

Grewia Flava Twigs Extracts: Phytochemical, Antioxidant, and Antimicrobial Evaluations

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Abstract

Background

Grewia flava infusions are consumed to assist with kidney problems and stomach ailments, however, there are no scientific data on the phytochemical profile or biological properties of the extract to validate its folklore use. Thus, the study aim was to assess the phytochemical profile, antioxidant, and antimicrobial activities of *Grewia flava* twigs extracts.

Results

The antioxidant activities of the extracts were assayed using 1,1-Diphenyl-2-picrylhydrazyl radical scavenging, reducing power, metal chelation, and total phenolic and flavonoid content assays. The agar well diffusion and microdilution methods were used for crude extracts and fractions (from 80% methanol extract) antimicrobial screening against *P. aeruginosa*, *S. aureus*, *E. coli*, *B. subtilis*, *A. niger*, and *R. oryzae*. The 80% methanol twig extract (250 ± 2 GAE/g) exhibited a high concentration of phenolic content followed by distilled water extract (192 ± 2 mg GAE/g) and the hexane extract (43.1 ± 0.2 mg GAE/g). Fraction 14 of the methanol twig extract exhibited MIC values of 0.21–0.31 mg/mL against all test microorganisms. The roots and twigs extracts exhibited significant antioxidant and antimicrobial activities, which were attributed to the extracts bioactive phytochemical compounds such as alkaloids, flavonoids, saponins, steroids, glycosides, anthraquinones, and tannins that were detected in the extracts. Also the roots and twigs non-polar extracts were subjected to gas chromatography–mass spectrometry analysis, which identified several bioactive compounds like betulin, β -amyirin, palmitic acid, lupenone, and phytol, highlighting the potential of the plant species as a botanical drug.

Conclusions

The study supports the traditional use of plant roots and twigs for treating various ailments, indicating its medicinal value. For sustainable harvesting of *Grewia flava*, twigs maybe used in place of roots; which to avoid killing the whole plant. However, a comparison of active compounds quantities in twigs relative to those in roots is crucial.

Background

Throughout history, medicinal plants have been utilized worldwide for treating various illnesses, particularly in developing countries rural communities where these plants are available and are considered to more effective and low cost when compared to synthetic drugs (Motamedi et al. 2010; Motlhanka and Nthoiwa 2013; Sinha et al 2015). Medicinal plants extracts have been shown to be constituted by bioactive phytochemicals such as tannins, terpenoids, alkaloids, anthraquinones, flavonoids, and saponins, which exhibit ethnopharmacological properties (Dhawan and Gupta 2017; Hutke and Naswale 2017). Herbal infusions and decoction are rich in phenolic compounds which have shown potent antioxidant properties. In the human body, antioxidant are known to work against both free radicals and reactive oxygen species. Synthetic antioxidants are commonly

used to prevent and treat chronic diseases, but their toxicity has led to a search for safer natural alternatives (Chiavaroli et al. 2011; Ahmed et al. 2019; Stobiecka et al. 2022).

Grewia flava, which is referred to as a raisin tree or brandy bush in English and commonly known as *moretlwa*, *moseme*, and *ntewa* in Tswana. It is a shrub from the Malvaceae family. It is widespread in the drier bush-land and deciduous woodlands in the northern, central, and eastern parts of Botswana, as well as in South Africa, Zimbabwe, Namibia, and Eswatini (Mashungwa et al. 2019). Typically growing up to 2–3 meters tall, *Grewia flava* has greyish-brown young branchlets that turn dark purplish to black as they age. It produces edible reddish-brown globular fruits (Lamola et al. 2017). According to folklore medicine, the plant's twigs are used to prepare herbal tea that is believed to help with kidney problems, while a mixture of stem bark, roots, and milk is thought to aid stomach problems caused by bacterial infections (Mashungwa et al. 2019). Dried berries are consumed in their natural form or can be used to make porridge and also be fermented to make a local Tswana wine called *khadi*, which is believed to have antioxidant properties (Motlhanka and Nthoiwa 2009; Motlhanka et al. 2018, 2020). Previous studies have focused mainly on ethnobotanical properties of roots, barks, leaves, and berries of *Grewia flava*, while this study profiled phytochemical properties of the plant by determining, antioxidant, and antibacterial activities of the twigs, which have been minimally researched. The use of *Grewia flava* twigs over roots would be more sustainable and is, therefore, recommended. This study determined the chemical profile, *in vitro* antioxidant, and antimicrobial activities of *Grewia flava* twigs.

Methods

Sample collection and preparation

G. flava twigs and roots were collected in October 2020 from Mmashoro village in the Central district of Botswana. Coordinates: 21° 48' 15.9" S 26° 27' 22.4" E, Botswana. The plant was identified at University of Botswana herbarium by Dr. Mbaki Muzila, voucher number MZ002_2022. The twigs and roots were washed and left to air dry for two weeks. Plant samples were placed into zip-lock bags after being powdered and stored at room temperature in a locker.

Extraction and Fractionation

Powdered twig and root samples of *G. flava* were macerated in *n*-hexane, acetone, distilled water (DW), and 80% methanol. In brief, 100 g of *G. flava* ground samples were extracted repeatedly with 500 mL of *n*-hexane (Hex), acetone (Ace), distilled water, and 80% methanol in water (80% MeOH). The Whatman No. 1 filter paper was used to filter the 24 hour extracts followed by solvent removal and storage at 4°C. Twig methanol extract was fractionated using a Merck silica gel, 60-80 mesh column packed with prepared in *n*-hexane. The column was subjected to different solvent systems of increasing polarity, starting with mixtures of *n*-hexane:ethyl acetate (EtOAc) (4:0, 4:1, 2:1, 4:3, 1:1) followed by mixtures of EtOAc:MeOH. (4:0, 4:1, 2:1, 4:3, 1:1). The column fractions R_f was monitored by Thin layer chromatography (TLC) (Silica gel 60, UV₂₅₄). Similar fractions based on TLC analysis were pooled together resulting in 24 column fractions.

Antioxidant activity assays

Total phenolic content (TPC)

The total phenolic content of the crude extracts was evaluated using the Folin-Ciocalteu assay as previously described (Lfitat et al. 2021; Maigoda et al. 2022). UV-Vis spectrophotometer was used to measure absorbance at 725 nm for the aliquots. Aqueous methanol (80%) was used as a blank. All the experiments were repeated three times.

Total Flavonoid content (TFC)

The aluminium chloride colorimetry method (Atere et al. 2018; Hmamou et al. 2022) to evaluate the total flavonoid content (TFC) of the crude extracts. Quercetin was used as the standard. TFC was expressed as mg quercetin equivalents per grams of the crude extract (mg QUE/ g of the crude extract).

DPPH radical scavenging assay

The 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assayed extracts antioxidant activity while ascorbic acid was used as a standard following a reported protocol (Hmamou et al. 2022) Methanol was used as a The blank was methanol and all measurements were repeated three times.

Ferric reducing antioxidant power assay

Ferric ion reducing capacity was measured using reported methods and was presented as mg ascorbic acid equivalents per grams of the extract dry matter (mg of AAE/g) (Kanmaz et al. 2020).

Metal chelation ability

Metal chelation ability was determined following a reported method (Saliu and Olabiya 2017) Ethylenediaminetetraacetic acid (EDTA) was used as a standard chelator and the experiment was done in triplicates.

Antimicrobial activity

Six different strains were selected for antibacterial and antifungal assays. The strains included Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*), Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacterial strains, and fungal strains (*Aspergillus niger* and *Rhizopus oryzae*), all obtained from the microbiology laboratory at the Department of Biological Sciences and Biotechnology at Botswana International University of Science and Technology (BIUST). Muller Hinton broth sub-cultured the bacteria while the fungal strains were cultured in Potato Dextrose broth. Nutrient Agar at 4°C was used as bacterial strains media during experimentation.

Agar well diffusion method

Kirby-Bauer agar diffusion method was adopted (Barberies et al. 2020). Briefly 50 mL of the Muller-Hinton agar was placed into petri dishes and allowed to set. A standardized inoculum (1.0×10^8 CFU/mL) of each strain (100 μ L) was introduced onto the surface of the set agar plate using a sterilized glass cell spreader. Using a 1000 μ L pipette tip wells (8 mm diameter) were made in the agar plate. The test sample (100 μ L of 10 mg/L extract) was introduced into the well and allowed to diffuse. The loaded plates incubation was at 37°C for 24 h for bacteria and at 30°C for 48 h fungi. The negative control was 10% dimethyl sulphoxide. The antibacterial test positive

control was chloramphenicol. After incubation, the zone of inhibition diameter (in millimeters) were measured and represented microbial growth inhibition (Rakholiya and Chanda 2014). The experiment was repeated thrice, and mean values were used $\pm$ SEM (standard error of the mean values).

Determination of minimum inhibitory concentration (MIC)

To determine the MIC, the micro broth dilution method was employed with a two-fold serial dilution of test samples in liquid medium. The extracts were selected based on their activity against the test strains in the Kirby-Bauer diffusion assay. The two-fold serial dilution of the selected extracts ranged from 2 mg/mL to 0.0313 mg/mL. The microbial strains were inoculated using the colony suspension method to obtain a suspension with log phase absorbance of 0.4 at 600 nm. The final concentration was adjusted to 5×10^5 CFU/mL. To each well, 50 μ L of Muller-Hinton broth, 25 μ L of test samples, and 25 μ L of the test organism suspension. The negative control used 10% dimethyl sulphoxide, while the positive control employed chloramphenicol for antibacterial assay. Bacterial plates were incubated for 24 h at 37°C while fungal plates were incubated for 48 h at 30°C. The microplate reader (MultiSkan FC, ThermoSci) measured the wells absorbances at 600 nm. The test sample lowest concentration that inhibited at least 80% of microbial growth was taken as the sample MIC compared to the growth control (Kowalska-Krochmal and Dudek-Wicher 2021; Zamakshshari et al. 2021).

Preliminary phytochemical screening

Literature procedures assessing the presence of tannins, saponins, flavonoids, steroids, glycosides, anthraquinones and alkaloids in the aqueous methanol root and twig extracts of *Grewia flava* were used for this qualitative phytochemical detection (Shaikh et al. 2020; Maigoda et al. 2022; Murugan et al. 2022).

GC-MS analysis

GC-MS analysis was performed on the *n*-hexane non-polar extracts using a HP-5 MS capillary column (Hewlett-Packard, CA, USA) (30m x 320 μ m x 0.25), 0.25 mm thickness in an Agilent 7890B GC system coupled to an Agilent 5977A mass detector. The helium carrier gas constant flow rate was set at 1 mL / min. The oven temperature initial temperature at 100 °C was held for 2 min. It was raised at a rate of 10 °C/min isothermally for 30 min. Sample of 0.3 mg/mL in dichloromethane were manually injected at 250 °C, at a volume of 1.0 μ L in the splitless mode. Mass spectra were obtained by EI at electron energy of 70 eV (Ahuchaogu et al. 2018; Phiri and Pheko 2021).

Phytochemical constituents were identified by matching their mass spectra with those found on the National Institute Standard and Technology (NIST 2012 version) database. The identification was also supported by reported studies. Peak areas were used to determine the relative percentage composition of each compound (Hamid et al. 2016; Akwu et al. 2019).

Statistical analysis

Statistical analysis for determining significant differences in active compounds quantities was based on one-way analysis of variance (ANOVA) followed by Tukey test. Values were regarded as statistically significant at $P < 0.05$.

Results

Extractions

Grewia flava roots and twigs were extracted using various solvents, and it was found that distilled water produced the highest extractible material from both plant parts (29.88% and 15.72% for the roots and twigs respectively), whereas acetone resulted in the lowest yield in both scenarios (Table 1). When choosing an extraction method, extractive yield should be considered because low yield is a drawback in natural products research. Non-polar compounds were extracted at higher yield from the twigs (6.97%) than from the root (0.38%) in hexane. The percentage yield of the extracting solvent showed that water was the best extraction solvent for both twig and roots, followed by 80% methanol, hexane and acetone.

Table 1 *Grewia flava* twig and root extracts yields, Total phenolic and flavonoid contents

Sample	Plant part	Extract yield (%)	Total phenolics (mg GAE/ g)	Total Flavonoids (mg QUE/ g)
Hexane	Root	0.38	28 ± 1 ^f	5.64 ± 0.01 ^f
	Twig	6.97	43.1 ± 0.2 ^b	9.106 ± 0.005 ^b
Acetone	Root	0.17	80 ± 0.8 ^c	16.22 ± 0.09 ^g
	Twig	0.53	86.9 ± 0.8 ^c	20.01 ± 0.05 ^c
80% methanol	Root	19.39	217 ± 2 ^e	56.16 ± 0.02 ^e
	Twig	10.32	250 ± 2 ^a	64.54 ± 0.04 ^a
Distilled water	Root	29.88	230 ± 3 ^g	51.15 ± 0.03 ^h
	Twig	15.72	192 ± 2 ^d	48.86 ± 0.09 ^d

All the results are represented as means of three individual experiments ± SEM (n=3). Results under each test that have a distinct superscript letter are statistically different ($p \leq 0.05$).

Phytochemical analysis

Phytochemical analysis was carried out on *G. flava* 80% methanol twig and root extracts. The screening exhibit the presence of alkaloids, flavonoids, saponins, steroids, glycosides, anthraquinones and tannins in both extracts.

Total phenolic content and flavonoid content

The biological activities of plant extracts have been linked to phenolic compounds and flavonoids (Akwu et al., 2019; Kumar et al., 2022) that constitutes plant extracts, hence why it was imperative to estimate the total phenolic content (TPC) and total flavonoid content (TFC) presented in Table 2. The aqueous methanol and distilled water recorded high concentrations of both TFC and TPC. For the twig extracts, methanol extract (250

± 2 GAE/g) exhibited high concentration of phenolic compounds followed by distilled water extract (192 ± 2 mg GAE/g), with the hexane extract (43.1 ± 0.2 mg GAE/g) exhibiting the lowest TPC value, and for the root extracts similar trend was observed except for the aqueous extract showing the high TPC compared to methanol extract. The same trend was also observed for the flavonoid estimation where 80% methanol extracts exhibited highest flavonoid content (Table 1).

Antioxidant activities

The study examined the ability *G. flava* extracts to scavenge free DPPH radicals. The results showed that all the extracts had a dose-dependent radical scavenging ability, with 80% methanol extracts showing the strongest ability under each plant part with IC_{50} values of 14.5 ± 0.7 and 98 ± 7 μ g/mL for the twig and root extract respectively (Table 2).

Table 2 Antioxidant activities of *Grewia flava* crude extracts.

Sample	Plant part	Metal chelation ability, IC_{50} (μ g/mL)	DPPH radical scavenging ability, IC_{50} (μ g/mL)	Reducing power capacity (mg AAE/g)
Hexane	Root	286 ± 1^a	382 ± 42^f	252 ± 1^a
	Twig	428 ± 15^a	70 ± 1^e	450 ± 6^g
Acetone	Root	268 ± 2^b	199 ± 42^b	281 ± 1^c
	Twig	208 ± 21^b	88.4 ± 0.9^f	433 ± 3^f
80% methanol	Root	179 ± 2^c	98 ± 7^c	637 ± 3^b
	Twig	110 ± 24^c	14.5 ± 0.7^g	745 ± 1^e
Distilled water	Root	265 ± 3^b	196 ± 16^b	247 ± 3^a
	Twig	141 ± 11^d	495.0 ± 0.7^h	183 ± 2^d
Ascorbic acid	-	-	23 ± 1^d	-
EDTA	-	70 ± 4^e	-	-

All the results are represented as means of three individual experiments $\pm$ SEM (n=3). Values with different superscript letters under each test are significantly different ($p \leq 0.05$), -: indicate not applicable.

The *n*-hexane and acetone twig extracts showed better activity, despite having relatively low concentrations of compounds (Table 1 and 2), which suggests that the antiradical activity was not exclusive to compounds.

The study employed the FRAP assay to evaluate the extracts' capability to donate electrons and convert ferric iron to ferrous iron. The FRAP results are presented in Table 2. The outcomes indicated that the aqueous methanol extracts had a stronger electron donating ability, with the twig extract exhibiting a reducing power of 745 ± 1 mg AAE/g, while the root extract displayed a reducing ability of 637 ± 3 mg AAE/g. This trend was

expected as the extract’s ability to reduce ions depends on the availability of phytochemicals that perform their antioxidant function by donating hydrogen or electrons, thus neutralizing free radicals. The observed pattern in electron donating ability was similar to the trend in DPPH scavenging ability, indicating a comparable mechanism for both.

The chelating ability of plant extracts and the reference standard tested are shown in Table 2. Antioxidants from the plant extract compete with *O*-phenanthroline to form complexes with Fe²⁺ ions. *O*-phenanthroline forms red-coloured complexes with Fe²⁺ which can be quantified spectrophotometrically. The assay showed that all extracts and EDTA had a dose-dependent response, with increased concentration leading to increased chelation ability. The aqueous methanol extracts showed strong chelation ability with IC₅₀ values of 98 ± 7 and 110 ± 24 µg/mL for the twig and root extracts respectively. However, EDTA had a stronger chelation ability. Twig extracts exhibited higher chelation ability than root extracts in all scenarios except for the aqueous extract.

The DPPH radical scavenging abilities of the 80% methanol twig extract column fractions are displayed in Table 3. Fraction 14, ethyl acetate (EtOAc, 100%) demonstrated the most effectiveness in DPPH radical quenching, (IC₅₀ of 10.6 ± 0.3 µg/mL) which was better than the parent extract. Fractions 1, 23, and 24 also showed significant radical quenching capacity (IC₅₀ of 15 ± 0.6 to 18 ± 2 µg/mL) in comparison to the reference standard (23 ± 1 µg/mL). All the fractions, except fraction 9, displayed a concentration dependent ability to scavenge DPPH radicals.

Table 3 Antioxidant activity of the twigs methanol extract column fractions

Column fraction	Eluent composition*	IC ₅₀ (µg/mL)
Fraction 1	Hex (100%)	18 ± 2 ^a
Fraction 2-13	Hex: EtOAc (4:1, 4:2, 4:3, 4:4	119-848± 1-4 ^{b,c,d,f,g,h,m}
Fraction 14	EtOAc (100%)	10.6 ± 0.3 ^k
Fraction 15-18	EtOAc: MeOH (4:1, 4:2)	44-491 ± 2-4 ^{m,g,n,o}
Fraction 19	EtOAc: MeOH (4:2)	77 ± 2 ^o
Fraction 20	EtOAc: MeOH (4:2)	27 ± 2 ⁱ
Fraction 21	EtOAc: MeOH (4:3)	37 ± 2 ^p
Fraction 22	EtOAc: MeOH (4:3)	49 ± 1 ^l
Fraction 23	EtOAc: MeOH (4:3)	15.1 ± 0.6 ^{a,k}
Fraction 24	EtOAc: MeOH (1:1)	18 ± 2 ^{a,k}
Ascorbic acid		23 ± 1 ^q

*Solvent system ratio was volume/volume

All the results are represented as means of three individual experiments $\pm$ SEM (n=3).

Significant differences exist between values with distinct superscript letters ($p < 0.05$).

Biological activities of the extracts and column fractions

The MIC was determined for samples with zones of inhibition ≥ 15 mm only. Samples with zones of inhibition ≤ 15 mm were considered intermediate.

Antimicrobial activity

The antimicrobial activity of methanol extracts of the twigs and roots of *G. flava* showed that both crude extracts were active against all organisms tested (Table 4 and 5).

The column fractions exhibited different degrees of activity against the microorganisms tested, with fractions 14 and 24 being the most effective. MIC values ranged from 0.02 to 1.70 mg/mL and extracts with MIC values less than 0.1 mg/mL exhibit good antibacterial activity (Malada et al. 2023). *P. aeruginosa* was susceptible to more column fractions compared to other bacterial strains. Gram-negative bacteria were resistant to plant extracts and this was due to their complex and multi-layered cell wall which acts as a barrier to many environmental substances including synthetic and natural antibiotics as previously reported (Rakholiya and Chanda 2014).

Table 4 Antibacterial activity of *Grewia flava* crude extracts and twig extract column fractions

Extracts	Zone of Inhibition (mm)					
	Bacterial strains				Fungal strains	
	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>A.niger</i>	<i>R. oryzae</i>
80% MeOH twig extract	30.0 ±0.6 ^a	31 ±2 ^a	28.3 ±0.3 ^a	20.3 ±0.7 ^a	24.3±0.3 ^a	22.7 ±0.7 ^a
80% MeOH root extract	27 ±1 ^{a, b}	24.3 ±0.9 ^b	18.3 ±0.7 ^b	24 ±2 ^{a, b}	16.0± 0.6 ^b	21.3 ±0.3 ^{a, b}
10% DMSO	ND	ND	ND	ND	ND	ND
Chloramphenicol	35.3 ±0.9 ^c	34.3 ±0.9 ^a	28.7±0.9 ^a	31.3 ±0.3 ^c	NT	NT
Fraction 1	ND	9.0± 0.6 ^c	ND	ND	ND	9.0± 0.6 ^c
Fraction 2	ND	19 ±2 ^b	ND	ND	14.0±0.7 ^{b, c}	14.3±0.7 ^d
Fraction 3	22.7 ±0.9 ^{b, d}	21.3 ±0.3 ^b	ND	ND	13.5±0.5 ^{c, d}	15.2±0.4 ^{d, e}
Fraction 4	20.3 ±0.9 ^d	19.3 ±0.9 ^b	ND	ND	15.2±0.4 ^{b, f}	15.5±0.3 ^{d, e}
Fraction 5	ND	9.3± 0.3 ^c	ND	ND	ND	10.7±0.3 ^{c, f}
Fraction 7	ND	ND	ND	ND	11.3±0.3 ^{d, e}	12.3± 0.3 ^{d, f}
Fraction 8	ND	ND	ND	ND	8.3± 0.3 ^d	9.3± 0.7 ^{c, f}
Fraction 9	ND	ND	ND	ND	ND	8.3± 0.3 ^c
Fraction 10	ND	ND	ND	ND	11.7± 0.3 ^{c, d, e}	10.7± 0.3 ^{c, f}
Fraction 12	ND	ND	ND	ND	8.3± 0.3 ^d	ND
Fraction 14	29 ±2 ^a	24 ±2 ^b	25 ±0 ^c	28±1 ^{b, c, d}	23±1 ^a	15.7±0.4 ^{d, h}
Fraction 15	ND	ND	ND	ND	14.7±0.7 ^b	11.5±0.3 ^f
Fraction 16	ND	ND	ND	19 ±2 ^a	ND	19±2 ^{b, e, h, i}
Fraction 6, 11, 13, 17-19, 21	ND	ND	ND	ND	ND	ND
Fraction 20	ND	ND	ND	20.2 ±0.6 ^a	ND	20.0±0.6 ^{a, i}

Fraction 22	8.7±0.3 ^e	10.7 ± 0.3 ^c	8± 0 ^e	ND	8.3± 0.3 ^d	ND
Fraction 23	10.7± 0.3 ^e	8±0 ^c	12.0± 0.6 ^f	8.7± 0.7 ^e	13.0± 0.6 ^{c, e}	ND
Fraction 24	30.7 ±0.3 ^{a, c}	21 ±2 ^b	23.3 ±0.3 ^c	25 ±2 ^{a, d}	21.3±0.7 ^{a, f}	20.2±0.4 ^{a, i}

The values in bold shows susceptibility (zone of inhibition ≥ 15 mm), ND: Not detectable at the tested concentrations. Negative control = 10% DMSO, Positive control = chloramphenicol. Significant differences exist between values with distinct superscript letters ($p \leq 0.05$). Fractions 1-24 were from the Twig 80% MeOH crude extract column chromatography.

GC-MS analysis

The study analyzed the *n*-hexane twig and root extract of *G. flava* using GC-MS and several bioactive compounds such as lupeol, hexadecanoic acid, β -sitosterol, α -amyrin, betulin, and phytol were identified, Table 6 and 7. The root extract contained 23 compounds, while the twig extract contained 28 compounds, with fatty acids being the major constituents in both the twig and root extracts, followed by triterpenes and fatty acid esters. The predominant phytochemical constituents in the root extract were lupeol (23.58%), *n*-hexadecanoic acid (14.58%), oleic acid (10.14%), hexadecanoic acid, methyl ester (9.75%), (*Z*)-9-O-octadecenamide (7.87%). From the twig extract major compounds were 9,12-octadecadienoic acid (25.85%), octacosane (17.98%), *n*-hexadecanoic acid (17.34%), hentriacontane (7.88%), hexadecanoic acid, and methyl ester (5.15%). Lupeol and γ -sitosterol were present in both plant part extracts, while phytol was only identified in the twig extract.

Table 5 MIC of methanol extracts and column fractions from *Grewia flava*.

Extracts	MIC (mg/mL)					
	Bacterial strains				Fungal strains	
	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>A. Niger</i>	<i>R. Oryzae</i>
80% MeOH twig extract	0.52± 0.06 ^a	0.26 ± 0.04 ^a	0.13±0.01 ^a	0.96± 0.02 ^a	0.17± 0.00 ^a	0.20± 0.02 ^a
80% MeOH root extract	0.52 ± 0.00 ^a	0.30± 0.03 ^a	0.11±0.00 ^a	0.86 ±0.13 ^a	0.18± 0.01 ^a	0.27± 0.01 ^{a, b}
Fraction 2	NT	0.7± 0.2 ^b	NT	NT	0.15± 0.00 ^a	0.56± 0.02 ^c
Fraction 3	0.47 ± 0.01 ^b	1.70 ± 0.06 ^c	NT	NT	0.42 ± 0.06 ^b	0.40± 0.01 ^{b, d}
Fraction 4	1.03 ± 0.06 ^c	1.4 ±0.2 ^c	NT	NT	0.49± 0.06 ^b	0.50±0.05 ^{c, d}
Fraction 14	0.30 ± 0.04 ^d	0.28 ± 0.02 ^a	0.02 ± 0.00 ^b	0.31± 0.04 ^b	0.29± 0.03 ^c	0.21± 0.03 ^a
Fraction 15	NT	NT	NT	NT	0.42± 0.05 ^b	NT
Fraction 16	NT	NT	NT	0.52± 0.01 ^c	NT	0.18± 0.08 ^a
Fraction 20	NT	NT	NT	0.921± 0.15 ^a	NT	NT
Fraction 24	0.25± 0.03 ^e	0.34± 0.02 ^a	0.06± 0.01 ^b	0.09 ±0.01 ^d	0.26± 0.02 ^{a, c}	0.03± 0.00 ^e

MIC values are average values of three individual determinations ±SEM. NT: “not tested”. Significant differences exist between values with distinct superscript letters ($p \leq 0.05$).

Discussion

The differences in extract yields in various studies can be attributed to factors such as the specific plant parts under study, season of sampling, geographic location of the plant, and extraction techniques employed (Senhaji et al. 2020). The occurrence of the various phytochemicals explains the application of the plant against different ailments, as they assist the body in combating illnesses and microbial invasion through their antioxidant abilities (Adebiyi et al. 2017; Aryal et al. 2019; Kaur et al. 2021). Common phenolic compounds are phenolic acids, hydroxycinnamic acid derivatives, anthocyanins, and flavonoids which have reported antioxidant, anti-inflammatory, antibacterial, and anti-carcinogenic effects (Adebiyi et al. 2017).

Table 6 Compounds identified from the *G. flava* root extract using GC-MS analysis.

S. No	Compounds	Rt/min ^a	Rel% ^b
1	2-(4-Pyridyl)-4-methylthiazole-5-carboxylic acid	12.528	2.50
2	4,5,6,7-Tetramethyl-2 <i>H</i> -isoindole	12.804	0.81
3	Diisobutyl phthalate	13.14	2.15
4	Hexadecanoic acid, methyl ester	13.651	9.75
5	1,4,4,5,7,8-Pentamethyl-6-chromanol	13.781	0.58
6	<i>n</i> -Hexadecanoic acid	14.051	14.58
7	1,1,7,7-Tetramethyl- <i>s</i> -hydrindacene	14.31	0.48
8	9,12-Octadecadienoic acid (<i>Z</i> , <i>Z</i>)-, methyl ester	15.287	2.85
9	11-Octadecenoic acid, methyl ester	15.34	3.06
10	Methyl stearate	15.557	0.87
11	9,12-Octadecadienoic acid (<i>Z</i> , <i>Z</i>)-	15.669	5.84
12	Oleic Acid	15.722	10.14
13	Octadecanoic acid	15.881	1.19
14	Hexadecanamide	16.075	4.76
15	Heptacosane	16.198	0.26
16	9-Octadecenamide, (<i>Z</i>)-	17.645	7.87
17	Octadecanamide	17.828	4.50
18	Diisooctyl phthalate	19.128	1.01
19	Squalene	21.174	0.82
20	γ-Sitosterol	25.445	0.90
21	Friedelan-3-one	25.721	0.90
22	Lupeol	26.627	23.58
23	Betulin	32.268	0.61

^aRetention times (Rt) on the HP 5MS column.

^b% composition based on peak areas calculated in GC on HP 5MS column.

Table 7 Compounds identified from the *G. flava* twig extract using GC-MS analysis.

S. No	Compounds	Rt/min ^a	Rel % ^b
1	Tetradecanoic acid	11.934	0.63
2	2-Pentadecanone, 6,10,14-trimethyl-	12.839	0.27
3	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	13.139	0.29
4	Hexadecanoic acid, methyl ester	13.651	6.99
5	n-Hexadecanoic acid	14.139	16.99
6	Chlorpyrifos	14.451	5.24
7	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	15.298	2.57
8	9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z,Z)-	15.363	1.94
9	Phytol	15.457	0.51
10	Methyl stearate	15.563	0.45
11	9,12-Octadecadienoic acid (Z, Z)-	15.780	27.19
12	Octadecanoic acid	15.957	3.51
13	7-Methyl-Z-tetradecen-1-ol acetate	16.204	0.53
14	Eicosanoic acid, methyl ester	17.316	0.37
15	12-Methyl-E, E-2,13-octadecadien-1-ol	17.598	0.39
16	9-Octadecenamide, (Z)-	17.639	0.39
17	Tetradecane, 2,6,10-trimethyl-	18.686	0.24
18	Diisooctyl phthalate	19.133	0.29
19	Tetratetracontane	20.192	0.37
20	Tetracosanoic acid, methyl ester	20.433	0.21
21	Hentriacontane	21.615	4.01
22	Octacosane	23.056	9.48
23	Campesterol	24.474	0.54
24	Stigmasterol	24.856	1.37
25	γ-Sitosterol	25.462	5.53
26	β-Amyrin	25.927	1.27
27	Lupenone	26.192	0.67
28	Lupeol	26.533	6.82

^aRetention times (Rt) on the HP 5MS column.

^b% composition based on peak areas calculated in GC on HP 5MS column.

Grewia species have been found to contain appreciable amounts of various phytochemicals, with flavonoids being the major group (Goyal 2012; Akwu et al. 2019; Kumar et al. 2022). The identified phytochemicals suggest a similar composition to other plants within the genus such as *G. Tenax* (Kumar et al. 2022), *G. tiliifolia* (Malhotra et al. 2020), *G. asiatica* (Sinha et al. 2015; Akram et al. 2019). The study confirms earlier findings on the occurrence of tannins, saponins, flavonoids, anthraquinones and alkaloids in *G. flava* extracts (Gololo et al. 2016) while also reporting the presence of glycosides and steroids.

The GC-MS analysis also the presence of terpenoids and phenolics such as lupeol and hexadecanoic acid in *n*-hexane twig extract which showed high antioxidant activity. Previous studies on other *Grewia* species isolated various flavonoids alkaloids, lignans sterols and terpenoids (Kumar et al. 2022). The identification of phenolic compounds in all extracts of *Grewia flava* suggests that the plant has potential for medicinal use in treating various conditions, which is consistent with its traditional use in folklore. The chelation ability was also attributed to the extracts' phenolics and flavonoids, which have been reported to form complexes with iron (II) ions (Sudan et al. 2014).

Crude extracts MIC values ranged from 0.11 to 0.96 mg/ml and this was attributed to the presence of flavonoids and phenolic compounds (Table 1). The study suggests that *G. flava* has the potential to be a source of antimicrobial agents with the ability to cross this barrier (Rakholiya and Chanda 2014). The activity of a plant extract exhibiting < 0.1 mg/ml MIC values are considered to be of pharmacological interest (Malada et al. 2023). Therefore, fractions 14 and 24 of the 80% methanol twig extract (MIC = 0.02-0.09 mg/ml) are worthy of a further study probe its use against microbial infections. Most of the plant extracts and fractions investigated exhibited moderate to good activities against test organisms thus validating the plant use in traditional medicine.

Many of the bioactive compounds identified by GC-MS have been reported for to show various antibacterial, antifungal and anti-inflammatory properties, which may further support the plant's traditional use (Agidew 2022; Kumar et al. 2022). Chlorpyrifos, a widely used insecticide, was unexpectedly identified in the twig extract, and contamination was suspected due to sampling in a widely farming area. Sampling from different areas is recommended to confirm the availability of this compound in the extract.

Conclusions

This study report on the antioxidant and antimicrobial activities of crude extracts and column fractions of *G. flava*. The extracts showed moderate activities attributed to their phytochemical composition. Some column fractions exhibited better activities than the crude extracts which merits further exploration of *G. flava* twig extract to isolate and identify bioactive compounds. *P. aeruginosa* was susceptible to more column fractions. The non-polar extracts GC-MS analysis identified important bioactive compounds such as 9,12-octadecadienoic acid (*Z, Z*) (5.84 and 27.19%), lupeol (23.58 and 6.82%), γ -sitosterol (0.9% and 5.53%) and *n*-hexadecanoic acid (14.58 and 16.99%) in twigs and root non-polar extracts respectively. The twig extract had comparable biological abilities to the root extract therefore its use in herbal preparation can be adopted for *G. flava* sustainable harvesting. The observed biological abilities and identified extracts bioactive compounds supports its use against microbial infections and folklore use. The study was limited in time and instrumentation to cover

isolations to identify extract active compounds. We therefore recommend the Liquid chromatography-mass spectrometry (LC–MS) profiling of the twig extracts components, their isolations and pharmacological studies.

Abbreviations

Hex	<i>n</i> -Hexane
Ace	Acetone
80% MeOH	80% Methanol
TLC	Thin layer chromatography
TPC	Total phenolic content
UV-Vis	Ultraviolet–visible
TFC	Total flavonoid content
QUE	Quercetin equivalents
DPPH	1,1-Diphenyl-2-picrylhydrazyl radical
FRAP	Ferric reducing antioxidant power
AAE	Ascorbic acid equivalents
EDTA	Ethylenediaminetetraacetic acid
MIC	Minimum inhibitory concentration
GC-MS	Gas chromatography–mass spectrometry

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing Interests

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' contributions

All Authors have read and approved the manuscript. GC conducted data collection, analysis and interpretation and manuscript writing. OM developed the study concept, data analysis and interpretation and manuscript writing supervision. KM and DL conducted the biological studies and data analysis. MM conduct plant identifications, collection, data analysis and manuscript editing.

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