaranthaceae	Treatment of diarrhoea, laxative, excessive menstruation	6.33
African heartvine	Mofotontsoane	<i>Pentarrhinum inspidum</i> E.	Asclepiadaceae	Treatment of skin conditions and inflammation	2.12
Black jack	Moshitji	<i>Bidens pilosa</i> L.	Asteraceae	Treatment of respiratory infections, wounds, digestive issues	12.51
Bitter gourd	Sebabi	<i>Momordica balsamia</i> L.	Cucurbitaceae	Treatment of skin ailments, diabetes, malaria	3.74
Devil's thorn	Tshehlo	<i>Dicerocaryum zanguebaricum</i> Lour.	Pedaliaceae	Treatment of inflammation and gum problems	5.68
Jute mallow	Thelele	<i>Corchorus tridens</i> L.	Tiliaceae	Treatment of reproductive issues, fever, pain	5.68
Narrow-leaved Vernonia	Lehlanye	<i>Vernonia fastigiata</i> O. and H.	Myrtaceae	Treatment of stomach pain and inflammation	6.38
Wild <i>Cucumis</i>	Monyaku	<i>Cucumis africanus</i> L.	Cucurbitaceae	Treatment of inflammation	2.62
Spider plant	Leroto	<i>Cleome gynandra</i> L.	Asparagaceae	Treatment of headaches, fever, joint inflammation	9.26
Rattle pods	Fore	<i>Crotalaria juncea</i> L.	Fabaceae	Treatment of skin conditions, anaemia, haemorrhage, menstrual disorders	6.48
Nightshade	Mofye	<i>Solanum retroflexum</i> Dunal	Solanaceae	Treatment of pain, wounds, inflammation	4.28
Red amaranth	Thepe	<i>Amaranthus cruentus</i> L.	Amaranthaceae	Aids digestion; anti-inflammatory	19.90
Puncture vine	Tshehlo	<i>Tribulus terrestris</i> L.	Zygophyllaceae	Treatment of infertility; controls cholesterol levels	8.33

TABLE 2 Ethnobotanical information on selected indigenous leafy vegetables (ILVs) used as food-based medicines in the Ga-Molepo area.

Family	Scientific name	Common name	Voucher specimen no.	Ailments treated	FI value
Capparaceae	<i>Cleome gynandra</i> L.	Spider plant (Leroto)	UNIN 1220288	Treatment of headaches, fever, joint inflammation	9.26
Fabaceae	<i>Crotalaria juncea</i> L.	Rattle pods (Fore)	UNIN 1220292	Treatment of skin conditions, anaemia, haemorrhage, menstrual disorders	6.48
Amaranthaceae	<i>Amaranthus cruentus</i> L.	Amaranth (Thepe)	UNIN 1220289	Aids digestion; anti-inflammatory	19.90
Zygophyllaceae	<i>Tribulus terrestris</i> L.	Puncture vine (Tshehlo)	UNIN 1220290	Treatment of infertility; helps control cholesterol levels; improves blood sugar	8.33
Asteraceae	<i>Bidens pilosa</i> L.	Black jack (Moshitsi)	UNIN 1220293	Treatment of respiratory infections, wounds, digestive issues	12.51

3.2.3 UHPLC-MS untargeted metabolites and multivariate analysis

More than 200 metabolites were identified across the six ILVs studied (Supplementary Table S1). These metabolites belong to several compound classes, including flavonoids, disaccharides, glycosides, esters, phenylpropanoids, and β glucosides, based on the identification generated from the obtained fragments, molar mass and chemical formulas (Supplementary Table S1). Table 3 lists the top 20 key metabolites contributing to sample similarities nor differences based on the multivariate analysis. As shown in Table 3, the majority of the detected metabolites fall within the flavonoid class.

To summarize the LC-MS untargeted metabolite profiling, chemometric analyses were conducted to illustrate similarities and differences in the overall metabolomic profiles of the studied ILVs (Mpai et al., 2022). Using data generated from UHPLC-QToF LC-MS, an unsupervised Principal Component Analysis (PCA) was applied (Figure 4) to reveal metabolite-level variation among the five ILVs. Five minor clusters corresponding to *A. cruentus*, *B. pilosa*, *C. juncea*, *T. terrestris*, and *C. gynandra* were observed, indicating that the composition of metabolite classes differs across species. Nonetheless, the PCA also grouped the vegetables into two major clusters: (*T. terrestris*, *C. juncea*, and *C. gynandra*) versus (*A. cruentus* and *B. pilosa*). This grouping is reflected in the horizontal PCA axis, which accounts for 38.1% of the overall

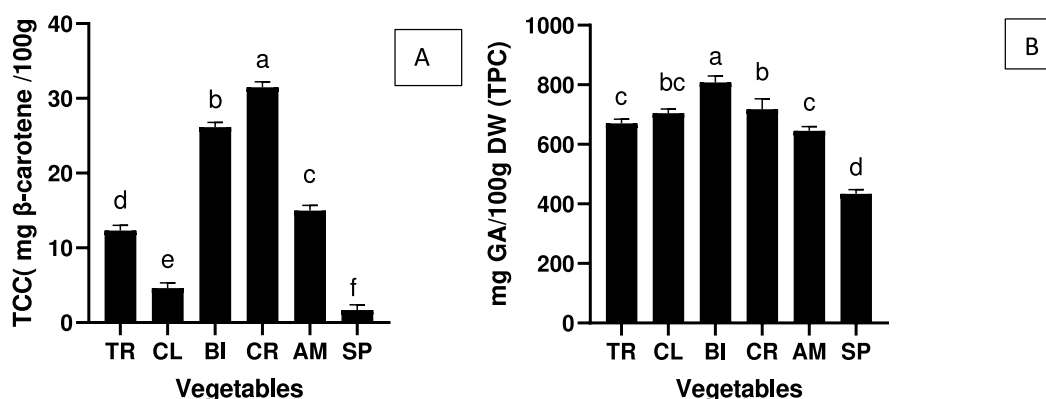


FIGURE 3
Total carotenoid content (A,B) Total phenolic content of selected leafy vegetables. Bars represent mean $\pm$ SE, and different letters indicate significant differences at $p \leq 0.05$. Vegetable codes: TR = *Tribulus terrestris*; CL = *Cleome gynandra*; BI = *Bidens pilosa*; CR = *Crotalaria juncea*; AM = *Amaranthus cruentus*; SP = *Spinacia oleracea*.

heterogeneity, while the vertical PCA explains an additional 26.5% of the variation.

A supervised scores plot (Figure 5) was also used to further separate the ILV clusters and illustrate metabolite-based relationships. Five distinct clusters—representing *A. cruentus*, *B. pilosa*, *C. gynandra*, *C. juncea*, and *T. terrestris*—are clearly visible, demonstrating pronounced metabolite differences among the ILVs. The horizontal axis of the supervised model explains 36% of the variation and likewise reveals two major groupings: (*C. juncea*, *T. terrestris*, and *C. gynandra*) versus (*A. cruentus* and *B. pilosa*).

Variable Importance in Projection (VIP) scores (Figure 6) were used to elucidate which metabolites contributed most to species separation. The metabolites with the strongest influence ($VIP > 1.0$) include typhaneoside, jaceidin, and reynoutrin, followed by isokaempferide, feruloyl malate, and santin. Metabolites with VIP scores below 1.0 exert a lesser impact on distinguishing the ILVs. These concentration patterns are visually reinforced in the biplot, where differences among species and their associated metabolites can be clearly observed.

The biplot (Figure 7) illustrates the relationships between the detected metabolites and the five ILVs. A clear separation of the species is evident, reflecting distinct metabolite distribution patterns across the vegetables. *Tribulus terrestris* is positioned separately from the other ILVs, primarily along the positive side of PC1, indicating a unique metabolite profile. In contrast, *Amaranthus cruentus* and *Bidens pilosa* are separated along the negative side of PC1. *C. juncea* and *C. gynandra* cluster closely together, demonstrating similarities in their metabolite composition, although each remains distinguishable as an individual species.

Regarding ILV–metabolite associations, *T. terrestris* shows a strong relationship with isokaempferide, jaceidin, and typhaneoside, as indicated by the arrows for these metabolites pointing in the same direction as the species cluster. This alignment suggests that these compounds occur at higher concentrations in *T. terrestris* relative to the other ILVs. *A. cruentus* is most closely associated with santin, feruloyl malate, and reynoutrin, reflecting their elevated levels in this species.

Cleome gynandra demonstrates strong associations with multiple metabolites, including santin, isokaempferide, jaceidin, and

typhaneoside. These relationships are supported by the VIP scores (Figure 6), which identify these compounds as key contributors to species separation. *Bidens pilosa* and *C. juncea* are predominantly associated with quercetin-3-O-hexosyl-(1-2)-deoxyhexosyl-7-O-hexoside, tiliroside, and schaftoside, indicating that these flavonoid derivatives occur at comparatively higher concentrations in these two ILVs. Metabolites pointing in similar directions in the biplot—for example, quercetin-3-O-hexosyl-(1-2)-deoxyhexosyl-7-O-hexoside and tiliroside—are positively correlated. Conversely, metabolites oriented in opposite directions, such as isokaempferide and feruloyl malate, exhibit negative correlations, reflecting contrasting concentration patterns across the ILVs.

Box-and-whisker plots (Figure 8) showed notable variation in metabolite concentrations across ILVs in both original and normalized datasets. *C. juncea* and *C. gynandra* exhibited the highest levels of quercetin-3-O-hexosyl-(1-2)-deoxyhexosyl-7-O-hexoside (Figure 8A). Normalized tiliroside concentrations were highest in *C. gynandra*, followed by *A. cruentus* and *B. pilosa* (Figure 8B). *C. juncea* had the highest concentrations of quercetin methoxy-7-O-hexosyl-deoxyhexoside (Figure 8C). Schaftoside was highest in *C. gynandra* after normalization (Figure 8D). Feruloyl malate normalized concentrations were highest in *C. gynandra* (Figure 8E). Similarly, reynoutrin was highest in *C. gynandra*, followed by *A. cruentus* (Figure 8F). Santin was abundant in *C. gynandra*, while normalized values peaked in *A. cruentus* (Figure 8G). Isokaempferide levels were highest in *C. gynandra* and *T. terrestris* before normalization but were highest in *C. juncea* after normalization (Figure 8H). Typhaneoside exhibited markedly increased normalized levels in *C. gynandra* (Figure 8I).

3.2.4 Antioxidant capacity (DPPH, ABTS, and FRAP assays)

Antioxidant activities of the leafy vegetables, as assessed using the DPPH, ABTS, and FRAP assays, showed significant differences ($p \leq 0.05$). In the DPPH radical scavenging assay (Figure 9A), *B. pilosa* exhibited the highest activity (72.45 $\mu\text{mol TE/g DW}$), followed by *C. gynandra* (53.97 $\mu\text{mol TE/g DW}$). No significant differences

TABLE 3 Tentative LC–MS identification of metabolites detected in the five indigenous leafy vegetables (ILVs).

A. <i>cruentus</i>	T. <i>terrestris</i>	C. <i>gynandra</i>	B. <i>pilosa</i>	C. <i>juncea</i>	Rt (min)	m/z	Compound name	Molecular formula	Compound class
X	X	X	X	X	1.15	387.1136	Turanose	C ₁₂ H ₂₂ O ₁₁	Disaccharide
X	X	X	X	X	7.51	771.1976	Quercetin-3-O-hexosyl(1-2)-deoxyhexosyl-7-O-hexoside	C ₃₃ H ₄₀ O ₂₁	Flavonoid
X	X	X	X	X	10.23	593.1495	Nicotiflorin	C ₂₇ H ₃₀ O ₁₅	Flavonoid
ND	X	X	X	X	12.91	299.0551	Isokaempferide	C ₁₅ H ₁₀ O ₆	Flavonoid
ND	ND	ND	X	X	13.80	343.0813	Santin	C ₁₈ H ₁₆ O ₇	Flavonoid
X	X	X	X	X	8.01	593.15	Vicenin-2	C ₂₇ H ₃₀ O ₁₅	Flavonoid
X	X	X	X	X	9.78	447.0917	Trifolin	C ₂₁ H ₂₀ O ₁₁	Flavonoid
X	X	X	X	X	11.81	593.1293	Tiliroside	C ₃₀ H ₂₆ O ₁₃	Flavonoid
X	X	X	X	X	22.29	311.1674	Thymol-β-D-glucoside	C ₁₆ H ₂₄ O ₆	Glycoside
ND	X	X	ND	X	9.32	769.2178	Typhaneoside	C ₃₄ H ₄₂ O ₂₀	Flavonoid glycoside
X	X	X	X	X	8.34	303.0501	Taxifolin	C ₁₅ H ₁₂ O ₇	Flavonoid
X	X	X	X	X	9.32	609.1454	Rutin	C ₂₇ H ₃₀ O ₁₆	Polyphenol/ Flavonoid glycoside
X	X	ND	X	X	9.58	433.1128	Reynoutrin	C ₂₀ H ₁₈ O ₁₁	Flavonoid
X	X	X	X	X	8.57	563.1392	Schaftoside	C ₂₆ H ₂₈ O ₁₄	Flavonoid
X	X	X	X	X	24.11	325.1161	(4E)-7-acetyloxy-6-hydroxy-2-methyl-10-oxo-2,3,6,7,8,9-hexahydrooxecin-3-yl (E)-but-2-enoate	C ₁₆ H ₂₂ O ₇	Macrocyclic/Ester
X	X	ND	X	X	8.72	309.0605	Feruloyl malate	C ₁₄ H ₁₄ O ₈	Phenylpropanoid (hydroxycinnamic acid derivative)
X	X	X	X	X	9.98	—	Kaempferol-3-O-rutinoside	C ₂₇ H ₃₀ O ₁₅	Flavonoid glycoside
X	X	X	X	X	12.05	—	Methoxy-quercetin-7-O-hexosyl-deoxyhexoside	C ₃₃ H ₄₀ O ₂₁	Flavonoid
ND	X	X	X	X	2.96	—	Gentisic acid 5-O-β-glucoside	C ₁₃ H ₁₆ O ₉	β-glucoside
ND	X	X	X	X	12.95	359.0764	Jaceidin	C ₁₈ H ₁₆ O ₈	Flavonoid

X = detected; ND = not detected.

were observed among *T. terrestris* (33.78 μmol TE/g DW), *A. cruentus* (reported values: 3.00 and 3.30 μmol TE/g DW), and *C. juncea* (32.80 μmol TE/g DW). *Spinacia oleracea* recorded the lowest DPPH scavenging activity (18.32 μmol TE/g DW). Results found on *B. pilosa* DPPH scavenging activity were similar to those reported on sweet potato leaves variety GSC_2 (Jia et al., 2022). Furthermore, the scavenging activity on *C. gynandra* were comparable to the those of *A. cruentus* species and higher than the activity found on *Coriandrum sativum* L. (Swain et al., 2024).

Similarly, the ABTS assay (Figure 9B) showed significant variation among the vegetables. *A. cruentus* (27.86 μmol TE/g DW) and *B. pilosa* (27.43 μmol TE/g DW) demonstrated the highest ABTS radical cation scavenging activities. Intermediate ABTS activities were observed for *C. gynandra* (24.29 μmol TE/g DW), *C. juncea* (21.88 μmol TE/g DW),

and *T. terrestris* (15.13 μmol TE/g DW). Again, *S. oleracea* showed the lowest activity (5.88 μmol TE/g DW). The ABTS activity in this *B. pilosa*, *C. gynandra*, and *C. juncea* were higher than the activity found on nightshade, and comparable to the activity of minimally processed (blanching) samples (Managa et al., 2020a). Furthermore, comparing with the Chinese cabbage, the ABTS activity in all the studied vegetables in this study showed higher activity than reported activity of Chinese cabbage (Managa et al., 2020b).

Results from the FRAP assay (Figure 9C) followed a similar trend. *B. pilosa* exhibited the highest ferric reducing antioxidant power (7.46 μmol TE/g DW), followed by *C. juncea* (5.39 μmol TE/g DW), *A. cruentus* (3.11 μmol TE/g DW), *C. gynandra* (2.78 μmol TE/g DW), and *T. terrestris* (2.11 μmol TE/g DW). *S. oleracea* again showed the lowest reducing capacity (0.22 μmol TE/g DW).

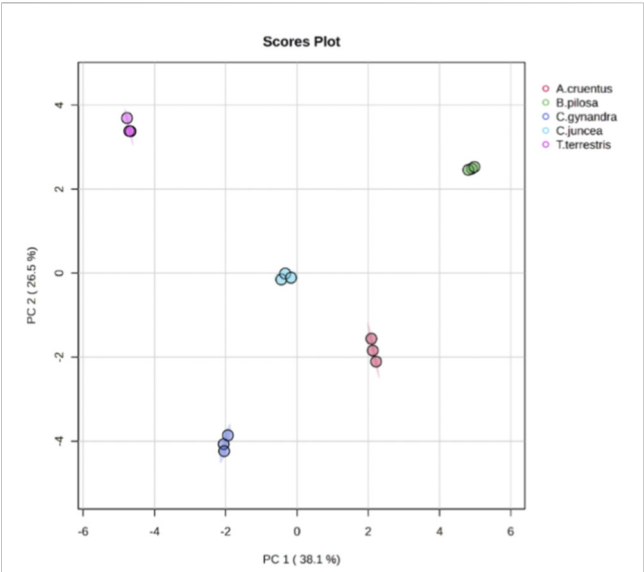


FIGURE 4
Principal component analysis (PCA) showing metabolite-based variation among the five indigenous leafy vegetables.

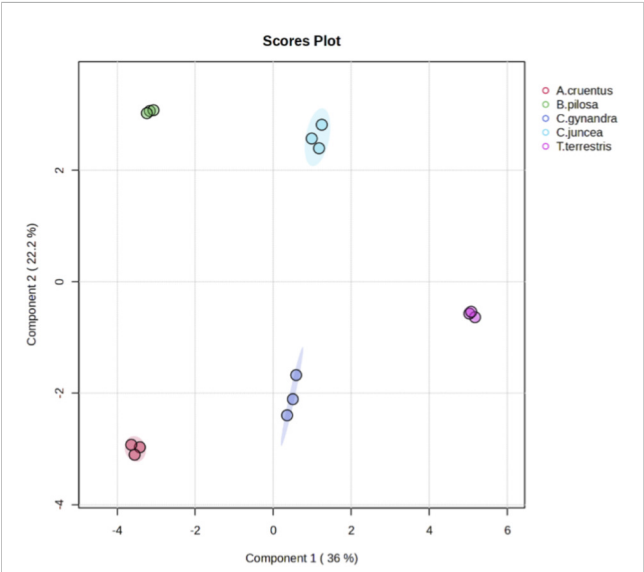


FIGURE 5
OPLS-DA scores plot illustrating the metabolic differentiation among the five indigenous leafy vegetables.

Indigenous leafy vegetables are known to exhibit high antioxidant activities, particularly during early growth stages (Cheptoo et al., 2019). This enhanced activity is often linked to their adaptation to local environmental conditions, which may stimulate the synthesis of bioactive compounds (Mungofa et al., 2022). Antioxidants are essential for human health, as they inhibit or delay oxidative reactions and reduce oxidative stress associated with chronic diseases such as hypertension, neurodegenerative disorders, and cancer (Apak, 2019; Muscolo et al., 2024).

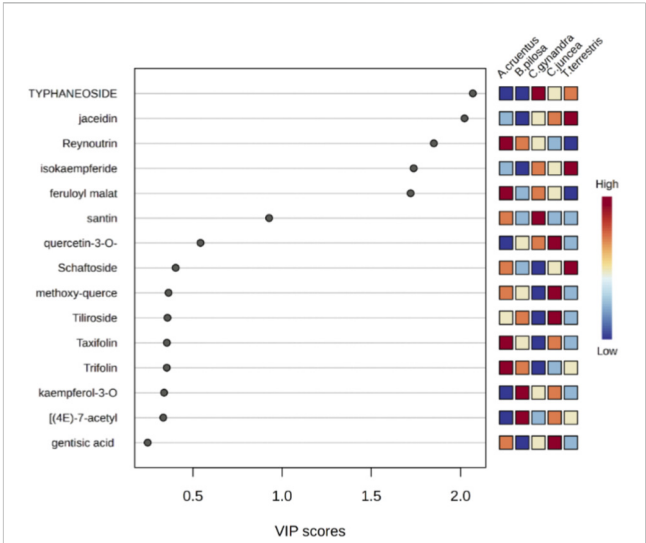


FIGURE 6
Variable Importance in Projection (VIP) scores for the five indigenous leafy vegetables and their detected metabolites. The VIP plot highlights metabolites that contribute most to species discrimination in the OPLS-DA model, with higher scores indicating greater influence on group separation.

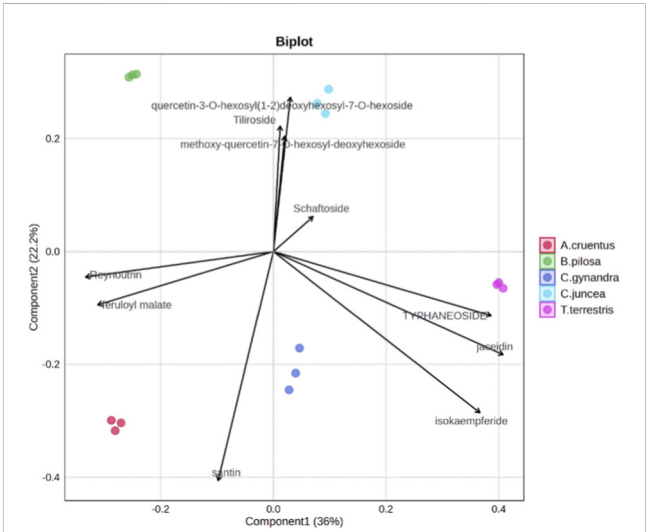


FIGURE 7
Biplot illustrating the relationships between the five indigenous leafy vegetables and their detected metabolites. The biplot displays species–metabolite associations based on UHPLC–QTOF–MS data, showing how metabolites contribute to the separation of *Amaranthus cruentus*, *Bidens pilosa*, *Cleome gynandra*, *Crotalaria juncea*, and *Tribulus terrestris*. Arrows indicate the direction and magnitude of metabolite influence, while clustering reflects similarities and differences among species' metabolic profiles.

The observed variability across the antioxidant assays may be attributed to differences in assay principles and antioxidant specificities. The DPPH assay measures free radical scavenging via reduction of a stable free radical, FRAP quantifies reducing power through the conversion of ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions,

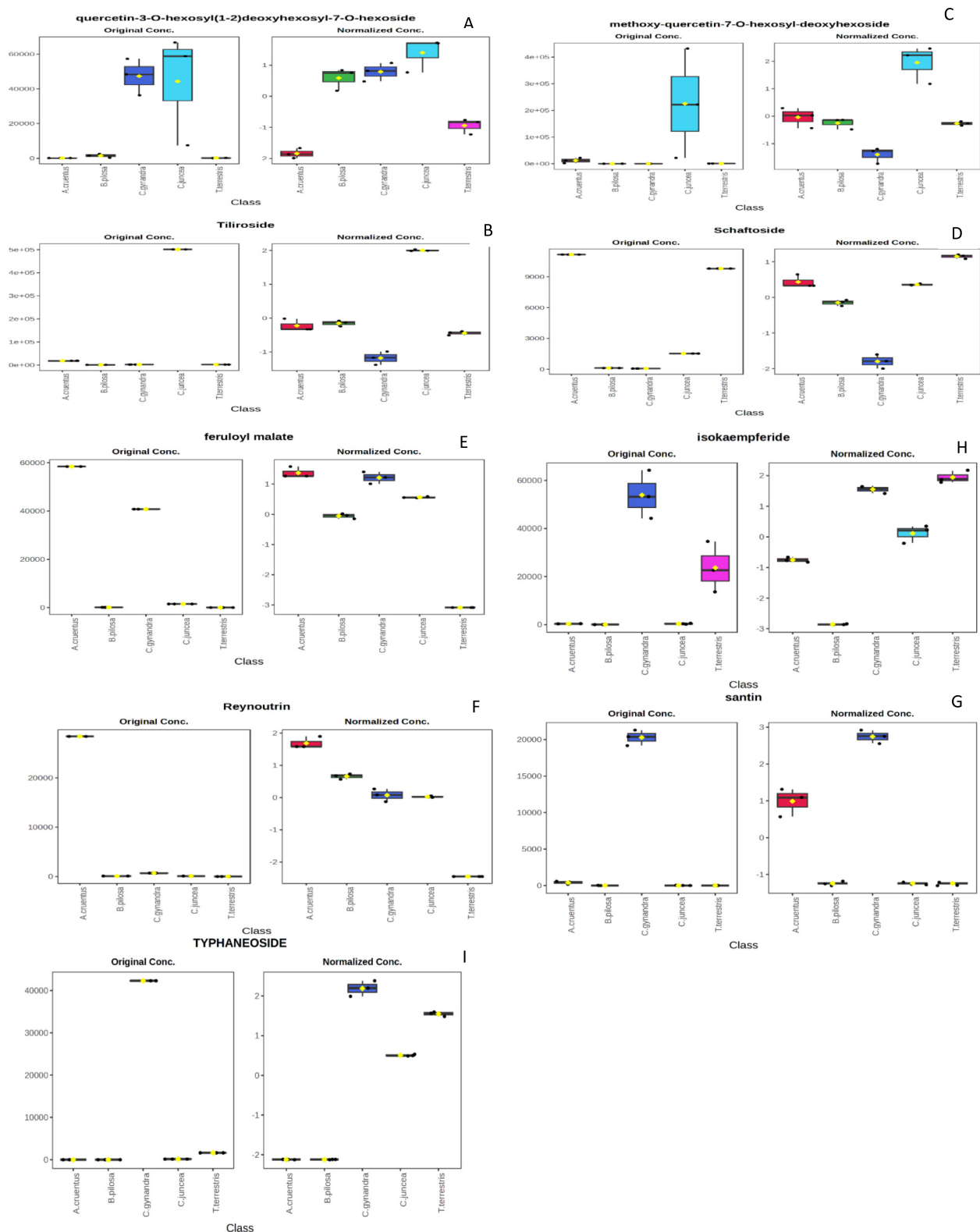


FIGURE 8

Box-and-whisker plots (A–I) showing concentrations of key metabolites across five ILVs: (A) quercetin-3-O-hexosyl-(1-2)-deoxyhexosyl-7-O-hexoside; (B) tiliroside; (C) quercetin-methoxy-7-O-hexosyl-deoxyhexoside; (D) schaftoside; (E) feruloyl malate; (F) reynoutrin; (G) santin; (H) isokaempferide; (I) typhaneoside. Values represent normalized UHPLC-QTOF-MS data; boxes show IQR, whiskers show variability, outliers are individual points.

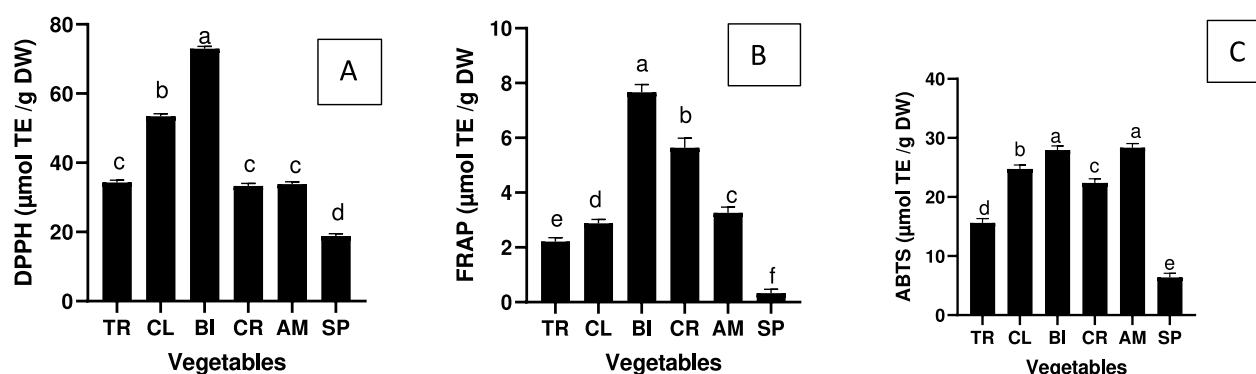


FIGURE 9 Antioxidant activity of indigenous leafy vegetables and spinach as measured by DPPH (A), ABTS (B), and FRAP (C) assays. Bars represent mean $\pm$ SE, and different letters indicate significant differences at $p \leq 0.05$. Vegetable codes: TR = *Tribulus terrestris*; CL = *Cleome gynandra*; BI = *Bidens pilosa*; CR = *Crotalaria juncea*; AM = *Amaranthus cruentus*; SP = *Spinacia oleracea*.

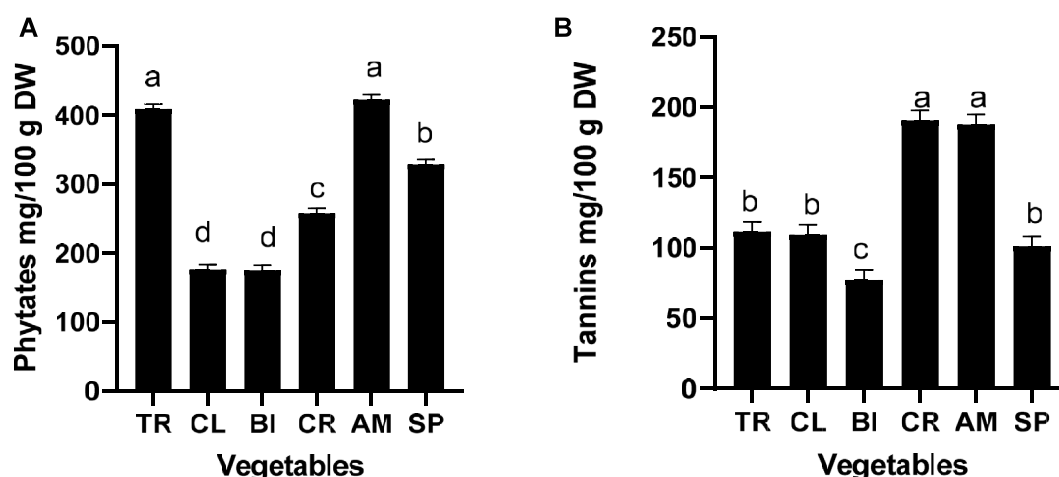


FIGURE 10 Phytate content (A) and tannin content (B) of the leafy vegetables analyzed. Bars represent mean $\pm$ SE, and different letters indicate significant differences at $p \leq 0.05$. Vegetable codes: TR = *Tribulus terrestris*; CL = *Cleome gynandra*; BI = *Bidens pilosa*; CR = *Crotalaria juncea*; AM = *Amaranthus cruentus*; SP = *Spinacia oleracea*.

and the ABTS assay assesses the ability of antioxidants to scavenge a pre-formed ABTS radical cation (Nurhayati et al., 2024). Consequently, DPPH primarily evaluates free radical scavenging capacity, FRAP reflects overall reducing potential, and ABTS provides a broader measure of radical scavenging activity (Nurhayati et al., 2024). Given these methodological differences, the use of multiple antioxidant assays is recommended to achieve a comprehensive evaluation of antioxidant potential (Chaves et al., 2020; Nurhayati et al., 2024). Furthermore, DPPH, FRAP, and ABTS assays differ significantly in their solvent compatibility and, consequently, their capacity to measure lipophilic or hydrophilic antioxidants (Danet, 2021). DPPH is hydrophobic (soluble in organic solvents) and best for lipophilic compounds, while FRAP is hydrophilic (aqueous-based) and suited for water-soluble compounds, with ABTS offering an amphiphilic nature that measures both types (Danet, 2021).

3.2.5 Antinutritional compounds

Antinutritional compounds represent an important class of bioactive constituents because they can interfere with the absorption and bioavailability of essential nutrients (Dahanayaka et al., 2026). The antinutritional components of the indigenous leafy vegetables (ILVs) and the reference spinach (*S. oleracea*) are presented in Figure 10, with specific focus on tannins and phytates.

Tannin concentrations in the examined ILVs (Figure 10A) ranged from 72.33 to 185 mg/100 g dry weight (DW). *B. pilosa* exhibited the lowest tannin content among all vegetables assessed. In contrast, *C. juncea* (185.67 mg/100 g DW) and *A. cruentus* (182.67 mg/100 g DW) showed the highest tannin levels, and these two did not differ significantly from each other. Intermediate tannin contents were recorded in *T. terrestris* (106.50 mg/100 g DW), *C. gynandra* (104.42 mg/100 g DW), and *S. oleracea* (96.25 mg/100 g DW).

Overall, the tannin concentrations found in the current study fall below those previously reported by [Abdi et al. \(2022\)](#), who observed tannin levels ranging from 138.27 to 531.97 mg/100 g DW in leafy vegetables. Condensed tannins are known to impair nutrient digestion and restrict the bioavailability of beneficial compounds ([Fonseca et al., 2023](#); [Zayed et al., 2025](#)). They exert these antinutritional effects by binding to proteins, minerals—particularly iron—and other nutrients, thereby reducing absorption and potentially inhibiting digestive enzymes ([Zayed et al., 2025](#)). The maximum tolerable limit for tannins in foods has been reported as 560 mg/100 g ([Ertop and Bektas, 2018](#); [Abdi et al., 2022](#)).

Phytate concentrations, also assessed as key antinutritional factors ([Figure 10B](#)), ranged from 170.25 to 417.75 mg/100 g DW. The highest phytate levels were recorded in *T. terrestris* (404.00 mg/100 g DW) and *A. cruentus* (417.75 mg/100 g DW), and these values did not differ significantly from each other. In contrast, *C. gynandra* (171.08 mg/100 g DW) and *B. pilosa* (170.25 mg/100 g DW) exhibited the lowest phytate concentrations among the vegetables assessed. Phytate content in *S. oleracea* (323.50 mg/100 g DW) was moderate and comparable to that of *C. juncea* (252.75 mg/100 g DW). Phytic acid is often criticized due to its strong chelating ability; it forms complexes with essential minerals such as iron, zinc, calcium, and magnesium in the gastrointestinal tract ([Sarkhel, and Roy, 2022](#)). These complexes are poorly absorbed, thereby reducing mineral bioavailability and potentially contributing to deficiencies, especially in populations that rely heavily on plant-based diets ([Nissar et al., 2017](#); [Zayed et al., 2025](#)).

3.3 Study limitations

Several limitations should be acknowledged. First, the study did not include micronutrient profiling (e.g., minerals and vitamins), which may provide further insight into the nutritional value of the ILVs. Second, untargeted metabolite profiling was non-quantitative, and not all compounds were confirmed using MS/MS fragmentation, limiting the certainty of metabolite identification. Third, total flavonoid content (TFC), a commonly used indicator of antioxidant potential, was not measured. Fourth, bioactivity assays focused only on antioxidant capacity; other pharmacological activities relevant to ethnomedicinal uses were not tested. These limitations should be addressed in future studies integrating quantitative metabolomics, pharmacological assays, and clinical validation.

4 Conclusion

This study documents the continued reliance on indigenous leafy vegetables as food-based medicines in the Ga-Molepo community and characterises five frequently used ILVs for their bioactive compounds. The combined ethnobotanical, phytochemical, and metabolomic approach demonstrates that ILVs such as *Bidens pilosa*, *Cleome gynandra*, and *Crotalaria juncea* possess high levels of phenolics, carotenoids, and antioxidant activity, supporting their traditional medicinal uses. While these findings underline the nutritional and therapeutic relevance of ILVs, further research is required to quantify micronutrients, confirm metabolite identities using advanced MS/MS techniques, and conduct targeted pharmacological and clinical investigations. Strengthening evidence for ILVs can contribute to

the development of nutraceuticals and sustainable dietary interventions against non-communicable diseases.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary Material](#), further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by University of Limpopo TREC committee (TREC/112/2024: PG). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

ML: Conceptualization, Investigation, Data curation, Formal Analysis, Methodology, Writing – original draft. SM: Data curation, Supervision, Writing – review and editing. TB: Supervision, Writing – review and editing. NM: Methodology, Writing – review and editing. AN: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review and editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/frfst.2026.1796647/full#supplementary-material>

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