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Potential therapeutic effects of *Hermannia depressa* N.E.Br. root extracts

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Background: *Hermannia depressa* is a medicinal plant species from the Malvaceae family, and it is traditionally used in the treatment of ailments in which microbial and inflammatory processes are involved.

Aim: This study aimed to validate the traditional medicinal claims of *H. depressa* through *in vitro* antimicrobial, anti-inflammatory, antioxidant, cytotoxicity, and phytochemical studies.

Setting: The roots of *H. depressa* were collected in Thaba 'Nchu, Free State province, South Africa.

Methods: The broth microdilution method, nitric oxide assay, the DPPH assay and Hoechst 33 342 nuclear dye and propidium iodide exclusion method were used to evaluate anti-microbial, anti-inflammatory, antioxidant activity and cytotoxicity, respectively. Qualitative and quantitative phytochemical screening methods were used to evaluate the bioactive compounds of the extracts.

Results: Methanol and acetone extracts from both plants showed antimicrobial activity against 13 microorganisms, with minimum inhibitory concentrations (MICs) ranging from 0.1 to 1.25 mg/mL, with *Candida albicans* and *Bacillus cereus* being the most inhibited isolates. Methanol and acetone extract further showed strong anti-inflammatory activity by inhibiting nitric oxide by more than 50%. Methanol (IC_{50} = 5.197 ± 0.10 µg/mL) and acetone (IC_{50} = 3.576 ± 0.44 µg/mL) extracts showed higher antioxidant capacity than ascorbic acid and Trolox. Acetone and methanol extracts demonstrated significant toxicity towards RAW 264.7 macrophages. Compounds with various pharmacological properties were detected from the H. depressa extracts

Conclusion: These findings support traditional use of H. depressa to treat ailments and has potential to be a source of therapeutic agents.

Contribution: This study contributes to the already existing knowledge on the pharmacological value of *H. depressa*.

Keywords: *Hermannia depressa*; antimicrobial activity; anti-inflammatory activity; antioxidant activity; cytotoxicity; Malvaceae.

Introduction

Inflammation is an immune response caused by many factors including, among others, microbial infections from bacteria, viruses, fungi, or other pathogens. While inflammation is a fundamental defence mechanism, chronic or dysregulated inflammatory processes can lead to the development of various disorders (Bennett et al. 2018). Importantly, both microbial infections and chronic inflammation can induce oxidative stress, a state characterised by an imbalance between the production of reactive oxygen species and the body's antioxidant defences. Oxidative stress, in turn, exacerbates inflammation and can cause cellular damage, potentially leading to a range of life-threatening diseases such as atherosclerosis, hypertension, heart failure, Alzheimer's disease, inflammatory disorders, cancer and many more (Kumar et al. 2022).

Non-steroidal anti-inflammatory drugs such as aspirin, naproxen, ibuprofen, diclofenac and many others are commonly prescribed for the management of various inflammatory disorders such as rheumatoid arthritis, asthma, chronic obstructive pulmonary, neurodegenerative, and autoimmune diseases, headaches, injuries, pain and many more (Jisha et al. 2019; Wongrakpanich et al. 2018). For microbial diseases, antimicrobial drugs such as penicillin, methicillin, streptomycin, vancomycin, chloroquine, and many others have been used as medication (Schellack & Fourie 2015).

Note: Additional supporting information may be found in the online version of this article as Online Appendix 1.

Similarly, to treat oxidative stress, many synthetic antioxidants have been developed and are currently being used. However, the high cost and the lack of availability of drugs continue to be significant obstacles in the fight against oxidative stress, inflammatory disorders, and microbial infections (Malekmohammad, Sewell & Rafieian-Kopaei 2019). Furthermore, the above-mentioned agents have reported side effects such as gastric ulcers, renal failure, induced nephrotoxicity, cardiovascular complications and gastrointestinal detriments, and development of antimicrobial-resistant strains (McEwen & Collignon 2018; Wongrakpanich et al. 2018). In addition, these drugs have shown limited success in treating many anti-inflammatory and microbial conditions, hence, there is a need to develop safe, affordable, accessible, non-toxic, and active novel drugs.

It has been recorded that 80% of the world's population relies on plants for their primary healthcare needs (Jamshidi-Kia, Lorigooini & Amini-Khoei 2018). People in developing countries such as South Africa rely on medicinal plants for the treatment of various diseases because of a lack of access to healthcare facilities and pricey drugs, as well as the negative side effects of synthetic medication (Ghlissi et al. 2016). Thus, plant usage has become a subject of interest in scientific research and industrial applications, such as medical, nutritional, and cosmetic applications, because plants have shown potential biological properties including, antimicrobial, antioxidant, and anti-inflammatory activities (Diniz do Nascimento et al. 2020).

Medicinal plants are currently being used to synthesise pharmacologically active compounds globally, especially in search of novel drugs (Alqahtani et al. 2019). Plants species contain active compounds with the potential to combat diseases caused by pathogenic microorganisms, inflammatory disorders, and oxidative stress (Chachad, Talpade & Jagdale 2016). These compounds include glycosides, saponins, flavonoids, steroids, tannins, alkaloids, and terpenes with the potential to treat diseases (Naz et al. 2017). The above-mentioned bioactive compounds are known to have various biological properties such as antioxidant, antimicrobial, anticancer and anti-inflammatory activities (Amit Baran 2016). Therefore, medicinal plants can be regarded as a good option to provide leads for the development of new drugs.

Hermannia depressa N.E.Br. is a dicotyledonous plant species belonging to the Malvaceae family and it is known as '*Seletjana*' in Sesotho. The plant is widely distributed in all South African provinces and grows in terrestrial habitat types. The plant has been traditionally used to address various health issues, including abdominal pains during pregnancy, nausea, diarrhoea, heartburn, stomachache, and as an emetic as well as for boosting appetite (Kose, Moteetee & Van Vuuren 2015; Reid et al. 2015). In addition, Ngobeni et al. (2023) reported the use of *H. depressa* to traditionally treat sexually transmitted infections (STIs). Given the above-mentioned traditional applications, this study evaluated the pharmacological potential of *H. depressa* extracts by investigating their antimicrobial, antioxidant, anti-inflammatory, cytotoxic activities, and phytochemical profiles.

Research methods and design

Plant collection and extracts preparation

Hermannia depressa roots were collected in Thaba 'Nchu, Free state, South Africa. Standard methods were used in plant material collection, drying, mounting, preparation, and preservation. The plant was identified first by its vernacular names and later validated by the South African National Biodiversity Institute (SANBI) personnel. Voucher specimen (B007) was deposited at the Central University of Technology, Centre for Applied Food Sustainability and Biotechnology. The roots were collected, washed, oven-dried at 40 °C for 72 h, and ground to powder. The dry, powdered material (50 g each) of the plants was weighed, pulverised, and soaked in 100% methanol, acetone, or water for 48 h on a rotary shaker. After filtering the extracts, further extraction with a new solvent was carried out until the solvent was clear. The solvents of extraction were evaporated at 40 °C, under a vacuum, using a Buchi Rotavapor, while the aqueous extracts were dried through lyophilisation. All extracts were stored in the fridge at 4 °C until required. All aqueous, methanol and acetone extracts were used throughout the experiments. The leaves, stems, and roots of Hermannia depressa can be seen in Figure 1.

Total phenolic content

The total phenolic content of the plant extracts was performed using the Folin-Ciocalteu method by Singleton, Orthofer and Lamuela-Raventós (1990) with modifications from Tahrani et al. (2019). A 100 µL of each extract solution at a concentration of 1 mg/mL, 100 µL of 50% Folin–Ciocalteu reagent and 2 mL of 7.5% sodium carbonate was prepared in triplicates. The mixture was incubated for 30 min at room temperature and the absorbance was measured at a wavelength of 720 nm. Gallic acid was used to formulate a standard curve (0–350 µg/mL; $y = 0.0029x - 0.00121$; $R^2 = 0.995$), and the total phenolic content of gallic acid equivalent (GAE) extracts was calculated using the following formula: C = c.V/m, where C is the total amount of phenolic compounds in mg/g of plant extract in

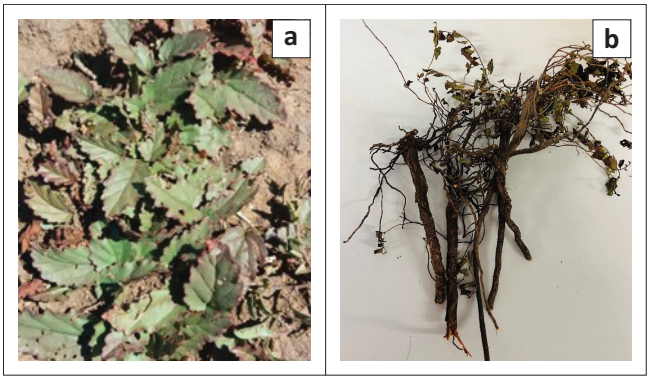


FIGURE 1: Leaves, stems, and roots of *Hermannia depressa* (a and b).

GAE, c is the concentration of gallic acid from the calibration curve (mg/mL), V is the extract volume (mL), and m refers to the weight of pure organic plant extract (g).

Total flavonoid compounds content

The total flavonoid content was determined using aluminium the colorimetric method by Pothitirat et al. (2009) with modifications from Vongsak et al. (2013). A 50 µL of crude extracts (1 mg/mL ethanol) was made up to 1 mL with methanol, mixed with 4 mL of distilled water and then 0.3 mL of 5% $NaNO_2$ solution; 0.3 mL of 10% $AlCl_3$ solution was added after 5 min of incubation, and the mixture was incubated for 10 min. Then, 2 mL of 1 mol/L NaOH solution was added and the final volume of the mixture was brought to 10 mL with double-distilled water. The absorbance of the mixture was measured at 415 nm against a blank sample without aluminium chloride using UV–VIS spectrophotometer. The content of total flavonoids was calculated as mean ± SD (n = 3) and expressed as grams of quercetin equivalents (IQE) in 100 g of the extract and dried powder.

Phytochemical analysis of the plant extracts using Liquid Chromatography with tandem mass spectrometry

Liquid Chromatography with tandem mass spectrometry (LC-MC/MS) analysis for the identification of active compounds from *H. depressa* crude extracts was carried out using the Waters Synapt G2, Electrospray Ionisation (ESI) positive or negative Cone Voltage 15 V, lock mass: leucine encephalin instrument. This is an Agilent 1100 LC system consisting of degasser, binary pump, autosampler and column heater. The column outlet was coupled to an Agilent MSD Ion Trap XCT mass spectrometer equipped with an ESI ion source. Data acquisition and mass spectrometric evaluation were carried out on a computer with an Acquity binary solvent manager instrument system. For the chromatographic separation, Waters BEH C18, 2.1 × 100 mm column was used. The column was held at 95% solvent A (0.1% formic acid in water) and 5% solvent B (0.1% formic acid in acetonitrile) for 1 min, followed by an 11 min step gradient from 5% B to 100% B; then it was kept for 4 min with 100% B; finally, the elution was achieved with a linear gradient from 100% B to 5% B for 2 min. The flow rate was 0.4 mL/min and the injection volume was 0.01 mL. The following parameters were used throughout all mass spectrometry (MS) experiments: for electrospray ionisation with negative ion polarity, the capillary voltage was set to 3 kV, the drying temperature to 350 °C and cone voltage of 15 V, the maximum nebulizer pressure to 15000 psi and the seal wash was 5 min. The total run time was 15 min, the scan speed was 26000m/z/s (ultra-scan mode) and the lock mass was Leucine enkephalin. The phenolics were identified using a combination of High-Performance Liquid Chromatography (HPLC) with diode array detection and liquid chromatography with atmospheric pressure chemical ionisation mass spectrometry (ESI-LC/MS/MS) based on their ultraviolet spectra, mass spectra and by comparison of the spectra with those of available authentic standards.

Antimicrobial activity

Microbial cultures

The antibacterial activity of the plant extracts was evaluated against *Staphylococcus aureus* (ATCC 11632), *Clostridium perfringens* (ATCC 13124), *Pseudomonas aeruginosa* (ATCC2 7853), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 13762), *Staphylococcus epidermidis* (ATCC 12228), *Streptococcus pneumonia* (ATCC 15909), *Streptococcus pyogenes* (ATCC 8668), *Neisseria gonorrhoeae* (ATCC 19424) and *Bacillus cereus* (ATCC 13061). The fungal species used were *Candida albicans* (ATCC 90028), *Candida krusei* (ATCC 6258) and *Candida parapsilosis* (ATCC 22019). All the control bacterial and fungal species were supplied by the National Health Laboratory Services (NHLS) and LANCET, Bloemfontein, South Africa. All the microbial species were maintained in Mueller–Hinton agar plates at temperatures of 4 °C. Prior to treatment with the plant extracts, the bacteria were inoculated in Mueller–Hinton broth, and incubated and shaken at 100 revolutions per min (rpm) for 24 h, to ensure purity and viability. The bacterial suspensions of test strains were adjusted to 0.5 McFarland standard, which equals 1-2 × 10^8 CFU mL^{-1} for bacteria and 1-5 × 10^6 CFU mL^{-1} for yeasts.

Determination of minimum inhibitory concentration

The antimicrobial activity of the extracts was investigated using the microdilution method. A total of 80 µL of the bacterial suspensions was pipetted into the 96 microwell plates already containing 80 µL of diluted plant extract to make a final volume of 160 µL in each well. The concentration of the plant extracts ranged from 0.16 mg/mL to 2.5 mg/mL. The control wells were, respectively, filled with culture medium only, bacterial suspension, 5% of the solvent extraction, and plant extract only. Chloramphenicol (0.125 mg/mL) was used as a positive control in bacteria, while amphotericin B (0.03 µg/mL – 1 µg/mL) was used for yeast. The microwell plates were incubated for 24 h for bacteria and 48 h for yeast. A total of 40 µL of 4 mg/mL iodonitrotetrazolium salt solution was added to each well. Growth was indicated by a change of colour ranging from pink to violet after 10 min to 30 min of incubation. All samples were tested in triplicates. The minimum inhibitory concentration (MIC) was recorded as the lowest concentration at which the plant indicated bacterial or fungal growth inhibition.

Anti-inflammatory activity

Nitric oxide (NO) production and viability of lipopolysaccharide (LPS)-activated RAW 264.7 macrophages were used to determine the anti-inflammatory potential of the selected plants. RAW 264.7 cell lines, seeded into 96-well plates at a density of 1 × 10^5 cells per well and allowed to attach overnight. The extracts were dissolved in Dulbecco's Modified Eagle's Medium (DMEN) complete medium to give final concentrations of 50, 100 and 200 µg/mL, respectively, and stored in room temperature until needed. To assess the anti-inflammatory activity, 50 µL of LPS (final concentration of 500 µg/mL) containing medium was added to the corresponding wells. Aminoguanidine (AG) was used as the positive control

for this experiment. Cells were incubated for a further 18 h. To quantify NO production, 50 µL of supernatant from each well of cell culture plates was transferred to a new 96-well plate and 50 µL Griess reagent was added. Absorbance was measured for 10 min at 540 nm, and the results were expressed relative to the appropriate untreated control. A standard curve using sodium nitrite dissolved in a culture medium was used to determine the concentration of NO in each sample. Percentage inhibition was calculated based on the ability of extracts to inhibit nitric oxide formation by cells compared with the control (cells in media without extracts containing triggering agents and dimethyl sulfoxide [DMSO]), which was considered as 0% inhibition (Dzoyem & Eloff 2015).

Antioxidant activity

The DPPH method was used to test the radical scavenging activity of the aqueous, methanol, and acetone extracts of *H. depressa*. The DPPH was dissolved in methanol to give 0.1 mM solution; 10 µL of extract samples in methanol (or methanol itself as blank control) was added to 175 µL of the methanol DPPH solution. For each extract, different concentrations were tested (200, 100, 50, 25, 12.5, 6.25, 0 µg/mL). The decrease in absorbance was measured at 520 nm after 20 min. The decrease in absorption induced by the test sample was calculated by subtracting that of the control. The antioxidant activity of each test sample was expressed as an IC_{50} value, that is, the concentration in µg/mL that inhibits DPPH absorption by 50% and was calculated from the concentration-effect linear regression curve. The percentage DPPH inhibition was calculated using the formula: (A0-A1)/A0*100 where A0 is the absorbance of the control and A1 is the absorbance of the test sample. Trolox was used for positive control. Trolox equivalents were calculated as the IC_{50} of the test sample relative to that of Trolox expressed as a dry mass.

Cytotoxicity screening

The cytotoxicity of plant extracts was carried out using the Hoechst 33342 nuclear dye and propidium iodide exclusion method. African green monkey kidney cell line was maintained at 37 °C in a humidified incubator with 5% CO_2 in 10 cm culture dishes. The complete growth medium consisted of 10% foetal bovine serum (FBS) supplemented by Dulbecco's Modified Eagle's Medium (DMEM). In each well, cells were seeded into 96 well microtiter plates with a density of 4000 cells/well using a volume of 100 µL. The microtiter plates were incubated for 24 h at 37 °C, 5% CO_2 and 100% relative humidity before adding test compounds to allow cell attachment. Cells were treated with 100, 200 and 400 µg/mL of 100 µL of each extract diluted in culture medium. The cells were also treated with the positive control called melphalan at 100 µg/mL. Cells were further incubated for 48 h. The treatment medium was aspirated from all wells and replaced with 100 µL of Hoechst 33342 nuclear dye (5 µg/mL) and incubated for 10 min at room temperature. Thereafter, cells were stained with propidium iodide (PI) at 100 µg/mL to enumerate the proportion of dead cells within the population. Cells were imaged immediately after the addition of PI using the ImageXpress Micro XLS Widefield Microscope (Molecular Devices) with a 10× Plan Fluor objective, DAPI and Texas Red filter cubes. The dye exclusion technique assumes that non-viable cells will absorb the dye, whereas viable ones would not. After 24 h of treatment with plant extracts, the total cell count was determined using a haemocytometer. Extracts with cytotoxic potential are identified by a 50% or greater reduction in viable cell count compared to untreated control cell cultures. The percentage growth inhibition was calculated using the following formula: % Inhibition = (Total cells-dead cells/Total cells) × 100.

The percentage inhibition versus concentration was plotted and the CTC50 (concentration needed to decrease viability by 50%) value was determined for the cell line. All experiments were carried out in triplicates. The results were calculated as mean ± standard deviation (SD) values.

Data statistical analysis

The values were presented as the mean ± standard deviation of three separate measurements. Correlation and regression analyses were performed using the EXCEL software from Microsoft Corporation, USA. The results were further analysed using one-way analysis of variance (ANOVA) and Tukey-Kramer post hoc test and statistical significance was determined by considering *p*-values less than 0.05 when comparing the different groups using EXCEL software from Microsoft Corporation, USA.

Ethical considerations

The study does not require ethical clearance since there was no direct contact with either human or animal subjects.

Results

Total phenolic and flavonoid content

Total phenolic content (TPC) was conducted to estimate the total amount of phenols present in each concentration of extracts of *H. depressa* shown in Table 1. The total phenol content was extrapolated from the gallic acid standard/calibration graph equation; y = 0.1942x – 0.0174, R2 = 0.9508 and was expressed as mg gallic acid equivalent (GAE)/g from the equation CV/m. The total phenolic content of *H. depressa* extracts exhibited a range of 2.93 to 8.45 mg GAE/g when assessed at a concentration of 1mg/mL for the extracts. The highest concentration of phenolics was found in acetone (8.45 ± 0.006 mg of GAE/g dried extract), followed by methanol and aqueous extracts of *H. depressa* (Table 1). The total flavonoid content (TFC) was calculated to estimate the total amount of flavonoids present in each concentration, as shown in Table 1. The TFC was calculated using the equation calibration curve of quercetin standard (y = 0.6961 x 0.0046, R^2 = 0.997) and was expressed as mg quercetin

equivalent (QE)/g from the equation C = c.V/m. The TFC of all the extracts ranged from 0.97 to 0.53 mg QE/g of extract in 1mg/mL of extract. *Hermannia depressa* aqueous extracts had the highest amount of flavonoids, while acetone was the second highest followed by methanol extracts.

Bioactive compounds analysis of Hermannia depressa extracts

The LC-MS/MS results showed spectral data of major bioactive compounds with important pharmacological properties identified in *H. depressa* methanolic, acetone and aqueous crude extracts detected with mass spectrometry (MS) in negative and positive modes (Table 2). The spectral data obtained from the peaks in the aqueous extracts matched the spectra of several compounds of various classes alkaloids, flavones, and flavonoids. Meanwhile in methanol extracts, compounds classified as steroids, flavonoids, and fatty acids were detected. Acetone extracts contained compounds that are classified as polyphenolics. The identification of the compounds was based on LC-MS/MS results and in comparison with literature.

Antimicrobial activity of Hermannia depressa extracts

Table 3 displays the results of antimicrobial activity of *H. depressa* extracts. The aqueous extracts of *H. depressa* showed no antimicrobial activity against all the microbial organisms except against *C. krusei*, where activity was at MIC of 1.25 mg/mL. *Hermannia depressa* methanolic extracts demonstrated significant antimicrobial activity against all the microorganisms tested, at MICs ranging from 0.3 to 1.25 mg/mL with *B. cereus* and *C. albicans* being the most affected. *Hermannia depressa* acetone extracts also demonstrated antimicrobial activity against 10 of 13 microbial species tested against at MICs ranging from 0.1 to 1.25 mg/mL with *C. albicans* being the most affected.

All experiments were conducted in triplicates.

Anti-inflammatory activity of Hermannia depressa extracts

The anti-inflammatory activity was expressed by the ability of plant extracts to inhibit the release of NO from live cells. A treatment plant is considered anti-inflammatory potent when

TABLE 1: Total phenolic and flavonoid content of *Hermannia depressa* extracts.

Plant name	Extracts	Total phenolic content (mg GAE/g of extract)	Total flavonoid content (mg QE/g of extract)
Hermannia depressa	Acetone	8.45 ± 0.006	0.58 ± 0.02
	Methanol	7.65 ± 0.004	0.53 ± 0.001
	Aqueous	5.66 ± 0.002	0.97 ± 0.01

Note: All experiments were conducted in triplicates; all the data were expressed as means ± standard deviation.

TABLE 2: Liquid Chromatography with tandem mass spectrometry spectral data of the most abundant phytochemical compounds in aqueous, acetone and methanolic extracts of *Hermannia depressa* detected with MS positive and negative modes.

Extracts	Peak	Proposed compound	Classification	m/z	Tf (min)	$[M-H]^-$	Molecular formula	Ref
Aqueous	1	Waltherione D	Alkaloid	364.272	3.88	+	$C_{22}H_{22}NO_4$	Arroyo et al. (2018)
	2	Isomer of Waltherione D	Alkaloid	364.271	4.32	+	$C_{22}H_{22}NO_4$	Cretton et al. (2020)
	3	Waltherione C	Alkaloid	348.275	4.85	+	$C_{22}H_{21}NO_3$	Silva et al. (2022)
	5	Buxifoliadine D	Accridone alkaloid	362.156	11.84	+	$C_{23}H_{23}NO_3$	Wu and Chen (2000)
	7	Quercetin	Flavonoid	301.093	5.06	-	$C_{15}H_{10}O_7$	Vacek et al. (2013)
	9	Tricin	Flavone	329.232	7.00	-	$C_{17}H_{14}O_7$	Bao et al. (2007)
	10	Gramrione	Flavone	329.231	7.73	-	$C_{17}H_{14}O_7$	Rahman et al. (2013)
Acetone	1	Waltherione D	Alkaloid	364.271	4.32	+	$C_{22}H_{22}NO_4$	Cretton et al. (2020)
	4	8-Dihydroantidesmone	-	344.281	6.00	+	$C_{19}H_{31}NO_3Na$	Essono Mintsa et al. (2022)
	6	Hibtherin A	-	349.202	8.20	+	$C_{22}H_{36}O_3$	Ma et al. (2009)
	7	2-Methoxy-5-octylaniline	-	236.166	9.93	+	$C_{15}H_{26}NO$	Zdorichenko et al. (2019)
	8	2-Methoxyestradiol	Steroid	303.234	10.17	+	$C_{15}H_{10}O_7$	Yuan et al. (2021)
	9	4'-Methoxynaringenin	Flavonoid	287.203	10.84	+	$C_{15}H_{10}O_6$	Yuan et al. (2021)
	1	Hexose or glucose	Carbohydrate	215.034	1.00	-	$C_6H_{12}OCl$	Sans et al. (2017)
	7	Jaceosidin	Flavonoid	329.232	6.99	-	$C_{17}H_{14}O_7$	Paje et al. (2022)
	8	Docosahexanoic acid	Fatty acid	327.216	7.35	-	$C_{22}H_{32}O_2$	Yuan et al. (2022)
	12	Vernolic acid	Fatty acid	295.227	10.11	-	$C_{18}H_{32}O_3$	Abdelhafez et al. (2020)
	13	6-Gingerol	Phenolic	293.211	10.48	-	$C_{17}H_{26}O_4$	Yuan et al. (2021)
Methanol	2	4-O-Caffeoylquinic acid	Polyphenolic	355.104	3.84	+	$C_{16}H_{18}O_9$	Yuan et al. (2021)
	2	Matairesinol	Lignans/Polyphenol	359.135	4.11	-	$C_{16}H_{22}O_9$	Yuan et al. (2022)
	3	Pinoresinol	Lignan/Polyphenol	359.135	4.18	-	$C_{16}H_{22}O_9$	Yuan et al. (2022)
	4	Medioresinol	Lignan/ Polyphenol	389.147	4.44	-	$C_{21}H_{24}O_{07}$	Irakli et al. (2021)
	5	7-Methoytaxifolin-3-glucoside	-	403.162	5.10	-	$C_{21}H_{22}O_{12}$	Jaiswal et al. (2014)
	7	Yunnaneic acid F	-	599.236	6.09	-	$C_{29}H_{26}O_{14}$	Barros et al. (2013)
	8	Salvigenin	-	329.103	9.16	-	$C_{20}H_{24}O_4$	Yuan et al. (2022)

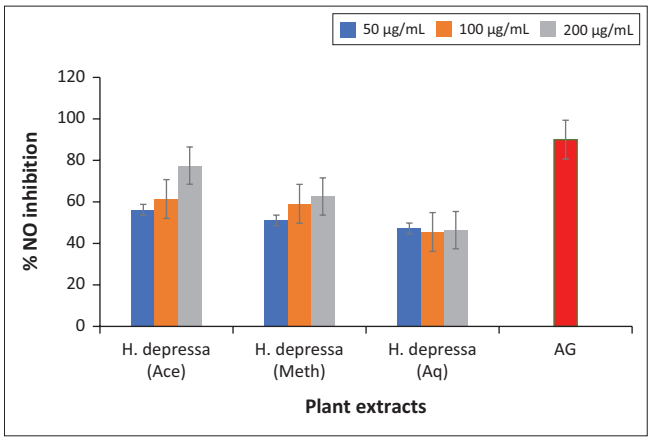
Note: Please see the full reference list of the article, Ngobeni, B., Manduna, I.T., Malebo, N.J. & Mashele, S.S., 2024, 'Potential therapeutic effects of *Hermannia depressa* N.E.Br. root extracts', *Journal of Medicinal Plants for Economic Development* 8(1), a239. https://doi.org/10.4102/jomped.v8i1.239, for more information.

TABLE 3: The minimum inhibitory concentration values of *Hermannia depressa* extracts against various microorganisms (mg/mL).

Microbial species	Gram (+ or -)	*H. depressa*			CHPL	AMPT B
		M	A	aq		
Candida albicans	N/A	0.30	0.10	-	-	< 0.1
Candida krusei	N/A	1.25	-	1.25	-	< 0.1
Candida parapsilosis	N/A	0.30	1.25	-	-	< 0.1
Bacillus cereus	+	0.30	1.25	-	< 0.1	-
Clostridium perfringens	+	0.60	-	-	< 0.1	-
Enterococcus faecalis	+	1.25	-	-	< 0.1	-
Escherichia coli	-	0.60	1.25	-	< 0.1	-
Neisseria gonorrhoeae	-	0.60	0.60	-	< 0.1	-
Pseudomonas aeruginosa	-	1.25	1.25	-	< 0.1	-
Staphylococcus aureus	+	0.60	1.25	-	< 0.1	-
Staphylococcus epidermidis	+	0.60	0.60	-	< 0.1	-
Streptococcus pneumoniae	+	0.60	0.60	-	< 0.1	-
Streptococcus pyogenes	+	0.60	0.60	-	< 0.1	-

aq, aqueous extracts; A, acetone extracts; M, methanol extracts; CHPL, chloramphenicol; AMPT B, amphotericin B; (-), No antimicrobial activity was found; N/A, Not applicable.

NO production inhibition from the cells is greater than 60% (Figure 2). Nitric oxide production inhibitory activity of *H. depressa* methanolic, acetone and aqueous plant extracts were tested using the liposaccharide (LPS)-activated malignant macrophage cell line RAW264.7. The acetone extracts of *H. depressa* demonstrated the highest level of potency in inhibiting NO, with a remarkable inhibition rate of 77.5% at the highest concentration. Similarly, the methanol extracts displayed significant inhibition of NO, reaching 62.6% at the highest concentration. *Hermannia depressa* aqueous extracts demonstrated less potency in inhibiting NO, thus no anti-inflammatory activity was displayed. An analysis of variance (one-way ANOVA) yielded significant variation among anti-inflammatory activity with different concentrations of the *H. depressa*, $F = 22.85$, $p = 1.22 \times 10^{-8} < 0.05$. According to Tukey's test nitric oxide percentage inhibition data were divided into 10 groups and the results are presented in supplementary data folder. The results suggest that *H. depressa* has anti-inflammatory activity by inhibiting NO production in a malignant macrophage cell line activated with LPS.



Aq, aqueous extracts; Ace, acetone extracts; Meth, methanol extracts

FIGURE 2: The nitric oxide percentage inhibition of *Hermannia depressa* extracts. One way analysis of variance between methanol, acetone and aqueous extracts confirms significant variation with $p > 0.05$.

Antioxidant activity of Hermannia depressa extracts

Free radical scavenging activities of methanolic, acetone and aqueous extracts of *H. depressa* were measured by the DPPH assay. Table 4 shows the IC_{50} and DPPH percentage inhibition of *H. depressa* extracts for antioxidant activity. The DPPH percentage inhibition of the extracts was tested at concentrations ranging from 6.25 to 200 µg/mL. *Hermannia depressa* methanolic, acetone, and aqueous extracts inhibited DPPH by more than 50% at the highest concentrations (100 µg/mL – 200 µg/mL), indicating strong free radical scavenging activity. When compared with synthetic antioxidant agents such as Trolox and ascorbic acid, the *H. depressa* acetone and methanol extracts demonstrated significant antioxidant activity, at IC_{50} values of 3.57 and 5.197 µg/mL, respectively. One-way ANOVA showed significant variation between antioxidant activity and different concentrations, $F = 480.6$, $p = 2.82 \times 10^{-33} < 0.05$. Tukey's test DPPH data were divided into 16 groups to determine the variance in activity between the extract groups and the positive control (Online Appendix 1).

Correlation between total phenolic content and antioxidant activities

A low correlation ($R^2 = 0.744$) was observed between the antioxidant activity data obtained through DPPH assays (IC_{50}) and the total phenolic contents of *H. depressa* extracts examined in the study (Figure 3). A significant correlation ($R^2 = 0.744$) was witnessed between antioxidant activity and total flavonoid content for the same extracts (Figure 4). This suggests that the flavonoid constituent of the extracts had an influence on the antioxidant properties of *H. depressa* extracts.

Cytotoxicity of Hermannia depressa extracts

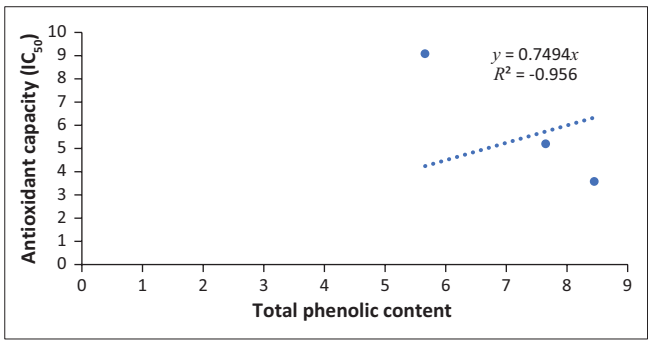
The cytotoxicity results are presented as the percentage of viable cells following exposure to the extracts at a specified range of concentrations (Figure 5). If the cell viability drops below 50% after being exposed to the highest concentration of the extract, the extract is considered toxic. The treatment of

TABLE 4: DPPH percentage of inhibition and IC_{50} values of *Hermannia depressa* extracts.

Concentration (µg/mL)	DPPH % inhibition				
	H. depressa			Ascorbic acid	Trolox
	Acetone	Methanol	Aqueous		
0	0.00	0.00	0.00	0.00	0.00
50	62.56	57.02	57.89	48.71	64.48
100	61.27	56.12	58.16	53.86	63.10
200	59.98	54.08	53.34	57.26	63.33
IC_{50} ± SD (µg/mL)	3.576 ± 0.44	5.197 ± 0.10	9.081 ± 1.94	6.569 ± 0.98	6.166 ± 0.43

All experiments were conducted in triplicates. All the data were expressed as means ± standard deviation. One way analysis of variance between methanol, acetone, aqueous extracts and the positive control confirms significant variation with $p < 0.05$.

SD, standard deviation



Source: Ngobeni, B., 2023, 'Ethnopharmacology of a selection of plants used for sexually transmitted infections in Thaba 'Nchu, Free State', Doctoral Thesis, Central University of Technology, Thaba Nchu

FIGURE 3: Correlation between the antioxidant capacity and total phenolic content of *Hermannia depressa* extracts.

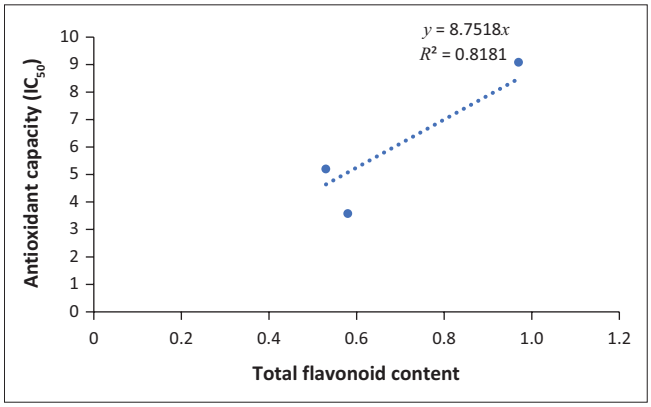
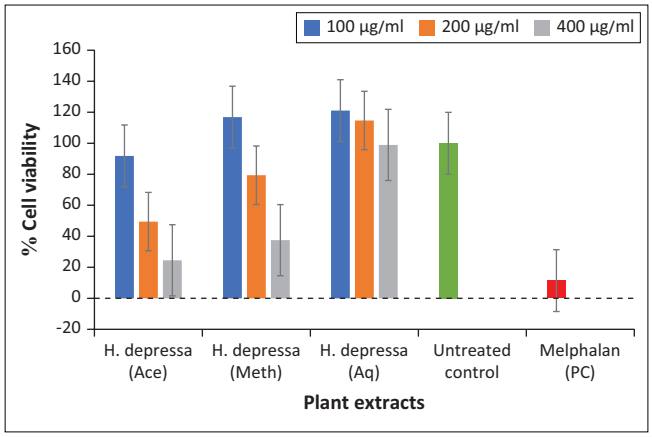


FIGURE 4: Correlation between antioxidant capacity and total flavonoid content of *H. depressa* extracts.

Vero cells with *H. depressa* acetone extracts at the highest concentration reduced the percentage cell viability to 24.52%, which is also an indication of the extract's toxicity towards the cells. When the Vero cells were exposed to *H. depressa* acetone and methanol extracts at the highest concentration, the percentage of viable cells decreased to 24.52% and 37.49%, respectively. Aqueous extracts showed no toxicity against Vero cells because the cell viability was increased above 50% after treatment with the extracts at the highest concentration. This decrease in cell viability at the highest concentration further confirms the toxic effects on the cells. An analysis of variance using one-way ANOVA showed significant variability among cell viability values with different concentrations of extracts, $F = 677.70$, $p = 3.98 \times 10^{-32}$. According to Tukey's test, cell viability data were divided into 10 groups to determine the variance in activity between the extract groups and the positive control (Online Appendix 1)



Source: Ngobeni, B., 2023, 'Ethnopharmacology of a selection of plants used for sexually transmitted infections in Thaba 'Nchu, Free State', Doctoral Thesis, Central University of Technology, Thaba Nchu

Note: One-way analysis of variance between methanol, acetone, aqueous extracts and the positive control confirms significant variation with $p < 0.05$.

Aq, aqueous extracts; Ace, acetone extracts; Meth, methanol extracts, PC, positive control

FIGURE 5: Cell viability of Vero cells after exposure to the positive control and different concentrations of *H. depressa* extracts.

Discussion

Screening for phytochemicals is the first stage in determining the medicinal value of any plant, as it provides a wide understanding of the nature of the compounds found in it (Sagbo et al. 2020). Various categories of phenolic compounds, including simple phenolics, phenolic acids, anthocyanins, hydroxycinnamic acid derivatives, and flavonoids are commonly identified in plants. These compounds exhibit diverse physiological functions, contributing to activities such as free radical scavenging, anti-mutagenic, anti-carcinogenic, antimicrobial, and anti-inflammatory effects (Adebiyi et al. 2017). In this study, methanolic, acetone, and aqueous extracts of *H. depressa* roots contained substantial quantities of phenolic compounds and low contents of flavonoids. These findings suggest that phenolics may be the major contributors to the antioxidant, anti-inflammatory and antimicrobial activity exhibited by *H. depressa* seen in this study as well as the plant's broad traditional use. The LCMS phytochemical analysis results revealed that *H. depressa* root extracts contain bioactive compounds of various classes such as flavonoids, flavones, lignans, ketones, alkaloids, quinic acids, biphenolic compounds, estradiol metabolites. The detected compounds have

various pharmacological properties such as antimicrobial, anti-inflammatory, anti-HIV, antimalarial, antioxidant, anti-virus, anti-cancer, and cytotoxic activities (Chu et al. 2019; Hwang et al. 2010; Jiang, Song & Jin 2020; Kim & Park 2018; Mansourabadi et al. 2016; Nageen et al. 2021; Nirmala & Sridevi 2021; Su, Cheng & Wink 2015; Wang et al. 2014; Zdunić et al. 2020). This could be an indication that the detected compounds are the major contributors to the therapeutic value of *H. depressa* and its traditional use in the treatment of various diseases. *Hermannia depressa* was tested for antimicrobial activity against Gram-positive, Gram-negative bacteria, and yeast. These findings of *H. depressa* antimicrobial effects are comparable to previously reported findings indicating the antibacterial activity of *H. depressa* roots against *E. coli*, *K. pneumoniae*, and *S. aureus* (Hlongwane 2016). This study is the first to report the antibacterial activity of *H. depressa* against *N. gonorrhoea*. The results affirm the traditional use of these plants for the treatment of sexually transmitted infections reported by Ngobeni et al. (2023). Given that gonorrhoea is the second most common sexually transmitted infection in the world, as well as the leading cause of mortality and morbidity (Oree et al. 2021). *Hermannia depressa* can be used as leads for the development of organic antimicrobial agents for the treatment of STIs. The study of Reid et al. (2005) has reported anti-inflammatory potential of *H. depressa* leaf aqueous, dichloromethane, and ethanol extracts inhibition against COX-1 and prostaglandin production. Their findings corroborate the findings of this study where *H. depressa* acetone and methanol extracts displayed strong anti-inflammatory effects. These results suggest that *H. depressa* can be exploited to develop anti-inflammatory drugs. The antioxidant effects of *H. depressa* could be linked to the presence of compounds such as phenolics, fatty acids, and glycosides detected in this study using LCMS. Flavonoids and tannins were previously reported from the root extracts of *H. depressa* (Vignesh, Selvakumar & Vasanth 2022). Phenolics are considered highly biologically effective because of their chemical structure, which includes various hydroxyl groups that function as scavengers and chelating agents. These properties are crucial for their antioxidant activities (Singh, Kaur & Silakari 2014). According to the findings of this study, *H. depressa* has antioxidant effects and the use of this under explored plant may play a role in preventing diseases such as cancer, cardiovascular diseases, and many other diseases caused by oxidative stress. However, even though *H. depressa* showed high antioxidant capacity, the linear correlation between the antioxidant activity and phenolic compounds was poor. The antioxidant activity of the plant may have been because of the synergistic interaction of phenolic compounds and other various non-phenolic compounds. Similar synergistic effects have been demonstrated in other studies as well (Hidalgo, Sánchez-Moreno & De Pascual-Teresa 2010; Skroza et al. 2015; Yin et al. 2012). Furthermore, non-phenolic compounds such as citric acid, ferrous sulphate, and d-glucose can interact with the Folin–Ciocalteu reagent, but they do not work well as free radical scavengers while potentially disrupting the relationship between total phenolic content (TPC) and the antioxidant effectiveness of extracts (Pasca et al. 2022). Moreover, antagonistic interactions among the antioxidant compounds may also have contributed to the poor linear correlation between phenolic compounds and antioxidant activity in this study (Chen et al. 2022). The low correlation results suggest that the antioxidative effects of *H. depressa* extracts cannot be solely linked to phenolic compounds. The source of the antioxidant activity in this study might be associated with various other secondary metabolites with antioxidative properties. While the observed biological activities of *H. depressa* may stem from detected phytocompounds, some of these have reported human and animal toxicity. Phytocompounds such as matairesinol, salvigenin, 6-gingerol and 4-O-Caffeoylquinic acid have shown cytotoxic effects against various cell lines (Su et al. 2015; Yamauchi et al. 2006; Wang et al. 2014). The same compounds were detected from *H. depressa* acetone and methanol extracts using LCMS, which is a suggestion that the cytotoxicity of *H. depressa* may be linked to the above-mentioned compounds. Although *H. depressa* extracts have shown to be hazardous to Vero cells, this does not imply that the use of this plant should be prohibited; however, it should be used with caution because the cytotoxicity is considered physiologically insignificant at concentrations tested in this study (Gertsch 2009).

Conclusions

Hermannia depressa showed significant antimicrobial, anti-inflammatory, and antioxidant activity and the plants contain crucial bioactive compounds that contribute to their biological activities, which validates the traditional uses of the plant. This plant has the potential to be used to create therapeutic agents for the treatment of microbial infections, pain, and human diseases caused by oxidative stress, such as cancer, cardiovascular disease, and premature ageing. As many people depend on plants for traditional medicinal purposes, human toxicity stands out as a paramount concern that necessitates careful consideration. *Hermannia depressa* acetone and methanol extracts showed toxicity against RAW 264.7 cell line; therefore, the biological activities demonstrated in this study may be attributed to the toxicity of the extracts. In addition, compounds with toxic properties were detected using LCMS; therefore, the toxicity of the plant extracts may be because of the detected compounds. While natural extracts are frequently considered insufficient for addressing clinical conditions, the findings of this research are encouraging. This study further provides an important basis for additional investigation into the isolation, characterisation and mechanism of cytotoxic compounds found in *H. depressa* extracts. Therefore, these plants have the potential to serve as a foundation for developing novel compounds in drug design aimed at fighting cancer.

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Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contributions

B.N. and I.T.M. conceived and designed the study. B.N. performed the experiments, analysed, and interpreted the data and wrote the article. I.T.M., N.J.M. and S.S.M. have read and approved the article.

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Data availability

The data that support the findings of this study are available on request from the corresponding author, B.N.

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