Natural Resources for Human Health

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Review

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Received 20 March 2026
Revised 26 May 2026
Accepted 20 July 2026
Available Online 28 August 2026

Edited by Prathapan Ayyappan

KEYWORDS:
Grewia monticola
Phytochemicals
Antioxidant activity
Antibacterial activity
α-Amylase inhibition
Organ-specific bioactivity

Natr Resour Human Health 2026; 6 (4): 530–541
https://doi.org/10.53365/nrfhh/226139
eISSN: 2583-1194

Organ-specific antioxidant, antibacterial, and α-amylase inhibitory activities of hydroethanolic extracts from *Grewia monticola* Sond

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ABSTRACT: Plants of the genus *Grewia* are widely used in traditional medicine and are recognized as important sources of bioactive secondary metabolites. However, the biological potential of Grewia monticola remains poorly explored. This study investigated the phytochemical composition, antioxidant capacity, antibacterial activity, and α-amylase inhibitory potential of hydroethanolic extracts obtained from the leaves, stem, and roots of *Grewia monticola*. Qualitative phytochemical screening and spectrophotometric quantification of total phenolic compounds (TPC), total flavonoid content (TFC), and total tannin content (TTC) were performed. Antioxidant activity was evaluated using DPPH radical scavenging, ferric reducing power (FRP), and phosphomolybdenum total antioxidant capacity (TAC) assays. Antibacterial activity was assessed by the disk diffusion method against Staphylococcus aureus, Salmonella typhimurium, Escherichia coli, and Enterococcus faecalis. α-Amylase inhibitory activity was determined using the 3,5-dinitrosalicylic acid (DNS) method. The extracts contained phenolics, flavonoids, and tannins with clear organ-specific distribution. Leaves exhibited the highest phenolic content, whereas stem extracts contained the highest levels of flavonoids and tannins. All extracts showed concentration-dependent antioxidant activity across all three assays. Antibacterial tests revealed notable inhibition of both Gram-positive and Gram-negative bacteria, with stem extracts producing the largest inhibition zones. In the α-amylase inhibition assay, root extracts showed the strongest activity (IC_{50} < 10 µg/mL), followed by leaves (30.10 ± 0.92 µg/mL) and stem (48.48 ± 1.54 µg/mL), all surpassing the positive control acarbose (IC_{50} = 63.57 ± 3.85 µg/mL). Overall, *G. monticola* represents a promising natural source of multifunctional bioactive compounds with antioxidant, antibacterial, and antidiabetic potential, warranting further phytochemical characterization and in vivo investigation.

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1. INTRODUCTION

Medicinal plants continue to play an important role in global healthcare systems, particularly in developing countries where traditional medicine often constitutes the first line of therapeutic intervention (Sofowora et al., 2013). The World Health Organization recognizes the importance of traditional and complementary medicine and encourages scientific research aimed at ensuring the quality, safety, and efficacy of natural products used therapeutically (WHO, 2013). In this context, the pharmacological validation of medicinal plants requires an integrated approach that combines ethnobotanical documentation, reproducible phytochemical characterization, and biological evaluation based on robust experimental designs (Maroyi, 2026).

In recent decades, the global epidemiological transition has been marked by a rising prevalence of non-communicable diseases, including type 2 diabetes mellitus, cardiovascular diseases, and chronic inflammatory disorders (Ahmad Khan & Ahmad, 2019). Many of these conditions are closely associated with oxidative stress, defined as an imbalance between reactive oxygen species (ROS) and endogenous antioxidant defense systems. This imbalance promotes oxidative damage of lipids, proteins, and nucleic acids, contributing to cellular dysfunction and disease progression (Lobo et al., 2010; Phaniendra et al., 2015). Oxidative stress has also been directly implicated in the pathogenesis of type 2 diabetes, where elevated ROS impair insulin signaling and accelerate pancreatic β-cell dysfunction (Phaniendra et al., 2015). Consequently, considerable scientific attention has been directed toward plant-derived metabolites with antioxidant properties, particularly phenolic compounds capable of modulating redox processes and inflammatory pathways associated with these diseases.

At the same time, antimicrobial resistance has emerged as a major global public health threat. The increasing mortality associated with drug-resistant bacterial infections highlights the urgent need to identify new antimicrobial agents and alternative therapeutic strategies (Tacconelli et al., 2018). Plant extracts rich in polyphenols and flavonoids have demonstrated antibacterial activity through multiple mechanisms, including disruption of bacterial membranes, inhibition of essential metabolic enzymes, and interference with quorum sensing systems and biofilm formation (Cushnie & Lamb, 2011). In this context, plants that combine antioxidant and antimicrobial potential represent particularly valuable candidates for pharmacological investigation.

In metabolic disorders such as diabetes, inhibition of digestive enzymes including α-amylase and α-glucosidase represents a well-established therapeutic strategy for controlling postprandial hyperglycemia (Wu et al., 2024). Several plant-derived phenolic compounds have demonstrated the ability to inhibit these enzymes in a structure-dependent manner, supporting ongoing research aimed at identifying natural enzyme inhibitors with potential antidiabetic applications (Tadera et al., 2006; Zhao et al., 2022). Thus, phenolic metabolites may exert multiple biological effects simultaneously, acting as antioxidants, antibacterial agents, and inhibitors of carbohydrate-digesting enzymes.

Within this scientific context, the genus *Grewia* (family Malvaceae) has attracted growing interest as a source of pharmacologically relevant phytochemicals. The genus comprises approximately 300–400 species distributed across tropical and subtropical regions of Africa and Asia (Suguna & Umesha, 2022). Phytochemical investigations have revealed that several species within this genus are rich in flavonoids, tannins, and triterpenoids — compounds associated with diverse pharmacological activities including antioxidant, antimicrobial, anti-inflammatory, and antidiabetic effects (Kumar & Pandey, 2013). Recent systematic reviews have reported the identification of more than 160 chemical constituents in different *Grewia* species, with flavonoids representing the most predominant class (Qamar et al., 2021).

Among these species, *Grewia monticola* Sond. has been reported in ethnobotanical studies as a plant traditionally used for the treatment of diarrhea, inflammation, wounds, and other gastrointestinal disorders (Maroyi, 2013, 2014; Shopo et al., 2022). In southern Mozambique, root decoctions are used to treat diarrhea, while fruits and seeds are applied topically to treat wounds and ear infections (Ribeiro et al., 2010). Similar uses have been reported in Angola, where bark preparations are used as antidiarrheal remedies (Urso et al., 2016). The consistency of these uses across different geographic regions underscores the significant ethnopharmacological relevance of this species. Despite this traditional importance, the available scientific evidence for *G. monticola* remains limited compared with other members of the genus. Recent studies have suggested potential biological activity, including anti-quorum sensing and anti-virulence effects of leaf extracts (Lebeloane et al., 2024); however, important gaps remain regarding the quantitative phytochemical characterization of different plant organs and the relationship between phenolic composition and biological activities.

Therefore, the present study aimed to (a) evaluate the antioxidant activity of hydroethanolic extracts from leaves, stem, and roots of *G. monticola* using three complementary assays (DPPH, FRP, and TAC); (b) assess antibacterial activity against clinically relevant Gram-positive and Gram-negative bacterial strains; and (c) determine α-amylase inhibitory activity as a proxy for antidiabetic potential. To the best of our knowledge, this is the first comparative, organ-specific biological and phytochemical evaluation of *G. monticola* extracts.

2. METHOD

2.1. Plant material collection and preparation

Different parts of *G. monticola* Sond. (roots, stem, and leaves) were collected in Matutuine District, Maputo Province, Mozambique, specifically in Bela Vista Administrative Post, Mudada neighborhood (26°24′00.0″ S; 32°36′00.0″ E). Taxonomic identification was carried out by specialists at the Herbarium of the Mozambique Agricultural Research Institute (IIAM) through comparison with reference specimens deposited in the botanical collection. A voucher specimen was deposited under accession number 63457.

After collection, the plant material was cleaned and air-dried at ambient temperature, protected from direct sunlight, for 105 days. Weight was monitored periodically until constant mass was reached. The dried material was ground using an electric mill (Skymsen®, model LS10-HD) and standardized by sieving through a 180 µm mesh. The powdered material was stored in hermetically sealed amber glass containers in a dry, light-protected environment until analysis.

2.2. Preparation of crude extracts

Hydroethanolic extracts were obtained by maceration. For each plant part, 50 g of powdered material was placed in glass containers with 250 mL of 80% ethanol (v/v). Extraction proceeded for 16 days with periodic agitation, and the solvent was renewed every 48 h to maximize recovery of soluble metabolites. After maceration, the extractive solutions were combined, and the plant residue was washed twice with 250 mL of the same solvent. The combined extracts were filtered under suction and concentrated under reduced pressure using a rotary evaporator (BÜCHI®, Germany) at 50°C. The dried extracts were stored in a desiccator, protected from moisture and light, until use.

2.3. Preliminary phytochemical screening

Qualitative phytochemical screening was performed using classical precipitation and colour reaction tests described by Harborne (1984). Alkaloids were detected using Dragendorff and Mayer reagents. Flavonoids were identified using the lead acetate test and the Shinoda reaction. Tannins were detected using gelatin and ferric chloride tests. Anthraquinones were assessed by reactions with concentrated sulfuric acid and potassium hydroxide. Terpenoids and steroids were evaluated using the Liebermann–Burchard and Salkowski tests, respectively. Cardiac glycosides were detected using Keller–Killiani and Baljet tests. Saponins were assessed by the foam test.

2.4. Quantitative phytochemical analysis

2.4.1. Total phenolic content

TPC was determined using the Folin–Ciocalteu method (Pérez et al., 2023; Singleton et al., 1999). Briefly, 0.5 mL of extract solution (1 mg/mL) was mixed with 0.5 mL of Folin–Ciocalteu reagent (10%, v/v). After 10 min, 1 mL of sodium carbonate (7.5%, w/v) and 2.5 mL of distilled water were added. The mixture was incubated for 2 h at room temperature. Absorbance was measured at 760 nm. All analyses were performed in triplicate (n = 3). Results were expressed as mg gallic acid equivalents per g dry extract (mg GAE/g).

2.4.2. Total flavonoid (TFC) content

TFC was determined by the aluminium chloride colorimetric method (Cumbane et al., 2022). Briefly, 500 µL of extract (1 mg/mL) was mixed with 2.5 mL of distilled water and 250 µL of sodium nitrite (5%). After 6 min, 250 µL of aluminium chloride (2%) was added. After 5 min, 1 mL of sodium hydroxide (1 M) was added. Absorbance was measured spectrophotometrically. Results were expressed as mg quercetin equivalents per g dry extract (mg QE/g).

2.4.3. Total tannin content (TTC)

TTC was determined by the protein precipitation method (de Amorim et al., 2012). Briefly, 0.5 g of casein was mixed with 5 mL of extract (1 mg/mL) and 5 mL of distilled water under agitation for 2 h. After filtration, the volume was adjusted to 10 mL. An aliquot of 0.5 mL of the filtrate was subjected to the Folin–Ciocalteu reaction under the same conditions as TPC. Absorbance was measured at 760 nm. Results were expressed as mg tannic acid equivalents per g dry extract (mg TAE/g).

2.5. Antioxidant activity assays

2.5.1. DPPH radical scavenging assay

Antioxidant activity was evaluated using the DPPH• radical scavenging assay (Cumbane et al., 2022). Extract solutions were prepared at concentrations of 20–100 µg/mL. For the assay, 1.0 mL of extract was mixed with 3.0 mL of DPPH solution and 1.0 mL of ultrapure water. The control replaced extract with 80% ethanol. Mixtures were incubated at room temperature in the dark. Absorbance was measured at 517 nm. Radical scavenging activity was calculated as Inhibition (%) = $[(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$.

2.5.2. Ferric reducing power (FRP) assay

FRP was assessed according to Oyaizu (1986), adapted by Vijayalakshmi & Ruckmani (2016). Briefly, 2.5 mL of extract was mixed with 2.5 mL of phosphate buffer (200 mM, pH

6.6) and 2.5 mL of potassium ferricyanide (1%). The mixture was incubated at 50°C for 30 min. Then, 2.5 mL of trichloroacetic acid (10%) was added and the mixture was centrifuged at 3,000 rpm for 10 min. An aliquot of 2.0 mL of supernatant was mixed with 0.5 mL of ferric chloride (0.1%). Absorbance was measured at 700 nm.

2.5.3. Total antioxidant capacity (TAC)— phosphomolybdenum method

TAC was determined by the phosphomolybdenum assay (Prieto et al., 1999). The reagent was prepared from sodium phosphate dodecahydrate, ammonium molybdate tetrahydrate, and concentrated sulfuric acid. For the assay, 2 mL of extract was mixed with 1 mL of reagent and 2 mL of purified water. The mixture was incubated at 90°C for 90 min. After cooling, absorbance was measured at 695 nm.

2.6. α-Amylase inhibitory activity

α-Amylase inhibitory activity was determined using the DNS colorimetric assay (Miller, 1959). The substrate was 1% (w/v) soluble starch in phosphate buffer (100 mM, pH 6.8). The enzyme solution was prepared at 2 U/mL in the same buffer. For the assay, 2 mL of extract, 500 µL of buffer, and 100 µL of enzyme were mixed and incubated at 37°C for 20 min. Then, 200 µL of starch solution was added and the mixture was incubated at 37°C for 30 min. The reaction was stopped with 1 mL of DNS reagent, followed by heating for 10 min. Absorbance was measured at 540 nm. Acarbose was used as positive control. Inhibition (%) = [1 – (A/B)] × 100, where A = absorbance with extract and B = enzymatic control absorbance.

2.7. Antibacterial activity

Antibacterial activity was evaluated by disk diffusion (Klančnik et al., 2010; Ushimaru et al., 2007). Four bacterial strains were used: E. coli ATCC 25922 and Salmonella typhimurium ATCC 14028 (Gram-negative); S. aureus ATCC 29213 and E. faecalis ATCC 29212 (Gram-positive). Bacterial suspensions were standardized to 0.5 McFarland (~1.5×10^8 CFU/mL). Mueller–Hinton agar plates were inoculated, and paper disks (6 mm) were impregnated with 10 µL of extract at concentrations of 100–1,250 µg/mL. DMSO was used as negative control and amoxicillin (30 µg/disk) as positive control. Plates were incubated at 37 °C for 24 h. Inhibition zone diameters were measured in mm. Experiments were performed in duplicate.

2.8. Statistical analysis

All experiments were performed in triplicate (n = 3) and results expressed as mean ± standard deviation (SD). IC_{50} values were determined by linear regression or interpolation from dose–response curves. One-way ANOVA followed by Tukey's post hoc test was used for multiple comparisons. Student's *t*-test was applied for pairwise comparisons. Pearson correlation analysis was performed to evaluate relationships between phytochemical content (TPC, TFC, and TTC) and biological activities (DPPH, FRP, TAC, and α-amylase inhibition). Statistical significance was set at $p < 0.05$. All analyses were performed using the GraphPad Prism.

3. RESULTS

3.1. Qualitative phytochemical profile

The qualitative phytochemical screening revealed an organ-specific distribution of secondary metabolites in *G. monticola* (Table 1). Phenolics and flavonoids were detected in all three organs, confirming their role as core constituents of this species. Condensed tannins were found exclusively in leaves, whereas hydrolysable tannins occurred in both stem and root extracts. Anthraquinones were detected in stem and root extracts, while terpenoids and steroids were mainly present in aerial parts. Alkaloids and saponins were

Table 1
Qualitative phytochemical screening of hydroethanolic extracts of *Grewia monticola.*

Sample	Phen	Flav	Hyd. tannins	Cond. tannins	Anthr	Terp	Ster	Alk	Sap
Leaves	+	+	–	+	–	+	+	–	–
Stem	+	+	+	–	+	+	+	–	–
Root	+	+	+	–	+	–	–	–	–

Phen = phenolics; Flav = flavonoids; Hyd. tannins = hydrolysable tannins; Cond. tannins = condensed tannins; Anthr = anthraquinones; Terp = terpenoids; Ster = steroids; Alk = alkaloids; Sap = saponins. (+) present; (–) absent.

absent from all tested organs. This organ-specific distribution is consistent with the different physiological and ecological roles of secondary metabolites in plant tissues (Mithöfer & Maffei, 2016) and aligns with phytochemical reports for other *Grewia* species (Qamar et al., 2021).

3.2. Quantitative phytochemical profile

Quantitative analysis revealed clear organ-specific differences in phenolic compound distribution (Table 2). TPC was highest in leaves (6.508 ± 0.180 mg GAE/g), followed by stem (6.293 ± 0.305 mg GAE/g) and roots (4.778 ± 0.058 mg GAE/g). TFC was highest in stem (9.143 ± 0.202 mg QE/g), followed by leaves (7.610 ± 0.163 mg QE/g) and roots (6.225 ± 0.190 mg QE/g). TTC was detected in stem (1.483 ± 0.055 mg TAE/g) and leaves (1.167 ± 0.047 mg TAE/g) but was not detected in root extracts, consistent with the qualitative results.

It is important to note that the Folin–Ciocalteu reagent may react with non-phenolic reducing substances (e.g., ascorbic acid and certain amino acids), potentially leading to slight overestimation of TPC (Everette et al., 2010; Pérez-Jiménez et al., 2010). Similarly, the aluminium chloride method predominantly detects flavones and flavonols (Nastasi, 2025); therefore, TFC values are most informative for relative comparisons within the same experimental framework. Overall, these results indicate that *G. monticola* is a relevant source of phenolic compounds, particularly flavonoids, providing a plausible biochemical basis for the biological activities evaluated in subsequent sections.

Table 2
Total phenolic (TPC), flavonoid (TFC), and tannin (TTC) contents of *Grewia monticola* extracts.

Sample	TPC (mg GAE/g)	TFC (mg QE/g)	TTC (mg TAE/g)
Leaves	6.508 ± 0.180[a]	7.610 ± 0.163[b]	1.167 ± 0.047[b]
Stem	6.293 ± 0.305[a]	9.143 ± 0.202[a]	1.483 ± 0.055[a]
Root	4.778 ± 0.058[b]	6.225 ± 0.190[c]	ND

Values are expressed as mean ± standard deviation (n = 3). TPC = total phenolic content expressed as mg gallic acid equivalents per g extract (mg GAE/g). TFC = total flavonoid content expressed as mg quercetin equivalents per g extract (mg QE/g). TTC = total tannin content expressed as mg tannic acid equivalents per g extract (mg TAE/g). Different superscript letters within the same column indicate statistically significant differences (one-way ANOVA followed by Tukey's test, $p < 0.05$).

3.3. Antioxidant activity

3.3.1. DPPH radical scavenging activity

All extracts showed dose-dependent DPPH radical scavenging activity across 20–100 µg/mL (Figure 1). Root extract showed the strongest activity (IC_{50} = 31.86 ± 1.11 µg/mL), followed by leaves (39.74 ± 0.92 µg/mL) and stem (45.63 ± 0.74 µg/mL; Table 3). Ascorbic acid (IC_{50} = 18.42 ± 0.33 µM) confirmed assay sensitivity. Significant differences were observed among all three organs ($p < .05$, Student's *t*-test).

Notably, although leaves had the highest TPC, root extract exhibited the lowest IC_{50}. This suggests that radical scavenging efficiency is governed by the structural characteristics of

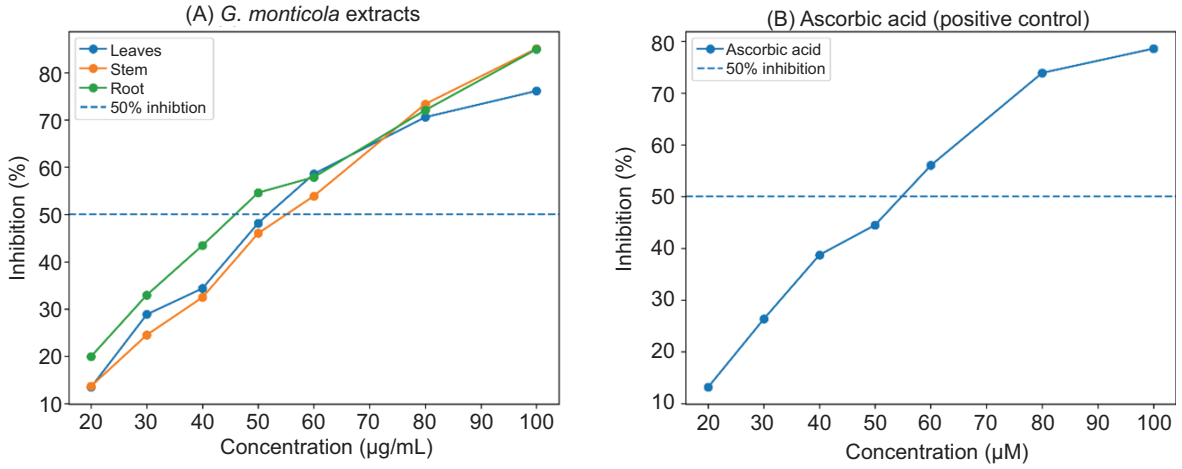

Figure 1. DPPH radical scavenging activity of hydroethanolic extracts of *Grewia monticola*. (A) Dose–response curves showing the DPPH• radical scavenging activity of leaf, stem, and root extracts of *G. monticola* at concentrations ranging from 20 to 100 µg/mL. (B) Dose–response curve of the positive control, ascorbic acid, at concentrations ranging from 20 to 100 µM. Values represent the mean ± standard deviation (n = 3). Radical scavenging activity was determined spectrophotometrically at 517 nm, with a decrease in absorbance indicating greater antioxidant activity.

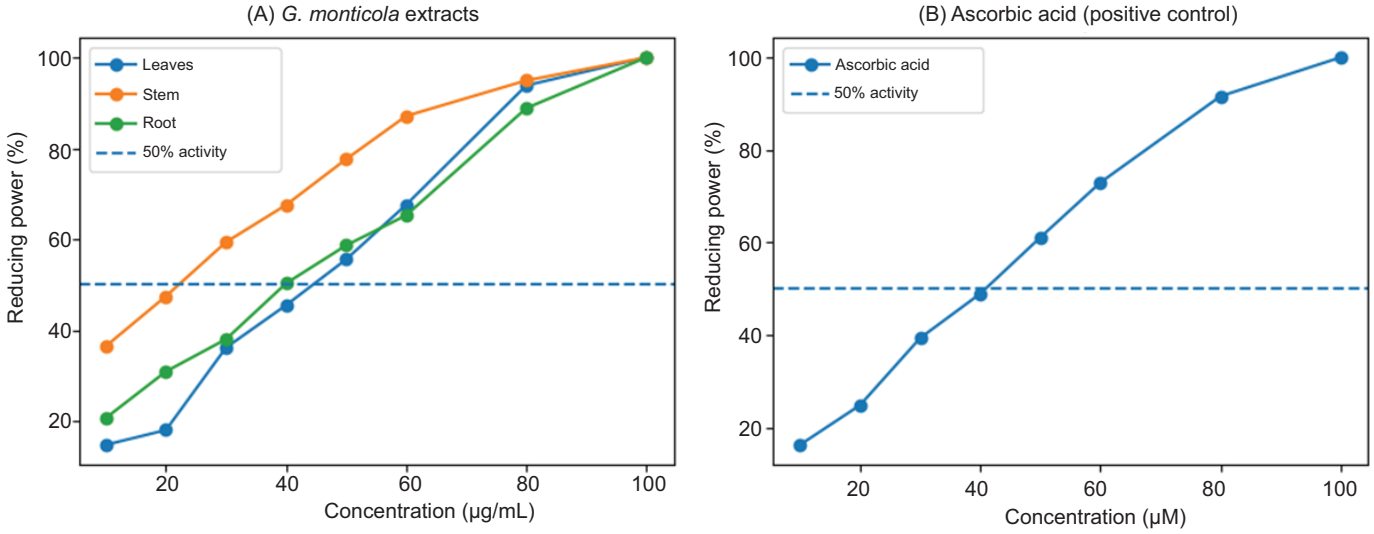

Figure 2. Ferric reducing power (FRP) of hydroethanolic extracts of *G. monticola*. (A) Dose–response curves showing the ferric reducing power of leaf, stem, and root extracts at concentrations ranging from 10 to 100 µg/mL (final concentration in the reaction mixture). (B) Dose–response curve of the positive control, ascorbic acid, at concentrations ranging from 10 to 100 µM. Values represent the mean ± standard deviation (n = 3). Increased absorbance indicates greater reducing power resulting from the reduction of Fe^{3+} to Fe^{2+}, as measured spectrophotometrically at 700 nm.

Table 3
Total phenolic (TPC), flavonoid (TFC), and tannin (TTC) contents of *Grewia monticola* extracts.

Sample	DPPH IC_{50} (µg/mL)	FRP IC_{50} (µg/mL)	TAC IC_{50} (µg/mL)
Leaves	39.74 ± 0.92[a]	47.68 ± 1.31[a]	27.12 ± 1.24[a]
Stem	45.63 ± 0.74[b]	33.91 ± 0.83[b]	37.92 ± 0.62[b]
Root	31.86 ± 1.11[c]	54.62 ± 0.71[c]	44.62 ± 0.62[c]
Ascorbic acid (µM)	18.42 ± 0.33	22.73 ± 0.28	43.74 ± 0.12

Values are expressed as mean ± standard deviation (n = 3). IC_{50} corresponds to the concentration required to achieve 50% antioxidant activity. Lower IC_{50} values indicate stronger antioxidant activity. Different superscript letters indicate statistically significant differences (ANOVA followed by Tukey test, $p < 0.05$).

individual phenolics — particularly hydroxylation patterns and aromatic conjugation — rather than total phenolic quantity alone (Re et al., 1999). Non-phenolic antioxidants present in root tissues, such as terpenoids, may also contribute independently to radical scavenging capacity.

3.3.2. Ferric reducing power (FRP)

All extracts showed dose-dependent ferric reducing activity across 10–100 µg/mL (Figure 2). Stem extract showed the strongest reducing activity (IC_{50} = 33.91 ± 0.83 µg/mL), followed by leaves (47.68 ± 1.31 µg/mL) and roots (54.62 ± 0.71 µg/mL; Table 3). Ascorbic acid (IC_{50} = 22.73 ± 0.28 µM) confirmed assay sensitivity. Differences among organs were statistically significant ($p < 0.05$).

The superior reducing activity of the stem extract is consistent with its higher levels of flavonoids and tannins, compounds recognized as effective electron donors through their aromatic hydroxyl groups and conjugated systems (Rice-Evans et al., 1996). The different organ ranking compared with the DPPH assay reflects the distinct chemical mechanisms underlying each method: the DPPH assay integrates both HAT and ET processes, whereas the FRP assay predominantly measures electron transfer (ET) to oxidized metal ions (Munteanu & Apetrei, 2021; Prior et al., 2005).

3.3.3. Total antioxidant capacity (TAC)

All extracts exhibited dose-dependent TAC across 10–100 µg/mL (Figure 3). Leaf extract showed the highest TAC (IC_{50} = 27.12 ± 1.24 µg/mL), followed by stem (37.92 ± 0.62 µg/mL) and root (44.62 ± 0.62 µg/mL; Table 3). The positive control ascorbic acid showed IC_{50} = 43.74 ± 0.12 µM. Differences among organs were statistically significant ($p < .05$).

The higher TAC in leaf extract is consistent with its greater total phenolic content. The differing rankings across the three assays (DPPH: root > leaf > stem; FRP: stem > leaf > root; TAC: leaf > stem > root) are commonly reported and expected, as each method evaluates distinct antioxidant mechanisms (Munteanu & Apetrei, 2021; Prior et al., 2005). These discrepancies also reflect the organ-specific distribution of metabolite classes with different redox properties,

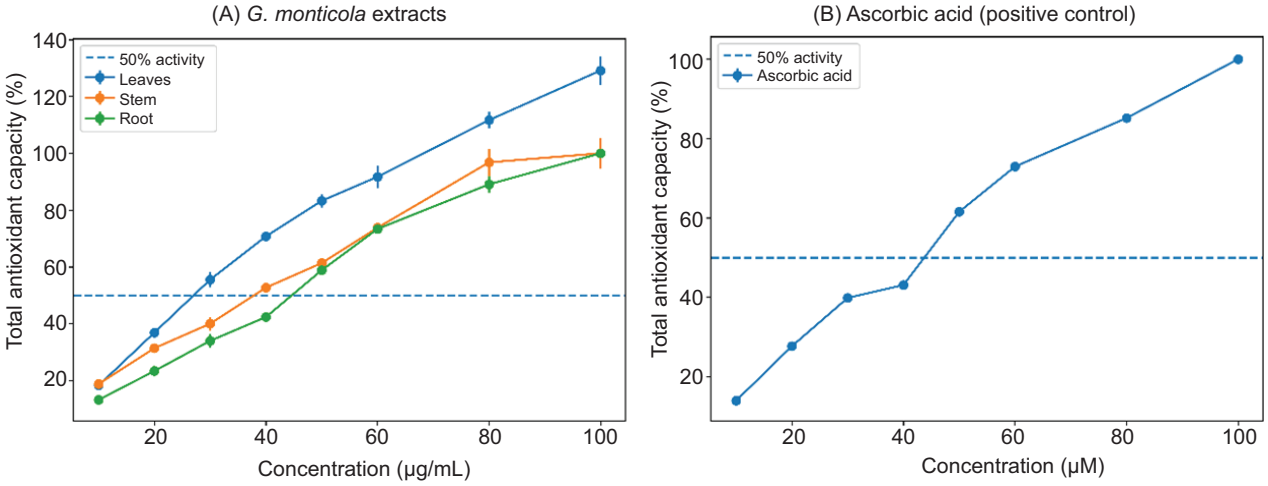

Figure 3. Total antioxidant capacity (TAC) of hydroethanolic extracts of *G. monticola*. (A) Dose–response curves showing the TAC of leaf, stem, and root extracts at concentrations ranging from 10 to 100 µg/mL. (B) Dose–response curve of the positive control, ascorbic acid, at concentrations ranging from 10 to 100 µM. Values represent the mean ± standard deviation (n = 3). Increased absorbance at 695 nm indicates greater antioxidant capacity resulting from the reduction of Mo(VI) to Mo(V).

underscoring the importance of using complementary assays in antioxidant evaluation.

3.4. α-Amylase inhibitory activity

All extracts showed dose-dependent α-amylase inhibition across 10–100 µg/mL (Figure 4). Root extract exhibited the strongest inhibitory activity (IC_{50} < 10 µg/mL), followed by leaves (IC_{50} = 30.10 ± 0.92 µg/mL) and stem (IC_{50} = 48.48 ± 1.54 µg/mL); all surpassed the positive control acarbose (IC_{50} = 63.57 ± 3.85 µg/mL; Table 4). Statistical comparisons confirmed significant differences between leaves and stem ($p < 0.001$), leaves and acarbose ($p < 0.01$), and stem and acarbose ($p < 0.05$). Root extract was excluded from this comparison as its IC_{50} fell below the tested concentration range.

The root extract showed inhibition values exceeding 57% at the lowest concentration tested and a plateau of approximately 70–71% at higher concentrations. Because inhibition already exceeded 50% at the lowest tested concentration (10 µg/mL), the reported IC_{50} represents an experimental upper limit; future studies including lower concentration ranges are recommended for more precise IC_{50} estimation.

The strong inhibitory activity of root extract may be associated with the presence of phenolic metabolites identified during phytochemical screening. Phenolics, including flavonoids and tannins, are recognized natural inhibitors of carbohydrate-digesting enzymes through hydrogen bonding and hydrophobic interactions with active site amino acid residues (Ćorković et al., 2022; Martinez-Gonzalez et al., 2017).

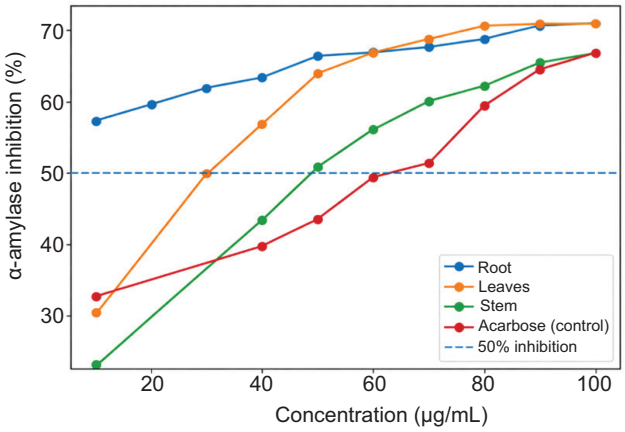

Figure 4. Dose–response curves for α-amylase inhibition by hydroethanolic extracts of *G. monticola* and the positive control, acarbose, as determined using the DNS assay. The dashed horizontal line represents 50% inhibition and was used to estimate IC_{50} values. Values represent the mean ± standard deviation (n = 3).

High-molecular-weight tannins may further contribute through enzyme–phenol complex formation (Dai & Mumper, 2010). Although specific compounds responsible for α-amylase inhibition were not identified in the present study, the literature suggests that quercetin, kaempferol, and chlorogenic acid — commonly reported in *Grewia* species — are potent α-amylase inhibitors (Dai & Mumper, 2010; Oboh et al., 2015).

3.5. Antibacterial activity

All *G. monticola* extracts exhibited concentration-dependent antibacterial activity against the four tested bacterial strains (Tables 5 and 6). Stem extract showed the most pronounced activity, producing the largest inhibition zones against *S. aureus*, *S. typhimurium*, and *E. coli* across the concentration range tested. At 6.25 µg/disc, the stem extract produced inhibition zones of 23.50 ± 0.04 mm (*S. aureus*) and 22.41 ± 0.04 mm (*E. coli*), approaching or exceeding the zones produced by amoxicillin (14.50 ± 0.02 mm and 21.11 ± 0.02 mm, respectively) under the same conditions. Root extract showed consistent dose-dependent activity, particularly against *E. coli* (21.07 ± 0.07 mm at 12.50 µg/disc). Leaf extracts exhibited more moderate effects. *Enterococcus faecalis* showed more limited sensitivity at lower doses, with significant inhibition only at higher concentrations for all extracts, consistent with the intrinsic resilience of this genus (García-Solache & Rice, 2019).

One-way ANOVA followed by Tukey's test confirmed statistically significant differences among plant organs for most bacterial strains ($p < 0.05$). It is important to emphasize that direct quantitative comparison of plant extracts with pure antibiotics by disk diffusion is methodologically inappropriate, since antibiotics are pure compounds with standardized diffusion properties, whereas plant extracts are complex mixtures (Balouiri et al., 2016; CLSI, 2019). Intra-assay comparisons among organs, however, are valid within this experimental framework.

Table 4
α-Amylase inhibitory activity of hydroethanolic extracts of *Grewia monticola*.

Sample	IC_{50} (µg/mL)	Maximum inhibition (%)	Activity ranking
Leaves	30.10 ± 0.92[a]	69.2 ± 1.4	Moderate
Stem	48.48 ± 1.54[b]	67.8 ± 1.1	Low
Root	< 10.00	71.0 ± 0.9	Very strong
Acarbose	63.57 ± 3.85[c]	74.5 ± 1.2	Reference

Values are expressed as mean ± standard deviation (n = 3). IC_{50} corresponds to the concentration required to inhibit 50% of α-amylase activity. Lower IC_{50} values indicate stronger inhibitory activity. Different superscript letters within the same column indicate statistically significant differences (one-way ANOVA followed by Tukey's post hoc test, $p < 0.05$). Because the root extract produced inhibition above 50% at the lowest tested concentration, its IC_{50} value is reported as < 10 µg/mL and was not included in the statistical comparison.

3.6. Integrated biological activity and correlation analysis

The results collectively demonstrate that *G. monticola* exhibits a multifunctional bioactive profile with clear organ-specific distribution (Tables 3–5). Leaves were the principal source of total antioxidant capacity (TAC); stems exhibited the strongest reducing power and antibacterial activity; roots showed the most potent α-amylase inhibitory and DPPH radical scavenging activities.

Pearson correlation analysis revealed a significant positive correlation between TFC and antibacterial activity (particularly for stem extracts; $r > 0.85$, $p < 0.05$) and between TPC and

Table 5
Antibacterial activity of hydroethanolic extracts determined by disk diffusion.

Extract	Dose (µg/disc)	*S. aureus*	*S. typhimurium*	*E. coli*	*E. faecalis*
Leaves	5.00	9.64 ± 0.37	9.77 ± 0.19	9.31 ± 0.28	–
	6.25	10.52 ± 0.19	10.43 ± 0.19	10.96 ± 0.37	–
	12.5	12.28 ± 0.19	11.29 ± 0.28	13.20 ± 1.87	–
	25.0	–	17.82 ± 0.93	–	–
Root	3.25	8.84 ± 0.00	9.44 ± 0.09	9.97 ± 0.09	9.04 ± 0.09
	5.00	9.57 ± 0.09	9.90 ± 0.19	11.68 ± 0.28	10.43 ± 0.19
	6.25	11.22 ± 0.19	11.35 ± 0.37	13.00 ± 0.28	11.95 ± 0.09
	12.50	15.43 ± 0.58	17.23 ± 0.11	21.07 ± 0.07	12.87 ± 0.43
Stem	3.25	11.81 ± 0.09	11.09 ± 0.19	11.42 ± 0.84	9.24 ± 0.19
	5.00	14.50 ± 0.03	12.40 ± 0.01	12.23 ± 0.07	10.49 ± 0.28
	6.25	23.50 ± 0.04	22.41 ± 0.04	22.41 ± 0.04	12.00 ± 0.02
	12.50	27.68 ± 0.06	25.04 ± 0.06	25.10 ± 0.04	19.16 ± 0.91
Amoxicillin	10.00	14.50 ± 0.02	13.38 ± 0.01	21.11 ± 0.02	27.75 ± 0.03

Values represent inhibition zone diameter (mm ± SD). "–" indicates no measurable inhibition.

Table 6
Comparative summary of antibacterial activity (MIC).

Extract	*S. aureus*	*S. typhimurium*	*E. coli*	*E. faecalis*
Leaves	~6.25 µg/disc	~6.25 µg/disc	~6.25 µg/disc	>12.50 µg/disc
Root	~6.25 µg/disc	~6.25 µg/disc	~5.00 µg/disc	~12.50 µg/disc
Stem	~5.00 µg/disc	~5.00 µg/disc	~5.00 µg/disc	~12.50 µg/disc

Amoxicillin was used as the positive control at a fixed dose of 10 µg/disc and was not included in the approximation of inhibitory thresholds.

TAC ($r > 0.90$, $p < 0.05$), supporting the hypothesis that flavonoids and phenolic compounds are major contributors to the observed biological activities. However, the absence of a strong correlation between TPC and DPPH activity in root extracts suggests the contribution of non-phenolic antioxidants or qualitative differences in phenolic composition among organs.

4. DISCUSSION

The present study provides the first comparative, organ-specific biological evaluation of hydroethanolic extracts from leaves, stem, and roots of *Grewia monticola*, contributing to the growing body of pharmacological evidence for this ethnobotanically relevant species.

The observation that different plant organs ranked differently across the three antioxidant assays (DPPH, FRP, and TAC) is mechanistically informative rather than inconsistent. Each method evaluates distinct aspects of antioxidant behavior operating through different chemical pathways. The DPPH assay captures both hydrogen atom transfer (HAT) and electron transfer (ET) mechanisms; the FRP assay predominantly evaluates ET capacity toward oxidized metal ions; and the phosphomolybdenum TAC assay reflects the overall reductive capacity of the extract under acidic conditions (Munteanu & Apetrei, 2021; Prior et al., 2005). This variability underscores the importance of employing multiple complementary assays, as adopted in the present study.

The superior DPPH radical scavenging activity of the root extract, despite its lower TPC compared with leaves, is most parsimoniously explained by qualitative differences in phenolic composition rather than total quantity. Structural factors — including degree of hydroxylation, extent of aromatic conjugation, and glycosylation state — are well established as primary determinants of antioxidant potency in individual phenolic compounds (Rice-Evans et al., 1996). Additionally, non-phenolic antioxidants such as terpenoids may contribute independently to radical scavenging capacity (Krishnaiah et al., 2011). Future studies employing LC-MS/MS or NMR-guided identification will be essential to elucidate the structural identity of the active antioxidant compounds in each organ.

The very strong α-amylase inhibitory activity of root extract ($IC_{50} < 10$ µg/mL) substantially surpassing the reference drug acarbose ($IC_{50} = 63.57$ µg/mL) is a highly significant finding with potential antidiabetic implications. Inhibition of α-amylase slows the rate of starch digestion, thereby reducing the postprandial glucose spike — a key therapeutic target in type 2 diabetes management (Gong et al., 2020; Kashtoh & Baek, 2023). Acarbose, the clinical reference inhibitor, acts as a competitive inhibitor of α-amylase; however, its use is associated with gastrointestinal side effects arising from microbial fermentation of undigested carbohydrates in the colon (Fernández-Bañares, 2022). Natural inhibitors capable of achieving moderate but sustained inhibition may therefore offer therapeutic advantages.

The antibacterial results of the present study are consistent with, and extend, the growing body of evidence for antimicrobial activity in the genus *Grewia*. For comparative context, *G. flava* acetone extracts demonstrated MIC values as low as 0.03 mg/mL against *S. aureus* (Lamola et al., 2017), while fractions from *G. flava* branches showed MIC values of 0.21–0.31 mg/mL against various pathogens (Coin et al., 2024). Although the present study did not determine MIC values, a significant limitation acknowledged below, the disk diffusion results for stem and root extracts are suggestive of potent antibacterial activity. The novelty of *G. monticola* relative to these better-studied congeners lies in its distinct geographic distribution, organ-specific activity profile, and the combination of antibacterial, antioxidant, and antidiabetic properties demonstrated here for the first time in an integrated manner.

Toxicological evaluation of *G. monticola* extracts was beyond the scope of the present study. However, safety assessment is an essential prerequisite for any therapeutic application. The traditional use of *G. monticola* across multiple African countries provides some degree of empirical support for safety; nevertheless, rigorous in vitro cytotoxicity assays (e.g., MTT assay against human cell lines) and in vivo acute and sub-chronic toxicity studies are required before therapeutic claims can be made.

Phytochemical composition in plants is known to vary with environmental factors including soil type, altitude, seasonal variation, rainfall patterns, and anthropogenic pressure (Gobbo-Neto & Lopes, 2007). Since samples were collected from a single site and season, the results may not fully represent the phytochemical diversity of *G. monticola* across its geographic range. Multi-site, multi-season studies are therefore recommended to assess phytochemical variability and confirm reproducibility of the biological activities reported.

The main limitations of this study are (a) phytochemical analysis was limited to spectrophotometric and qualitative

assays, which lack compound-level specificity and may overestimate phenolic content; (b) antibacterial assessment relied solely on disk diffusion — the absence of MIC and MBC data precludes quantitative benchmarking against standard antibiotics; (c) specific phenolic compounds responsible for α-amylase inhibition were not identified; (d) toxicological and stability data are lacking; and (e) the study used a single geographic source and season. Future investigations should prioritize LC-MS/MS metabolomic profiling, bioassay-guided fractionation to isolate active compounds, broth microdilution assays for MIC/MBC determination, enzyme kinetic studies, in vitro cytotoxicity evaluation, and in vivo pharmacological studies.

5. CONCLUSION

This study demonstrates, for the first time in a comparative and organ-specific manner, that hydroethanolic extracts of *Grewia monticola* exhibit significant antioxidant, antibacterial, and α-amylase inhibitory activities. These activities were strongly organ-dependent, reflecting the differential accumulation of bioactive secondary metabolites across plant tissues. Root extracts showed the strongest radical scavenging (DPPH) and α-amylase inhibitory activities; stem extracts showed the highest ferric reducing power (FRP) and antibacterial effects; and leaf extracts showed the greatest total antioxidant capacity (TAC).

Taken together, the results position *G. monticola* as a promising candidate for the development of natural antioxidant, antimicrobial, and antidiabetic agents. Future studies focusing on compound identification, mechanism elucidation, toxicological profiling, and in vivo validation will be essential to translate these findings into applied pharmacological or nutraceutical contexts.

AUTHOR CONTRIBUTIONS

Conceptualization was done by Nhanrave, A., Cumbane, P., and Raice, R. Methodology was carried out by Nhanrave, A. and Cumbane, P. Formal analysis and writing — original draft were done by Nhanrave, A. Writing — review & editing were done by Cumbane, P. and Raice, R. Supervision was performed by Raice, R. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

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

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Instituto Superior de Ciências e Tecnologia de Moçambique (ISCTEM) for providing laboratory infrastructure and technical support during the experimental phase of this study.

FUNDING

No funding was received for this study.

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