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Antioxidative and antibacterial activity of indigenous edible wild fruit species from Bushbuckridge Local Municipality, South Africa

Sinorita Chauke[1], Lungisile Tshitshi[1], Maropeng Erica Matlala[1], Wilfred Otang-Mbeng[1], Bongisiwe Gladys Shelembe[1,2], Peter Tshepiso Ndhlovu[1*]

[1]School of Biology and Environmental Sciences, University of Mpumalanga, Private Bag X11283, Mbombela 1200, South Africa
[2]Agricultural Research Council, Vegetable, Industrial and Medicinal Plants, Private Bag X293, Pretoria 0001, South Africa

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ABSTRACT

Introduction: Antimicrobial drug resistance and unwanted side effects of conventional medicines necessitate research on medicinal plants' use as alternatives. This study investigated the antibacterial and antioxidant properties of *Carissa spinarum*, *Diospyros mespiliformis*, *Euclea crispa*, *Ficus thonningii*, *Strychnos madagascariensis*, and *Strychnos spinosa*.

Methods: Hexane, acetone, and methanol leaf extracts were tested for antimicrobial activity against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* using disc diffusion and microdilution assays, and for antioxidant activity using 2,2-diphenyl-1-picryhydrazyl (DPPH) and ferric-reducing power assays.

Results: Methanol yielded the highest quantities of crude. The extracts of *D. mespiliformis*, *E. crispa*, and *F. thonningii* showed strong antimicrobial activity against *P. aeruginosa* with inhibition zones up to 28 mm and minimum inhibitory concentrations (MICs) from 0.781–1.563 mg/mL. The acetone extract of *D. mespiliformis* also exhibited activity against *K. pneumoniae* (MIC: 3.125 mg/mL). The methanol extract of *E. crispa* displayed potent antioxidant activity, achieving a half-maximal inhibitory concentration (IC_{50}) of 1.42 µg/mL, which was comparable to ascorbic acid at concentrations of 62.5–250 µg/mL ($P > 0.05$). Acetone extracts of *S. spinosa* and methanol extracts of *C. spinarum* demonstrated good ferric-reducing power; however, all the plant extracts were significantly different from ascorbic acid and butylated hydroxytoluene at 250 µg/mL ($P<0.05$).

Conclusion: The leaves of wild plant species exhibited significant antimicrobial and antioxidant activities, making them possible sources of novel compounds for combating infections and oxidative stress.

Implication for health policy/practice/research/medical education:
The leaf extracts of *Carissa spinarum*, *Diospyros mespiliformis*, *Euclea crispa*, *Ficus thonningii*, *Strychnos madagascariensis*, and *Strychnos spinosa* showed antibacterial activities against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. In addition, the leaves of *C. spinarum*, *D. mespiliformis*, *E. crispa*, *S. madagascariensis*, and *S. spinosa* demonstrated potent antioxidant activities. Therefore, these wild plants may serve as potential sources of antibacterial compounds to treat infections caused by *K. pneumoniae* and *P. aeruginosa* and antioxidant compounds suitable for use in skincare products.

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Introduction

There is a significant burden on global healthcare systems and economic well-being due to the prevalence of both communicable and non-communicable diseases (1,2). Globally, these diseases have been linked to the death of thousands of people (3), with non-communicable diseases accounting for 71 % of deaths per year (4). Furthermore, Manyazewal et al (5) found that in Africa, the number of deaths caused by communicable and non-communicable diseases is on the rise. Oxidative stress generates free radicals associated with aging, inflammatory, and degenerative disease (6). Degenerative

***Corresponding author**: Peter Tshepiso Ndhlovu,
Email: Tshepiso.Ndhlovu@ump.ac.za

diseases are regarded as non-communicable diseases (7). Inflammation, cancer, and skin irritations can all be attributed to high levels of free radicals oxidizing biomolecules, resulting in tissue damage and cell death (8). Antioxidants aid in the prevention of oxidation by scavenging free radicals in the human system (9).

Degenerative diseases and disorders are often associated with or accompanied by microbial infections. For instance, atopic dermatitis is a skin condition linked to oxidative stress and microbial infections in the cutaneous tissue (10). *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are some of the common bacterial pathogens affecting the human skin; they are also associated with antimicrobial drug resistance (11). Importantly, skin diseases cover a wide range of conditions that present major challenges in healthcare delivery and management, impacting people of all ages and demographics worldwide (12). Additionally, microbial pathogens can be triggers of degenerative diseases due to the overproduction of free radicals during an infection (13). Moreover, microbial infections are among the most common causes of death across the globe; the choice of antimicrobial treatment is limited owing to the occurrence of antimicrobial drug-resistant strains (14). This has caused researchers to resort to the utilisation of natural products as sources of novel antimicrobial drugs (15). Plant-based antioxidants are used to hinder and manage degenerative maladies (16). Plant-based antioxidants include anthocyanins, beta-carotene, flavonoids, lutein, polyphenols, organosulfur compounds, and vitamins A, C, and E (17).

Plant secondary metabolites have been used as treatments for a wide variety of diseases since ancient times. Plant secondary metabolites have also been used as drug precursors, prototypes, and probes for pharmacology (18). Firoozbahr et al (19) also highlighted the importance of plants as a significant source of novel antimicrobials. Six plant species were selected in this study due to their reported traditional uses in managing various skin ailments, including *Carissa spinarum* L. (Apocynaceae) (20), *Diospyros mespiliformis* Hochst. ex A.DC, *Euclea crispa* (Thunb.) Gürke (Ebenaceae) (21), *Ficus thonningii* Blume (Moraceae) (22), *Strychnos madagascariensis* Poir, and *Strychnos spinosa* Lam. (Loganiaceae) (23). Chauke et al (24) also emphasized the importance of the six plant species as a source of medicine in the Mpumalanga province. Hawas et al (25) reported that *D. mespiliformis* leaves had antioxidant and antimicrobial activities against *Escherichia coli* and *Staphylococcus aureus. Euclea crispa* leaves have antioxidant activity (26) and antimicrobial activities against *Haemophilus influenzae* and *S. aureus* (27). A previous study showed that *F. thonningii* root had potent antioxidant activity and antimicrobial properties against *E. coli* and *Salmonella typhi* (28). *Diospyros mespiliformis*, *S. spinosa,* and *S. madagascariensis* fruit have good antioxidant activity (29-31). This study introduces new insights into the antioxidant and antimicrobial properties of selected plant species from Mpumalanga province, highlighting the influence of environmental and climatic conditions on plant phytochemistry. Unlike most research, which often focuses on fruit, this study examines the antioxidant potential of leaves. The study evaluated the antimicrobial activity of *C. spinarum*, *D. mespiliformis*, *E. crispa*, *F. thonningii*, *S. spinosa*, and *S. madagascariensis*, determining the minimum concentrations at which they are effective. Their antioxidant properties, including respective half-maximal inhibitory concentrations are also evaluated. The findings highlight the potential of indigenous plants from Mpumalanga province as sources of antibacterial agents and antioxidants, offering promising applications in addressing antimicrobial resistance, combating oxidative stress, and managing dermatological conditions such as atopic dermatitis and skin aging.

Materials and Methods

The leaves of *C. spinarum*, *D. mespiliformis*, *E. crispa*, *F. thonningii*, *S. madagascariensis*, and *S. spinosa* were collected from various locations in the Bushbuckridge local municipality, under Chief Mnisi's Tribal Council. Leaves were selected for this particular study since they were easier to process using a blender compared to other plant parts used traditionally such as the stem or bark and roots. Furthermore, while other plant parts such as barks and roots are traditionally used, exploring leaves adds a layer of scientific inquiry into whether different plant parts could also yield useful medicinal properties, contributing to a broader understanding of the plant's therapeutic potential. The village leaders, the Tribal Council and the Mpumalanga Tourism and Parks agency permitted the collection of plant species in the area. The plant species identities were confirmed at the University of Mpumalanga with the help of Dr L.J Ramarumo. Voucher specimens for each plant species were prepared and labelled with the following voucher numbers: *S. spinosa* (SC001), *C. spinarum* (SC002), *F. thonningii* (SC003), *D. mespiliformis* (SC004), *E. crispa* (SC005), and *S. madagascariensis* (SC006) and stored in the Indigenous Flora Research Laboratory at the University of Mpumalanga. The following consumables, manufactured by Sigma-Aldrich Germany, including iodonitrotetrazolium chloride, Nutrient agar, Mueller-Hinton broth, and agar were purchased from Merck Life Science (Pty) Ltd, Johannesburg, South Africa. The solvents hexane, acetone, and methanol were purchased from LabChem, Gauteng, South Africa. The chemicals potassium ferricyanide, trichloroacetic acid, iron(III) chloride, L-ascorbic acid, butylated hydroxytoluene, and amoxicillin trihydrate manufactured by Thermo Scientific, China were purchased from LabChem, Johannesburg, South Africa. A HemTron Strike 280 rotary evaporator (WIGGENS, China) was used to remove the solvents.

Plant extraction

Plant leaves were washed with distilled water, dried at room temperature, and ground into a fine powder with a heavy-duty blender (Zhongshan City Haitai Electrical Co. Ltd, China). Powdered plant material has an increased surface area that allows efficient contact of the extraction solvent with the target phytochemicals (32). The ground plant material (100 g) was sequentially extracted with solvents in order of increasing polarity as described by Ahmed et al (33) with minor modifications. The ground plant material was macerated in 400 mL of acetone, methanol, and hexane. Extraction involved shaking the plant material for 48 hours at room temperature using an orbital shaker. The extract was filtered using a Whatman's No. 1 filter paper and a rotary evaporator was used to remove the solvent. The resulting crude extract was weighed and kept at room temperature until use.

Determination of antimicrobial activity

Bacterial strains and inoculum quantification

The plant extracts were screened against the gram-negative American Type of Culture Collection (ATCC) of *Klebsiella pneumoniae* (ATCC-700603) and *Pseudomonas aeruginosa* (ATCC-27853) obtained from Davies Diagnostics (Pty) Limited, Gauteng. Standard strains were used to ensure reproducibility and facilitate result comparison, given that susceptibility can vary significantly among different isolates of the same microbial species (34). The microbial strains were resuscitated in a Nutrient agar medium and incubated overnight at 37 °C before biological assays. For disc diffusion, the overnight bacterial cultures were spread into a Mueller-Hinton (MH) agar and for microdilution assay, the overnight cultures were transferred into the MH broth and quantified to a 0.5 McFarland standard. The inoculum was adjusted to roughly 5×10^5 CFU/mL for the microdilution assay. Furthermore, acetone served as the negative control, and amoxicillin trihydrate was the positive control. Amoxicillin is a broad-spectrum antibiotic used against various organisms including *K. pneumoniae* and *P. aeruginosa* (35).

Disc diffusion assay

The assay was conducted according to a previous study with some modifications (36). This technique has been applied as a preliminary step to measure the inhibition diameter produced around the disk. Petri dishes were loaded with 25 mL of MH agar medium, allowed to solidify at room temperature in a biosafety cabinet, and then inoculated with overnight cultures of selected test bacteria using the spread plate method. Sterile Whatman filter paper discs (4 mm- diameter) were dipped into 10 µL of 100 mg/mL of each plant extract and allowed to stand for a few seconds to remove the excess extract. The discs were placed equidistant on the MH agar inoculated with the pathogen and incubated overnight at 37 °C. The diameter of the inhibition zone, including the disc's diameter, was measured in millimetres (mm) around the disc.

Determination of the minimum inhibitory concentration (MIC) using microdilution assay

The MICs of plant extracts were evaluated using the serial microplate method developed by Eloff (37) and Masoko et al (38). Plant extracts with a concentration of 100 mg/mL were serially diluted two-fold from well A to H of a 96-well microtiter plate. Each plant extract was tested in triplicate. A hundred microlitre (100 µL) of bacterial culture was added into the wells and incubated at 37 °C overnight. After incubation, 40 µL of Iodonitrotetrazolium chloride (Sigma Aldrich, USA) dye at 0.2 mg/mL was added to the wells for visual assessment of microbial viability. The MIC was recorded as the lowest concentration of extract that inhibited bacterial growth.

Determination of antioxidant activity

DPPH (2,2-diphenyl-1-picryhydrazyl) free radical scavenging activity

The antioxidant potential of plant extracts was determined in triplicate according to Mwinga (39) with slight modifications. A volume of 750 µL of 0.1 mM DPPH solution dissolved in methanol was added to plant extracts dissolved in their respective solvents at 250, 125, 62.5, 31.3, and 15.6 µg/mL. The total volume of the mixture of plant extract and DPPH solution was 5 mL. The control was prepared similarly, replacing the plant extract with methanol. The reaction mixture was kept in the dark at room temperature for 30 minutes, after which the absorbance was measured at 517 nm using an E-SP1100-UV-P spectrophotometer (Biocom Biotech, Gauteng, South Africa). Antioxidant activity was evident in the decrease in absorbance (40). L-ascorbic acid was used as a standard and a solvent in which each plant extract was dissolved as a blank. Equation (1) was used to calculate the percentage of free radical scavenging activity (% RSA).

$$\%RSA = \left[\frac{(Absorbance\ of\ control - Absorbance\ of\ test\ sample}{Absorbance\ of\ control}\right] \quad (1)$$

Where RSA is free radical scavenging activity.

The RSA was graphed against the concentration of the plant extract, and the half-maximal inhibitory concentration (IC_{50}) was calculated from the normalised logarithmic regression curve.

Ferric-reducing antioxidant power (FRAP) assay

The reducing power of plant extracts was assessed following a method described previously (41). Various concentrations of each plant extract (250, 125, 62.5, 31.3, and 15.6 µg/mL) were combined with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL potassium ferricyanide (1 % w/v). The solution was incubated for 20 minutes at 50 °C, after which 2.5 mL trichloroacetic acid

(10 % w/v) was added. The mixture was centrifuged for 10 minutes at 300 rpm. A 2.5 mL volume of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of Iron (III) chloride (0.1 % w/v) and the absorbance was measured at 700 nm. Butylated hydroxytoluene (BHT) and L-ascorbic acid were used as positive controls.

Data analysis

The disc diffusion, microdilution, DPPH, and FRAP assays were performed in triplicate, with the outcomes expressed as the mean and standard deviation of these three replicates. Significant differences in the DPPH and FRAP results (inhibition percentages and absorbance values, respectively) were analysed using one-way ANOVA followed by Tukey's post hoc tests in SPSS version 27, with a significance threshold set at $P < 0.05$. The comparisons between positive controls and different solvent extracts (acetone, hexane, and methanol) were conducted independently for each plant species and for each assay to evaluate differences in antioxidant activity between the solvent extracts within the respective assay.

Results

Dried leaves of *C. spinarum*, *S. spinosa*, *E. crispa*, *F. thonningii*, *S. madagascariensis*, and *D. mespiliformis* collected from the Mpumalanga province were serially extracted using, acetone, hexane, and methanol. The extraction efficiency of different solvents from the dried leaves is presented in Table 1. Methanol extracted the highest quantity of crude from *D. mespiliformis* (10.39%), *E. crispa* (10.09%), and *C. spinarum* (5.79%). This suggests that methanol has a strong affinity with compounds present in these plants compared to acetone and hexane. Acetone and hexane extracted low quantities, which was less than 5% in all plant samples.

The disc diffusion assay was employed to assess the antimicrobial properties of dried leaves of *C. spinarum*, *D. mespiliformis*, *E. crispa*, *F. thonningii*, *S. madagascariensis*, and *S. spinosa* against *P. aeruginosa,* and *K. pneumoniae.* As detailed in Table 2, the majority of the plant extracts did not exhibit activity against *K. pneumoniae*, as indicated by "NA" (No activity). These results suggest that these plant extracts do not have antimicrobial activity against this strain. In contrast, *P. aeruginosa* was susceptible to all the plant extracts, especially hexane extracts of *D. mespiliformis* and *E. crispa* with the highest inhibition zone of 28 mm, and *C. spinarum* acetone extract and *S. madagascariensis* acetone, hexane, and methanol extracts with inhibition of 25 mm. Amoxicillin showed strong antimicrobial activity with inhibition zones of 35 mm and 45 mm for *K. pneumoniae and P. aeruginosa,* respectively.

The acetone and methanol extracts of *D. mespiliformis* showed high antibacterial activity against *K. pneumoniae*, with MICs of 3.125 mg/mL and 6.25 mg/mL, respectively. In contrast, the hexane extract of *F. thonningii*, *E. crispa*, and *D. mespiliformis*, along with the acetone and methanol extracts of *C. spinarum*, showed no inhibitory effect on *K. pneumoniae*. However, all plant extracts displayed varying levels of inhibitory activity against *P. aeruginosa*. Particularly, the acetone extract of *D. mespiliformis* demonstrated excellent activity with an MIC of 0.781 mg/mL. Additionally, other extracts, including the methanol extract of *D. mespiliformis* and the methanol, hexane, and acetone extracts of *E. crispa* and *F. thonningii*, showed potent antibacterial activity with a MIC of 1.563 mg/mL. Amoxicillin, a positive control showed strong antimicrobial activity with low MIC values (0.781 and 0.781 mg/mL) for *K. pneumoniae and P. aeruginosa,* respectively.

The dose-dependent radical scavenging activity of methanol, acetone, and hexane leaf extracts of six wild fruit plants is illustrated in Figure 1 and their relative half-maximal inhibitory concentration (IC_{50}) in Table 3. Plant extracts with the lowest IC_{50} have the greatest radical scavenging effect. The IC_{50} of the most potent extracts were in the order: L-ascorbic acid < *E. crispa* methanol extract ≤ *D. mespiliformis* methanol extract < *C. spinarum* methanol extract < *D. mespiliformis* acetone extract < *E. crispa* acetone extract < *S. madagascariensis* methanol extract < *S. spinosa* methanol extract (Table 3). None of the plant extracts demonstrated antioxidant activity higher than L-ascorbic acid, which showed 96.93% inhibition at a concentration of 250 µg/mL and the lowest IC_{50} of 0.07 µg/mL. However, some plant extracts demonstrated antioxidant activities comparable to ascorbic acid. Notably, the methanol extracts of *C. spinarum* and *D. mespiliformis* exhibited a perfect similarity ($P = 1.00$), followed by the methanol extract of *E. crispa* ($P = 0.995$). The methanol extracts of *S. madagascariensis* ($P = 0.450$) and *S. spinosa* ($P = 0.0859$) also showed comparable antioxidant potential.

The curves (Figure 2) show the dose-response reducing powers of methanol, hexane, and acetone extracts of selected plant species. The higher the absorbance of the

Table 1. Extraction yield of the leaves of six wild edible fruit species using various solvents

Solvent	Yield (%)					
	Carissa spinarum	*Diospyros mespiliformis*	*Euclea crispa*	*Ficus thonningii*	*Strychnos madagascariensis*	*Strychnos spinosa*
Hexane	2.12	0.9	1.22	1.38	4.19	2.04
Acetone	2.06	2.8	2.77	1.84	1.41	1.06
Methanol	5.79	10.39	10.09	1.53	2.99	1.96

Table 2. Antibacterial activity of plant extracts against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* assessed through disc diffusion and broth microdilution assays

Plant species	Extractants	*Klebsiella pneumoniae*		*Pseudomonas aeruginosa*	
		Disc diffusion Inhibition zone (mm)[a]	Broth microdilution MIC (mg/mL)	Disc diffusion Inhibition zone (mm)[a]	Broth microdilution MIC (mg/mL)
Carissa spinarum	Hexane	NA	50	22	6.25
	Acetone	NA	NA	25	3.125
	Methanol	NA	NA	15	6.25
Diospyros mespiliformis	Hexane	NA	NA	28	6.25
	Acetone	NA	3.125	20	0.781
	Methanol	NA	6.25	19	1.563
Euclea crispa	Hexane	NA	NA	28	1.563
	Acetone	NA	12.5	22	1.563
	Methanol	NA	12.5	21	1.563
Ficus thonningii	Hexane	NA	NA	20	6.25
	Acetone	NA	25	21	1.563
	Methanol	NA	50	22	3.125
Strychnos madagascariensis	Hexane	NA	100	25	12.5
	Acetone	NA	25	25	3.125
	Methanol	NA	100	25	12.5
Strychnos spinosa	Hexane	NA	50	18	3.125
	Acetone	NA	25	19	3.125
	Methanol	NA	100	20	12.5
Amoxicillin		35	0.781	45	0.781

NA, no activity; MIC, minimum inhibitory concentration. Results are expressed as the mean of three independent measurements and the standard deviation (SD) was zero; [a] Diameter of inhibition zone including disc diameter of 4 mm (10 µL of 100 mg/mL).

reaction mixture at 700 nm the stronger the reducing power. The reducing power of the plant extracts and standards were as follows at 250 µg/mL: BHT > *S. madagascariensis* acetone extract > *S. spinosa* acetone extract > L-ascorbic acid > *C. spinarum* methanol extract > *E. crispa* methanol extract > *D. mespiliformis* methanol extract. The hexane, acetone, and methanol extracts of *C. spinarum*, *D. mespiliformis*, *E. crispa*, *F. thonningii*, and *S. spinosa* showed statistically significant variations ($P<0.001$) when evaluated independently within each plant species and compared to the positive controls, ascorbic acid and BHT. However, there was no significant difference between the hexane and acetone extracts of *S. madagascariensis* ($P=0.058$).

Table 3. Half-maximal inhibitory concentration (IC_{50}) of acetone, hexane, and methanol extracts of the leaves of six wild edible fruit species

Plant species	Extractants	IC_{50} (µg/mL)
L-ascorbic acid	Methanol	0.07
Carissa spinarum	Hexane	>250
	Acetone	>250
	Methanol	1.51
Diospyros mespiliformis	Hexane	>250
	Acetone	1.92
	Methanol	1.42
Euclea crispa	Hexane	82.27
	Acetone	3.19
	Methanol	1.42
Ficus thonningii	Hexane	49.40
	Acetone	20.49
	Methanol	11.14
Strychnos madagascariensis	Hexane	34.81
	Acetone	13.07
	Methanol	3.29
Strychnos spinosa	Hexane	134.29
	Acetone	10.38
	Methanol	3.60

Discussion

Extraction yield is affected by the type of solvent, the solvation power, and the affinity (42,43). Polar compounds are most commonly extracted using a polar solvent such as methanol (44). Similar to the results of the present study, previous studies have reported methanol as the best solvent for high plant extract yield (45).

Contrary to the current findings, a previous study (46) found that the methanol extract of *C. spinarum* leaves had antimicrobial activity against *K. pneumoniae* with an inhibition zone of 11.0 ± 0.23 mm at 100 mg/mL. The butanol fraction of *E. crispa* leaves at 10 mg/mL had an inhibition zone of 18 mm against *K. pneumoniae* and 16 mm against *P. aeruginosa* (47) which is comparable to the 21 mm inhibition zone obtained from the methanol and acetone extracts of *E. crispa* (Table 2). A study conducted by Ijoma et al (48) revealed that the hexane and methanol leaf extracts of *F. thonningii* had an inhibition zone of 19.2 and 20 mm respectively against *K. pneumoniae*, which is contrary to the findings of this study (Table 2), wherein the plant extracts showed no activity against *K. pneumoniae*. The variances in the antimicrobial activities in the current study as compared to the literature may be

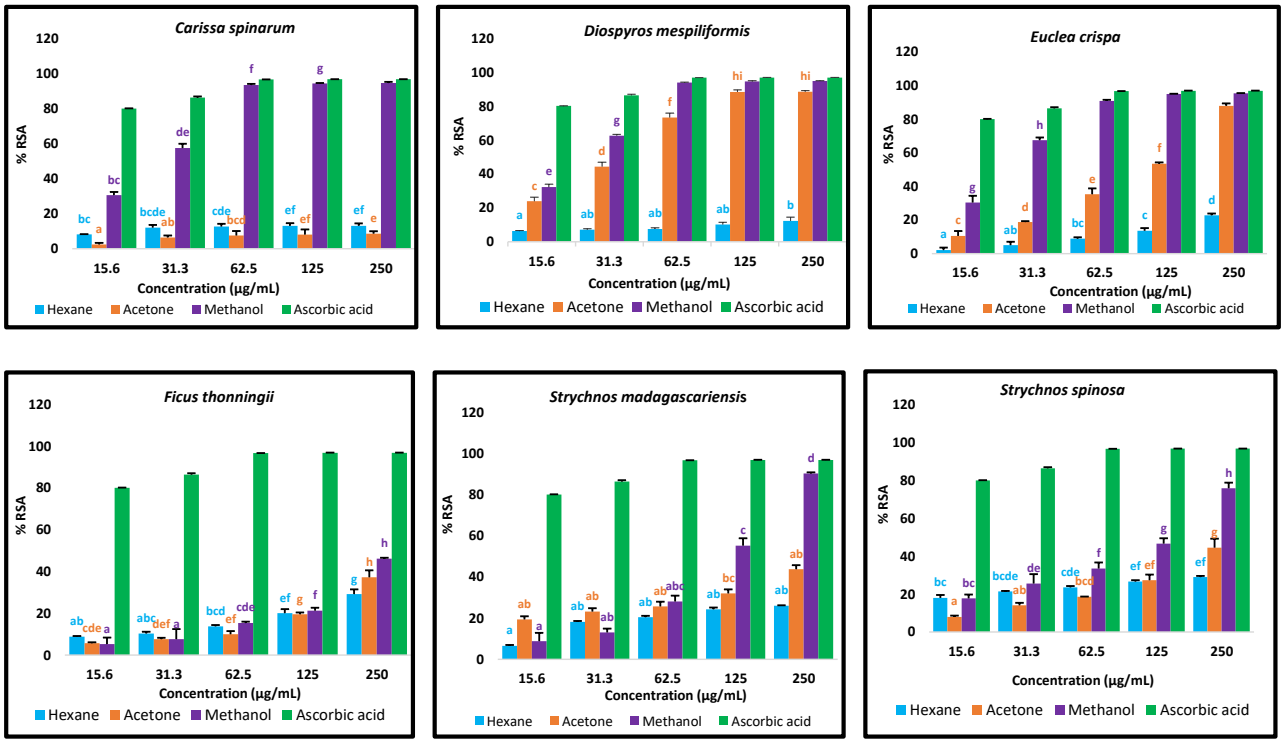

Figure 1. Antioxidant activity of hexane, acetone, and methanol leaf extracts from *Carissa spinarum, Diospyros mespiliformis, Euclea crispa, Ficus thonningii, Strychnos spinosa,* and *Strychnos madagascariensis*, evaluated against the 1,1-diphenyl-2 picrylhydrazyl (DPPH) radical. Letter labels on the bar graphs indicate significant differences ($P < 0.05$) in the antioxidant activities of each plant extract compared to the positive control (ascorbic acid). Different letters indicate a significant difference ($P < 0.05$) between the antioxidant activities of plant extracts.

resulting from differences in the geographical location, seasonality, and climatic conditions from which the plant species were collected since geographical and climatic conditions affect the phytochemistry of plants and thereby their biological activity (49). Furthermore, different types of cultures of the same bacterial strain may have different susceptibilities to plant extracts. For instance, a previous study (47) found differences in the antibacterial activity of *E. crispa* leaves against *K. pneumoniae* (ATCC 13047) and *K. pneumoniae*.

Hexane leaf extracts of *D. mespiliformis* demonstrated strong antibacterial activity against *P. aeruginosa* with MICs ranging from 156.25 to 312.5 µg/mL (50) like the hexane, acetone, and methanol leaf extracts (Table 2). The current study found that the methanol and acetone extracts of *E. crispa* had much higher MICs of 12.5 mg/mL against *K. pneumoniae*. Interestingly, the methanol, hexane, and acetone extracts of *E. crispa* (Table 2) each had an MIC of 1.563 mg/mL against *P. aeruginosa* which may indicate that all the plant extracts contain antimicrobial phytochemicals whose mechanism of action needs to be studied. Ultimately, *E. crispa* and *D. mespiliformis* had very similar activities with MIC of 1.563 mg/mL against *P. aeruginosa* which may result from the fact that both species belong to the Ebenaceae family and may contain similar compounds. For instance, a previous study (51) identified a flavonoid with antibacterial activity known as rutin from the leaves of *E. crispa*. A previous study (25) also isolated rutin from the leaves of *D. mespiliformis*. The current research indicated that the methanol and acetone extracts of *F. thonningii* had poor antimicrobial activities with MICs of 25 and 50 mg/mL against *K. pneumoniae*. The findings of the current study also displayed that the acetone and methanol extracts of *S. madagascariensis* and *S. spinosa* had similar activity against *P. aeruginosa* (Table 2), due to shared phytoconstituents responsible for antimicrobial activity among plants of the same family and genus (52).

The disc diffusion method was used for preliminary screening to obtain qualitative results in the form of inhibition zones while the microdilution assay was used to determine the quantitative results in the form of the MIC of plant extracts (53). The current study's findings proved the microdilution assay to be the most sensitive method of determining the antibacterial activities of plant extracts. Similarly, a study (54) found that plant extracts did not show antifungal activity in disc diffusion but showed activity in microdilution assay. Scorzoni et al (54), further elaborated that the antimicrobial effectiveness of different samples may not always be accurately detected due to variances in physical properties like solubility, volatility, and diffusion in agar. Moreover, factors such as the agar volume, microbial strains used, adsorption by the disk, disk size, quantity of compound applied, type and strength of agar, and pH, can all impact the size of inhibition zones. The disc diffusion method is a simple, cost-effective

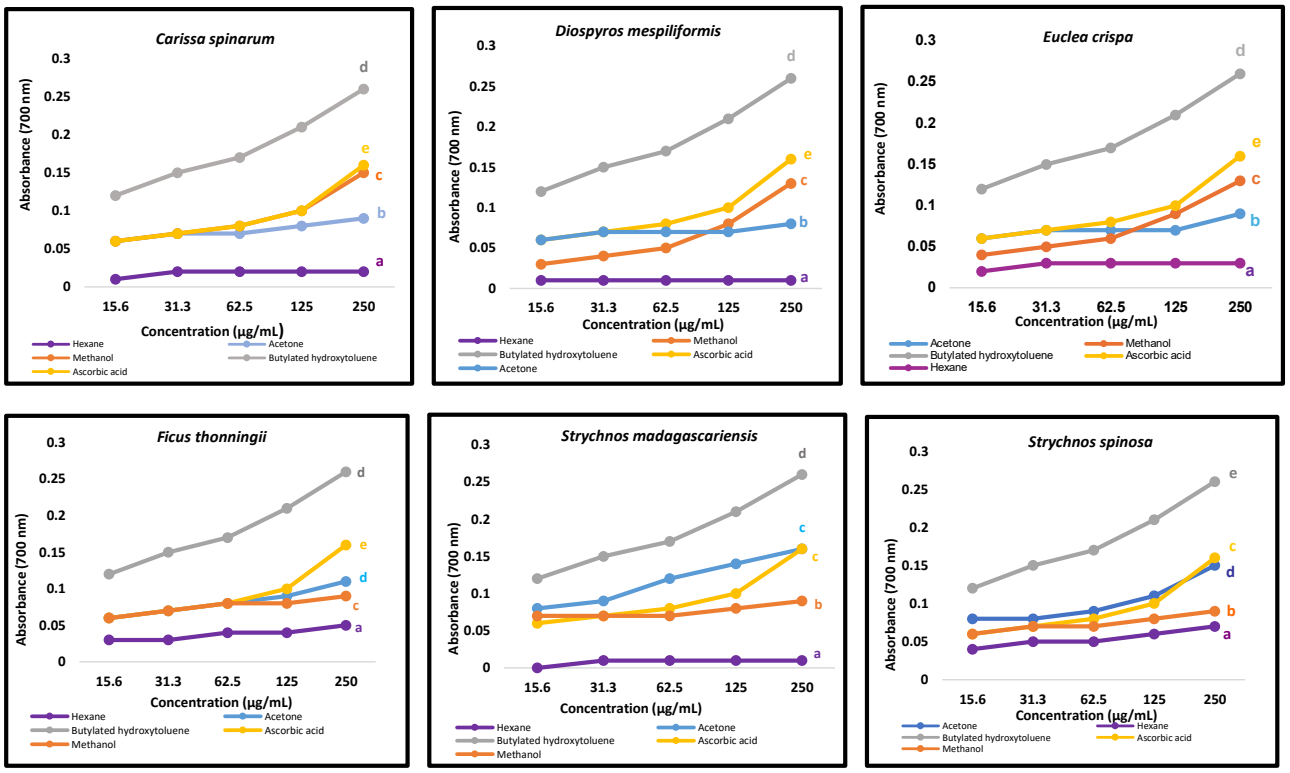

Figure 2. Ferric reducing power of hexane, acetone, and methanol leaf extracts from *Carissa spinarum, Diospyros mespiliformis, Euclea crispa, Ficus thonningii, Strychnos spinosa,* and *Strychnos madagascariensis*. Distinct letters on the line graphs indicate significant differences ($P < 0.05$) in the reducing power of each extract compared to the positive controls (ascorbic acid and butylated hydroxytoluene) at 250 µg/mL, whereas the same letter denotes no significant difference ($P > 0.05$).

technique that allows multiple antimicrobial agents to be tested on a single plate, with results easily interpreted through visible bacterial growth since bacterial growth can be directly observed on the plate (55).

Diospyros mespiliformis Hochst. ex A.DC and *Euclea crispa* (Thunb.) Gürke are species of the Ebenaceae family with excellent antioxidant activities. Previous studies show that Ebenaceae plant species have good antioxidant activities including *Diospyros ebenum* (56). The results (Figure 1) indicate that methanol extracts of *E. crispa*, *C. spinarum*, *D. mespiliformis*, and *S. madagascariensis* had excellent antioxidant activities at >90% which is comparable to ascorbic acid, which was the most potent with 96.93% radical scavenging ability. Furthermore, the results (Figure 1) show that the acetone extracts of *D. mespiliformis* and *E. crispa* had considerable antioxidant activities with 88.51% and 87.9%, respectively. Hexane extracts had the least antioxidative effect in each plant species with the highest antioxidative effect with IC_{50} of 34.81 µg/mL observed in *S. madagascariensis*. The current findings (Table 3) indicate that *D. mespiliformis* methanol and acetone extracts of leaves have good antioxidant activity with an IC_{50} of 1.42 and 1.92 µg/mL, respectively. Hegazy et al (57) also found that the fruit of *D. mespiliformis* had good antioxidant activity with percentage inhibition of up to 87.36% comparable to the 88.51% inhibition by the leaves obtained in the current study (Figure 1). *E. crispa* also showed great antioxidant activities with IC_{50} of 1.42 µg/mL (Table 3). However, Palanisamy et al (26) found a much higher IC_{50} value of 135.4 ± 0.7 µg/mL from the ethanolic extract of *E. crispa* leaves. *C. spinarum* methanol extract also showed notable activities against DPPH with an IC_{50} of 1.51 µg/mL. Liu et al (58) isolated ten phytochemicals from the ethanol fraction of the root bark of *C. spinarum* that displayed antioxidant activities against DPPH, especially one unidentified compound with an IC_{50} value of 16.5 ± 1.2 µM. The methanol extracts of two plant species belonging to the Loganiaceae family, *S. spinosa* and *S. madagascariensis* had IC_{50} of 3.29 and 3.60 µg/mL, respectively. The IC_{50} values are almost equal, which may be because they belong to the same family and genus (*Strychnos*). Isa et al (59) evaluated the antioxidant potential of the leaf of *S. spinosa* using DPPH radical assay and the results showed free radical scavenging activities of acetone, methanol, and dichloromethane/methanol extracts with IC_{50} values ranging from 33.66–230.15 µg/mL. From their study, methanol extract had the most noteworthy activity with IC_{50} of 36.56 µg/mL which is much lower compared to the current findings. *F. thonningii* had the least antioxidant activity with the highest scavenging activity of 46.19% exhibited by methanol extract at 250 µg/mL. However, Fongang et al (60) reported that the methanolic stem roots extract has good free radical scavenging activity (68.30, 75.20, and

81.26%) at 10, 50, and 100 µg/10 µL, respectively.

Ferric-reducing power (FRAP) assay measures the ability of plant extracts to reduce ferric ions (Fe^{3+}) to ferrous (Fe^{2+}) due to their reductive properties. When iron (III) chloride ($FeCl_3$) is added to a solution containing the ferrous (Fe^{2+}) form, a chemical reaction whereby the Fe^{3+} ions from $FeCl_3$ react with the Fe^{2+} ions to form Prussian, blue-colored complex forms. Thus, the extent of reduction can be assessed by measuring the formation of Perl's Prussian blue at 700 nm. Greater absorbance signifies a stronger ferric-reducing power (8). In the current study (Figure 2), hexane extracts showed the least ferric-reducing power in all the selected plant species. However, the positive control (BHT) had the most potent reducing power incomparable to the rest of the plant extracts. Interestingly, the reducing power of *S. madagascariensis* was comparable to ascorbic acid. The present study also showed a dose-dependent relationship between the plant extract concentration and the ferric-reducing power. The current study showed that the ferric-reducing power of plant extracts was good at 125 and 250 µg/mL. The ferric-reducing abilities of *S. madagascariensis*, *S. spinosa*, *D. mespiliformis,* and *E. crispa* were closely related since they belong to the Loganiaceae and Ebenaceae families, respectively.

The current study revealed that some plant extracts exhibited a high scavenging effect against DPPH but low reducing power. For example, methanol extracts of both *S. madagascariensis* and *S. spinosa* showed high DPPH radical scavenging activity but weaker ferric-reducing power than acetone extracts. Conversely, methanol extracts of *D. mespiliformis*, *E. crispa*, and *C. spinarum* demonstrated strong DPPH radical scavenging activity and ferric-reducing power. In a previous study (61) some plant extracts exhibited good DPPH radical scavenging activity and a good ferric-reducing power. Additionally, high antioxidant activity is linked to high total phenolic content (62). Methanol extracts showed the best activity in both assays compared to acetone and hexane extracts (Figures 1 and 2), indicating that methanol was a good solvent for extracting antioxidant compounds. Hexane extracts, however, showed less activity in both assays.

This study faced several limitations including plant samples collected solely from one region in Mpumalanga, which may limit the applicability of findings, as medicinal properties can vary by geography and environmental factors. Additionally, using ATCC strains instead of clinical ones may not accurately represent the plant extracts' effectiveness against real-world infections. The study was restricted to two gram-negative bacterial species, limiting insight into the broader antimicrobial potential of plant extracts. The research conducted *in vitro* may not fully predict *in vivo* efficacy (63).

Conclusion

The leaves of the tested wild plant species possessed antimicrobial and antioxidant properties, which were influenced by the solvents used for extraction. These findings provide scientific support for the traditional use of these plants in treating infectious skin conditions and oxidative stress. Consequently, these wild plant species represent a valuable reservoir of therapeutic compounds and require additional exploration. Future studies should prioritize identifying the active compounds responsible for the antimicrobial and antioxidant effects. Research should also isolate and characterize these bioactive compounds, which have potential applications in conventional healthcare, dermatology, and skin care product development. Additionally, *in vivo* studies are needed to validate the efficacy observed *in vitro*, and testing should be expanded to include a broader range of bacterial strains for a more comprehensive understanding of the antimicrobial potential. Cytotoxicity assessments are essential to confirm that these plant extracts can be used safely without harmful effects.

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Authors' contribution

Conceptualization: Sinorita Chauke, Bongisiwe Gladys Shelembe.
Data curation: Sinorita Chauke.
Formal analysis: Sinorita Chauke.
Funding acquisition: Wilfred Otang-Mbeng.
Investigation: Sinorita Chauke.
Methodology: Sinorita Chauke, Bongisiwe Gladys Shelembe, Lungisile Tshitshi.
Supervision: Bongisiwe Gladys Shelembe, Peter Tshepiso Ndhlovu, Wilfred Otang-Mbeng.
Writing–original draft: Sinorita Chauke.
Writing–review & editing: Peter Tshepiso Ndhlovu, Lungisile Tshitshi, Maropeng Erica Matlala, Wilfred Otang-Mbeng, Bongisiwe Gladys Shelembe.

Conflict of interests

The authors declare no conflict of interest.

Ethical considerations

The ethical clearance was approved by the University of Mpumalanga ethics board with the ethics reference: UMP/Chauke/230013937/MSc/2024. Permission to collect plant species was issued by the Mpumalanga Tourism and Parks Agency and the Mnisi Traditional Council of Bushbuckridge Local Municipality.

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