J Herbmed Pharmacol. 2025; 14(3): 348-357.

http://www.herbmedpharmacol.com doi: 10.34172/jhp.2025.53002

Journal of Herbmed Pharmacology

Phytochemical analysis and evaluation of antimicrobial, anti-inflammatory, antioxidant, and cytotoxic properties of *Berkheya onopordifolia*

Brian Ngobeni[1*], Idah Tichaidza Manduna[2]

[1]Department of Clinical Sciences, Faculty of Health and Environmental Sciences, Central University of Technology, Free State, Bloemfontein, South Africa
[2]Centre for Applied Food Sustainability and Biotechnology, Faculty of Health and Environmental Sciences, Central University of Technology, Free State, Bloemfontein, South Africa

ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 5 Mar. 2025
Revised: 15 Apr. 2025
Accepted: 20 Apr 2025
epublished: 1 Jul. 2025

Keywords:
Berkheya onopordifolia
Asteraceae
Cytotoxicity
Anti-inflammatory activity
Antimicrobial activity
Antioxidant activity

ABSTRACT

Introduction: *Berkheya onopordifolia* and other *Berkheya* species are used traditionally to treat fever, malaria, skin conditions, sexually transmitted infections, gastrointestinal issues, and inflammation in many regions of Southern Africa. This study aimed to validate its traditional uses through in vitro analysis of methanol, acetone, and aqueous extracts for antimicrobial, anti-inflammatory, antioxidant, and cytotoxic properties.

Methods: The nitric oxide (NO) assay was used to evaluate anti-inflammatory activity, while cytotoxicity was determined using Hoechst 33342/PI staining method. Antioxidant activity was assessed through 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric ions reducing antioxidant power (FRAP) assays, and antimicrobial activity using the broth microdilution technique. Bioactive compounds were examined using different qualitative and quantitative techniques.

Results: Flavonoids, alkaloids, steroids, glycosides, diterpenes, and dicarboxylic acids were identified in the extracts. Antimicrobial activity was observed in both methanol and acetone extracts, at minimum inhibitory concentrations (MICs) between 0.6 to 1.25 mg/mL against *Candida albicans*, *Candida parapsilosis*, and several gram-negative and positive bacteria, including *Escherichia coli*, *Neisseria gonorrhoeae*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*. Acetone extracts inhibited NO release by over 50%, indicating strong anti-inflammatory potential, but showed cytotoxicity to Vero cells at 400 µg/mL. It also exhibited moderate antioxidant activity (IC_{50} = 9.83 ± 0.31 µg/mL), significantly different ($P \leq 0.001$) from Trolox (6.17±0.43 µg/mL) and ascorbic acid (6.57±0.98 µg/mL).

Conclusion: Despite low selectivity and linear correlation values, the acetone extract demonstrated notable antimicrobial, anti-inflammatory, and antioxidant activities. These findings support the traditional medicinal use of *B. onopordifolia* and highlight the need for further studies on its isolated bioactive compounds.

Implication for health policy/practice/research/medical education:
The results of this study provide scientific evidence for understanding the antimicrobial, anti-inflammatory, antioxidant, cytotoxic effects and the bioactive compounds identified from *Berkheya onopordifolia*. The results highlight the need for further studies on its isolated bioactive compounds.

Please cite this paper as: Ngobeni B, Manduna IT. Phytochemical analysis and evaluation of antimicrobial, anti-inflammatory, antioxidant, and cytotoxic properties of *Berkheya onopordifolia*. J Herbmed Pharmacol. 2025;14(3):348-357. doi: 10.34172/jhp.2025.53002.

Introduction

The current global healthcare landscape is marked by escalating challenges such as an increase in microbial infections, antibiotic resistance, inflammatory disorders, and the ongoing quest for effective anticancer agents (1). Moreover, synthetic drugs employed in treating microbial infections, inflammatory disorders, and ailments associated with oxidative stress come with a set

***Corresponding author**: Brian Ngobeni,
Email: bngobeni@cut.ac.za

of disadvantages. These drugs may exhibit undesirable side effects, ranging from mild discomfort to severe complications, potential toxicity, allergic reactions, and the financial burden associated with the production and acquisition (2-4). Considering these setbacks, there is a growing interest in exploring alternative and more holistic approaches to address health issues, emphasizing the need for sustainable and safer therapeutic options from medicinal plants.

An estimation of 75%-80% of the world's population relies on medicinal plants for basic health care, largely because they are more culturally accepted, better tolerated by the body and tend to cause minimal adverse effects (5). Moreover, plants provide a base for discovering compounds that may contribute to the production of notable and novel drugs. For centuries, herbal remedies have long been utilized to treat various ailments because they contain diverse compounds with potential therapeutic and pharmacological effects (6,7). The important compounds include tannins, flavonoids, alkaloids, terpenoids, saponins, as well as phenolic compounds. The health benefits of these compounds include antioxidant, antimicrobial, analgesic, anticancer, anti-diabetic and many other activities (8). The presence of these compounds has influenced research on medicinal plants for their pharmacological effects to find promising leads for future drug discovery and development.

Berkheya onopordifolia (DC.) O.Hoffm. ex Burtt Davy, belonging to the Asteraceae family indigenous to South Africa, has long been valued for its traditional medicinal uses. Plants from the *Berkheya* genus have been used in conventional medicine to manage numerous ailments such as fever, malaria, skin conditions, STIs, gastrointestinal issues, inflammatory conditions, respiratory problems, and reproductive problems (9,10). Despite the genus's traditional use and medical claims, not many studies especially on *B. onopordifolia* have been conducted to support these claims. As scientific interest in medical plants grows, *B. onopordifolia* emerges as an important subject to be investigated due to its ethnopharmacological history and the need for innovative therapeutic solutions in modern healthcare.

Against the above circumstances, exploring the untapped pharmacological potential of medicinal plants becomes imperative. *Berkheya* genus has a well-documented historical use and proven effectiveness and emerges as a compelling subject for thorough scientific investigation due to the pharmacological potential it holds. Understanding the traditional uses and ethnopharmacological significance of *B. onopordifolia* provides a crucial context for this research. The documented traditional knowledge, passed down through generations, serves as the starting point for our scientific inquiry into the potential therapeutic applications of this plant (11). Thus, the objective of this study was to systematically evaluate the pharmacological properties of *B. onopordifolia*, focusing on anti-inflammatory, antimicrobial, antioxidant, and cytotoxic properties, and concurrently conducting a thorough phytochemical analysis on crude extracts.

Materials and Methods

Chemicals and materials

Solvents for extract preparation were sourced from Lasec Group (South Africa), while RAW 264.7 mouse macrophages were procured from Cellonex (South Africa). Lipopolysaccharide (LPS), Greiss reagent, aminoguanidine, Folin-Ciocalteu reagent, $NaNO_2$, NaOH, iodonitrotetrazolium salt, $AlCl_3$ and Mueller Hinton agar and broth, DPPH, $FeSO_4$, Melphalan, Chloramphenicol, Amphotericin B were sourced from Sigma-Aldrich (USA). Microbial strains were obtained from Lancet Laboratories (South Africa). Absorbance values were recorded using a multi-well plate spectrophotometer (Spectra Max 340 microplate reader). Trolox, ascorbic acid as well the reagents used to prepare the FRAP reagent were purchased at Fisher scientific. Propidium iodide, Dulbecco's modified eagle's medium (DMEM), ferric chloride, fetal bovine serum (FBS), 2,4,6-tripyridyl-S-triazine (TPTZ), and Hoechst 33342 were obtained from Thermo Fisher Scientific.

Plant collection and extracts preparation

Roots of *B. onopordifolia* (Figure 1C) were harvested in Thaba 'Nchu, located in the Free State province of South Africa. Taxonomic identification was confirmed by SANBI, and a voucher specimen (B0020) was registered at the Central University of Technology's CAFSaB (Centre for Applied Food Sustainability and Biotechnology) herbarium. The harvested roots were cleaned, oven-dried at 40℃, and ground into powder. Fifty grams of the powder were extracted separately with methanol, acetone, and distilled water for 48 hours on a shaker. The solvents were then removed at 40 °C using a rotary evaporator and lyophilized for aqueous extracts.

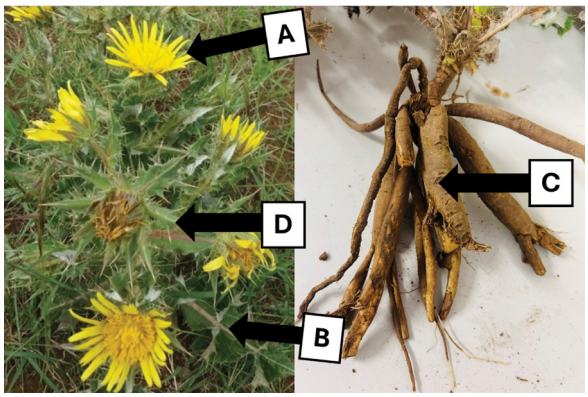

Figure 1. *Berkheya onopordifolia* flowers (A), leaves (B), thorns(D), and roots (C).

Phytochemical analysis of the plant extracts

Estimation of total phenolic content (TPC)

The Folin-Ciocalteu assay described by Agbo with slight modifications was used to measure the TPC of the extracts (12). The Folin-Ciocalteu method is an electron transfer based assay that measures the reducing capacity of a sample, which is expressed as phenolic content. Each extract solution (1 mg/mL) was prepared in triplicate by mixing 100 µL 50% Folin-Ciocalteu reagent and 2 mL of 7.5% sodium carbonate. After incubating the mixture for 30 minutes at 25 °C, the absorbance was measured at a wavelength of 720 nm. The mean absorbance values recorded at various concentrations of gallic acid were utilized to plot the calibration curve.

Estimation of total flavonoid content (TFC)

The TFC was measured using a modified version of Dirar's method (13). A 50 µL aliquot of crude extract (1 mg/mL) was diluted to 1 ml with methanol, combined with 4 mL distilled water, and 0.3 mL of 5% $NaNO_2$. After 5 minutes, 0.3 ml of 10% $AlCl_3$ was added, followed by an additional 10 minutes of incubation. Then, 2 ml NaOH (1 mol/L) was added and the adjusted to 10 ml with distilled water. The absorbance was recorded at 415 nm using a UV–VIS spectrophotometer, with a blank for reference. The results were presented as grams of quercetin equivalent gram per 100 g of extract.

Analysis of plant extracts phytochemicals through Liquid Chromatography-Mass Spectrometry (LC-MS)

Active compounds from *B. onopordifolia* extracts were identified using LC-MS with a Waters Synapt G2 instrument and electrospray ionization (ESI) in positive and negative settings. Chromatographic separation was achieved on a Waters BEH C18 column (2.1 × 100 mm), particle size 2.5 µm, and pore size 130 Å. The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile), starting at 95% and 5% for 1 minute. A gradient elution was then applied, reaching 100% within 11 minutes, maintained for 4 minutes, and then returned to 5% B over the next 2 minutes. The flow was set at 0.4 mL/min, with an injection volume of 10 µL and a total runtime of 15 minutes. Compound identification was conducted using high-performance liquid chromatography (HPLC) coupled with diode array detection and tandem mass spectrometry via ESI-LC/MS/MS (Electrospray ionization-liquid chromatography-tandem mass spectrometry).

Antimicrobial activity analysis of the extracts

Microbial cultures preparations

The crude extracts were assessed for their antibacterial activity against various bacterial and fungal species. The microbial strains were preserved on Mueller Hinton (MH) media plates at a temperature of 4 °C. The bacteria or yeast were first cultured in MH broth and incubated for 24 hours before exposure to the plant extracts. The microbial strains were then adjusted to a 0.5 McFarland standard, corresponding to an optical density (OD_{600}) of approximately 0.08 to 0.13, which is equivalent to 1 – 2 × 10^8 CFU/mL for bacteria and 1 – 5 × 10^6 CFU/mL for yeasts.

Determination of the lowest concentration that inhibits microbial growth

Microdilution method was utilized to evaluate the antimicrobial efficacy of the extracts. A stock solution of the plant extract was created using 2% Dimethyl sulfoxide, after which it was serially diluted to obtain various concentrations. A mixture of 80 µL bacterial or yeast suspension and 80 µL of diluted plant extract (ranging from 0.16 to 2.5 mg/mL) was prepared in 96-well plates. Control wells contained culture medium, bacterial or yeast suspension, solvent (2% DMSO), or extract alone. Chloramphenicol (0.125 mg/mL) served as a positive control for bacterial strains, while amphotericin B at concentration ranging from 0.03–1 µg/mL served as positive control for yeast. After incubating the 96 well plates for 24 hours (bacteria) and 48 hours (yeast), 40 µL of Iodonitrotetrazolium salt (4 mg/mL) was introduced to each well. Microbial growth was confirmed by visible colour change, whereas the absence of colour changes indicated that the extracts had inhibited growth.

Selectivity index (SI)

The SI was determined by calculating the logarithmic ratio of cytotoxicity concentration (CTC_{50}) to the minimum inhibitory concentration for each microbial strain (SI = log [CC_{50}]/[MIC]). This index was used to determine the relationship between cytotoxicity and antimicrobial effectiveness. Selectivity index scores lower than 1 indicate greater toxicity to Vero cells and lower selectivity for microbial species, whereas values greater than 1 represent greater selectivity against species of microorganisms than Vero cells (14).

Evaluation of the anti-inflammatory effects of the plant extracts

Nitric oxide (NO) assay

To assess anti-inflammatory effects, NO levels were measured in RAW 264.7 macrophages after exposure to the extracts. Cells were cultured in 96-well plates and allowed incubate overnight. Cells were treated with extracts at concentrations between 50-200 µg/mL, while inflammation was triggered using LPS (500 ng/mL), with aminoguanidine serving as positive control. After 18 hours, NO levels were measured by transferring 50 µL of supernatant to a new plate, adding Griess reagent, and reading absorbance at 540 nm. Inhibition of NO production was calculated relative to untreated controls

(considered 0% inhibition) (15).

Investigation of the antioxidant activity of the plant extracts

DPPH radical scavenging method

The radical scavenging activity of water, methanol, and acetone extracts from *B. onopordifolia* was evaluated using the DPPH method. A 0.1mM DPPH solution in methanol was prepared, and 10 µL of extract (or methanol for control) was added to 175 µL of the DPPH solution. The extract was tested at concentrations ranging from 200-6.25 µg/mL. Absorbance was recorded at 520 nm after 20 minutes, and the reduction was determined by comparing it to the control. Antioxidant activity was expressed as the IC_{50} value (concentration inhibiting DPPH by 50%). Trolox was the positive control, and Trolox equivalents were calculated based on the test sample's IC_{50}.

Ferric ions reducing antioxidant power (FRAP) method

The FRAP assay was used to further assess the antioxidant activity of the plant extracts with modifications (16). This method measures the ability of the treatment sample to reduce ferric iron to ferrous form. The FRAP reagent was prepared by combining ferric chloride (20mM), acetate buffer (pH 3.6), 2,4,6-tripyridyl-s-triazine (TPTZ) (10mM) and water. For each sample, 10 µL of extract or control (Trolox or Ascorbic acid) was mixed with 200 µL FRAP reagent, vortexed, and incubated for 30 minutes at 37 °C. Absorbance was measured at 593 nm using a UV–Visible spectrophotometer. Results were presented as ferrous equivalent (FE) in µM, with $FeSO_4$ used for calibration.

Cytotoxicity screening of the plant extracts

Hoechst 33342 and propidium iodide (PI) nuclear staining technique

The toxic effects of the plant extracts were assessed using Hoechst 33342 and PI staining technique according to Bellamakondi et al (17). Vero cells were maintained at 37 °C in a humid incubator containing 5% CO_2 and DMEM supplemented with 10% FBS. A total of 4000 cells per well were plated in 96-well plates and allowed to incubate for 24 hours. The cells were treated with extracts at concentrations ranging from 100-400 µg/mL and Melphalan (100 µg/mL) as a control was added, followed by 48-hour incubation. Cells were dyed with Hoechst 33342 at 5 µg/mL and PI at 100 µg/mL, then imaged using the ImageXpress Micro XLS microscope. Extracts were deemed cytotoxic if live cell counts were ≤50% of untreated controls.

Statistical analysis

Data were expressed as the mean ± standard deviation, based on three separate experiments. Microsoft Excel (USA) was used to analyse the correlation and regression of the results. Additional analysis involved one-way ANOVA, accompanied by Duncan and Tukey-Kramer post hoc tests. A *P* value of less than 0.05 was considered statistically significant for comparisons between groups.

Results

Total phenolic and flavonoid content

The TPC and TFC of *B. onopordifolia* extracts were measured to estimate the amounts of these compounds. The calibration curve for phenolic content, using gallic acid, showed maximum absorbance at 765 nm (Y= 0.0029x-0.0121; R^2= 0.995). The results were presented as gallic acid equivalents (GAE) per gram of dry extract in Table 1. The TPC values were between 0.49 to 2.5 mg GAE/g, with methanol extracts showing the highest levels while aqueous and acetone extracts were at the lowest levels. The calibration curve for flavonoids, using quercetin, showed maximum absorbance at 415 nm (y = 0.6961x - 0.0046; R^2 = 0.997). The results were presented as quercetin equivalents (QE) per gram (Table 1). TFC values were ranged between 0.1 to 24.3 mg QE/g, with acetone extracts containing the highest concentration, followed by methanol extracts, while aqueous extracts had the lowest flavonoid levels. Moreover, the TPC and TFC showed no statistically significant difference for the investigated extracts for acetone, methanol, and aqueous, respectively.

TPC, TFC and antioxidant capacity correlation.

Table 2 presents the correlation results between the antioxidant activity assessed through DPPH and FRAP IC_{50} values, TPC and TFC values of *B. onopordifolia*

Table 1. Antioxidant capacity, along with total phenolic and flavonoid contents of *Berkheya onopordifolia* extracts

Extracts	DPPH IC_{50} (µg/mL)	FRAP IC_{50} (µg/mL)	TPC (mg GAE/g)	TFC (mg QE/g)
Acetone	9.83±0.31^c	ND	0.49±0.008^a	24.3±0.008^a
Methanol	172.01±6.18^b	17.34±0.88^b	2.5± 0.003^a	11.77±0.081^a
Aqueous	302.13±28.87^a	28.83±2.25^a	0.49±0.007^a	0.176±0.008^a
Trolox	6.17±0.43^c	1.00±0.02^c	ND	ND
Ascorbic acid	6.57±0.98^c	ND	ND	ND
P value	0.00	0.00	0.81	0.07

Mean values in the same column with different superscripts are statistically significantly different; ND: not determined; GAE: Gallic acid equivalent, QE: Quercetin equivalent; TPC: Total phenolic content; TFC: Total flavonoid content; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric ions reducing antioxidant power.

Table 2. Linear relationship between the antioxidant activities and the phenolic and flavonoid contents of *Berkheya onopordifolia* extracts

Quantitative method	Correlation coefficients	
	DPPH	FRAP
Total phenolic content	y = 86.07x (R^2=0.4173)	y=3,1775x (R^2 = 0.0609)
Total flavonoid content	y=8.8561x (R^2 = 0.4497)	y=1.5095x (R^2 = 0.279)

DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric ions reducing antioxidant power.

extracts examined in this study. An R-value of less than 0.7 indicated a weak correlation between the total phenolic and flavonoid contents and DPPH IC_{50} values. The correlation between FRAP IC_{50} values and total phenolic as well as flavonoid contents also showed low correlation as indicated by the R-value below 0.7.

Analysis of phytochemical content of *Berkheya onopordifolia* extracts through LC-MS

LC-MS chromatogram analysis of *B. onopordifolia* aqueous, methanol, and acetone extracts revealed various bioactive compounds with significant pharmacological properties. Table 3 displays the peak numbers, proposed compounds, retention times (RT), analysis mode (+ or -), molecular weight, and formulas of the most abundant compounds. Aqueous extracts identified pharmacologically important compounds, including skimmin and the pyrrolizidine alkaloid 14-methylmonocrotaline, along with formononetin (18). Acetone extracts revealed the flavone 3',4'-Dimethoxyluteolin and, in negative mode analysis, showed azelaic acid and carnosic acid, a major oxidized derivative of carnosol, a diterpene found in Lamiaceae species like Salvia and Rosmarinus (19). Methanol extracts detected 4'-methoxynaringenin (Isosakuranetin) and the natural flavone Jaceosidin.

The cytotoxicity of *Berkheya onopordifolia* extracts

Cytotoxicity is presented in Figure 2 as percentage viability of Vero cells after treatment with *B. onopordifolia* extracts at concentrations between 100-400 µg/mL. Extracts are considered toxic if cell viability falls below 50% at the highest concentration. Exposure to acetone extracts reduced cell viability to below 50%, indicating toxicity. In contrast, methanol and aqueous extracts maintained over 50% viability, suggesting lower toxicity.

Antimicrobial properties of *Berkheya onopordifolia* extracts

Table 4 presents the antimicrobial activity of *B. onopordifolia* extracts, measured by minimum inhibitory concentrations. Methanolic, acetone, and aqueous extracts were tested against gram-negative, gram-positive bacteria, and yeast. Aqueous extracts exhibited poor antimicrobial activity across all species. Methanolic extracts were effective against 10 of 13 microorganisms, with MICs ranging from 0.6-1.25 mg/mL, notably affecting *S. aureus*, *E. coli, S. epidermidis*, *S. pneumoniae*, *N. gonorrhoeae*, *S. pyogenes*, *C. perfringens*, *and C. parapsilosis*. Acetone extracts showed activity against five species, all with a MICs of 0.6 mg/mL.

Selectivity index of *Berkheya onopordifolia* extracts

Selectivity index results are presented in Table 5 determined by dividing cytotoxicity (IC_{50}=mg/mL) by MIC (mg/mL). All selectivity index values for all microorganisms were below 1, which indicates that none of the plant extracts indicated selectivity against all the microbial species utilized in this study. The antimicrobial activity demonstrated against the selected microbial species may have been influenced by the plant's cytotoxicity.

Table 3. The most abundant bioactive compounds identified in *Berkheya onopordifolia* extracts by LCMS analysis

Extract	Peak	Proposed compound	Class	Mass-to-charge ratio (m/z)	Retention time (min)	Mode	Molecular formula	Ref.
Aqueous	1	Skimmin	Coumarin or glycoside	325.116	1.00	+	$C_{15}H_{16}O_8$	(20)
	2	14-Methylmonocrotaline-like	Alkaloid	362.155	11.84	+	$C_{17}H_{25}NNaO_6$	(21)
	3	Formononetin	Flavonoids	267.025	5.76	-	$C_{16}H_{12}O_4$	(22)
Acetone	2	3',4'-Dimethoxyluteolin	Flavonoids	315.087	7.83	+	$C_{17}H_{14}O_6$	(23)
	1	Azelaic acid	Dicarboxylic acid	187.097	5.39	-	$C_9H_{16}O_4$	(24)
	6	Carnosic acid	Diterpene	331.191	10.45	-	$C_{20}H_{26}O_4$	(25)
	8	Naringenin	Flavonoids	271.226	11.13	-	$C_{15}H_{12}O_5$	(26)
Methanol	6	2-Methoxyestradiol	Steroid	303.233	10.17	+	$C_{15}H_{10}O_7$	(27)
	7	4'-methoxynaringenin	Flavonoids	287.203	10.84	+	$C_{15}H_{10}O_6$	(27)
	9	Jaceosidin	Flavonoids	329.231	7.00	-	$C_{17}H_{14}O_7$	(28)

LCMS, liquid chromatography mass spectrometry.

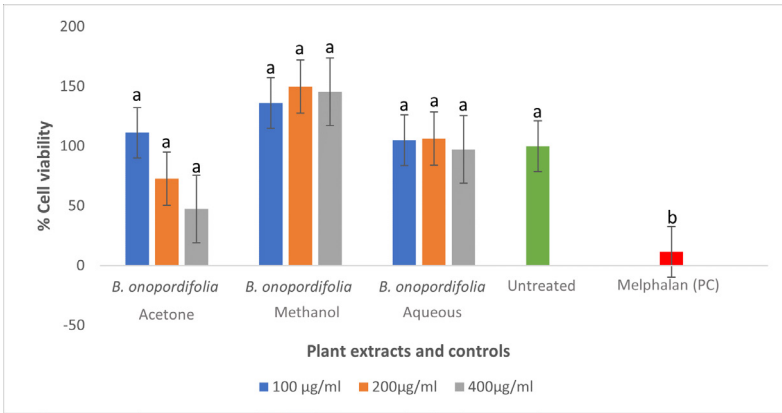

Figure 2. Vero cells' viability after exposure to different concentrations of Berkheya onopordifolia extracts or positive control. a: Insignificantly different from each other and b significantly variant to the positive control ($P < 0.05$).

Anti-inflammatory effects of *Berkheya onopordifolia* extracts

The anti-inflammatory effects of the extracts were considered significant when NO inhibition was >60% (Figure 3). *B. onopordifolia* acetone extracts demonstrated notable anti-inflammatory activity with over 50% inhibition of NO production at higher concentration. In contrast, methanol and aqueous extracts exhibited less than 50% inhibition of NO production even at the maximum concentration, thus, indicating no anti-inflammatory activity.

Antioxidant activity *Berkheya onopordifolia* extracts using DPPH and FRAP

B. onopordifolia antioxidant activity results are presented in Table 1, presenting DPPH IC_{50} values for methanolic, acetone, and aqueous extracts which is defined as concentration required to reduce DPPH colour by 50%. The extracts DPPH IC_{50}s were 9.83±0.31 µg/mL (acetone), 172.01±6.18 µg/mL (methanol), and 302.13±28.87 µg/mL (aqueous). Both methanol and aqueous extracts had lower efficacy compared to synthetic antioxidants like Trolox (IC_{50} = 6.17±0.43 µg/mL) and ascorbic acid (IC_{50} = 6.57±0.98 µg/mL). In contrast, acetone extracts showed moderate effectiveness when compared to the positive controls. The FRAP assay assess the ability of the antioxidant to reduce Fe^{3+}-TPTZ complex to Fe^{2+}-TPTZ under acidic conditions (pH 3.6). A sample is a strong antioxidant if its FRAP value is similar to or lower than Trolox. Methanol and aqueous extracts exhibited poor Ferric reducing capacity, with FRAP values significantly higher than Trolox, consistent with the DPPH results. The DPPH and FRAP IC_{50} values showed a statistically significant variance in all the explored extracts using the Duncan and Tukey-Kramer post hoc test (Table 1).

Table 4. Minimum inhibitory concentration levels of *Berkheya onopordifolia* extracts against different microorganisms (mg/mL)

Microorganisms	ATCC number	Gram (+ or -)	*B. onopordifolia* extracts			CHPL	AMPT B
			Methanol	Acetone	Aqueous		
Candida albicans	90028		0.6	0.6	-	ND	<0.1
Candida krusei	6258		-	-	-	ND	<0.1
Candida parapsilosis	22019		0.6	0.6	-	ND	<0.1
Bacillus cereus	13061	+	1.25	-	-	<0.1	ND
Clostridium perfringens	13124	+	0.6	-	-	<0.1	ND
Enterococcus faecalis	29212	+	-	-	-	<0.1	ND
Escherichia coli	13762	-	0.6	-	-	<0.1	ND
Neisseria gonorrhoeae	19424	-	0.6	0.6	-	<0.1	ND
Pseudomonas aeruginosa	27853	-	-	-	-	<0.1	ND
Staphylococcus aureus	11632	+	0.6	0.6	-	<0.1	ND
Staphylococcus epidermidis	12228	+	0.6	0.6	-	<0.1	ND
Streptococcus pneumoniae	15909	+	0.6	-	-	<0.1	ND
Streptococcus pyogenes	8668	+	0.6	-	-	<0.1	ND

CHPL: Chloramphenicol, AMPT B: Amphotericin B; All experiments were conducted in triplicates; (-): No antimicrobial activity was found; ND: not determined.

Table 5. Cytotoxicity and selectivity index of *Berkheya onopordifolia* extracts against 13 microbial species calculated by dividing cytotoxicity (CTC_{50} = mg/mL) by MIC (mg/mL)

	***B. onopordifolia* extracts**		
	Methanol	**Acetone**	**Aqueous**
Cytotoxicity (CTC_{50} [mg/mL])	**0.15**	**0.06**	**0.10**
Candida albicans	0.25	0.1	-
Candida krusei	-	-	-
Candida parapsilosis	0.25	0.1	-
Bacillus cereus	0.12	-	-
Clostridium perfringens	0.25	-	-
Enterococcus faecalis	-	-	-
Escherichia coli	0.25	-	-
Streptococcus pyogenes	0.25	-	-
Neisseria gonorrhoeae	0.25	0.1	-
Pseudomonas aeruginosa	-	-	-
Staphylococcus aureus	0.25	0.1	-
Staphylococcus epidermidis	0.25	0.1	-
Streptococcus pneumoniae	0.25	-	-

(-): not determined; CTC_{50}: Half maximal cytotoxic concentration; MIC: Minimum inhibitory concentration.

Discussion

Results from quantitative phytochemical analysis of *B. onopordifolia* roots showed to have more flavonoid contents as compared to phenolics. Flavonoids are compounds known for their anti-inflammatory, antimicrobial, antioxidant and many other therapeutic properties (29). Therefore, the flavonoids found may have contributed to the traditional medicinal benefits of *B. onopordifolia* roots in treating various ailments. The LC-MS analysis of *B. onopordifolia* root extracts identified bioactive compounds belonging to various classes. The classes include alkaloids, glycosides, flavonoids, flavones, isoflavones, fatty acids, diterpenes, steroids, and dicarboxylic acids. The phytochemical constituents found in the root extracts exhibit various pharmacological effects ranging from anti-inflammatory, antidiabetic, antimicrobial, antibacterial, anticancer, cytotoxic and antiviral effects (30). This indicates that the identified bioactive compounds may play a key role in the pharmacological effects and traditional uses *B. onopordifolia* in treating various health conditions. Although there are many advantages associated with traditional medicine, the main concern has consistently been about their safety with regards to human health (31). Bioactive compounds found in plants are presumed to be the main contributors to their pharmacological properties. However, the same compounds are also known to be contributors to the toxic effects produced by medicinal plants. Therefore, before any medicinal plant can be considered for therapeutic use, it is crucial to ensure that it does not have harmful effects on human cells (31). The observed toxic effects demonstrated by *B. onopordifolia* acetone extracts in this investigation may be ascribed to the presence of azelaic acid, carnosic acid, and 3',4'-dimethoxyluteolin, as their toxic effects have been indicated in prior studies (18,32-34). Similarly, plants belonging to the *Berkheya* genus, like *Berkheya setifera*, harbour various compounds classified as alkaloids, phenolics, saponins, glycosides, and terpenoids (7). While these compounds are recognized for their therapeutic properties in treating diverse ailments, they are also acknowledged for their potential toxic effects on human cells (35,36). Furthermore, *B. setifera* leaf and root aqueous extracts were found to exhibit toxic effects using the brine shrimp lethality assay, aligning with the toxicity

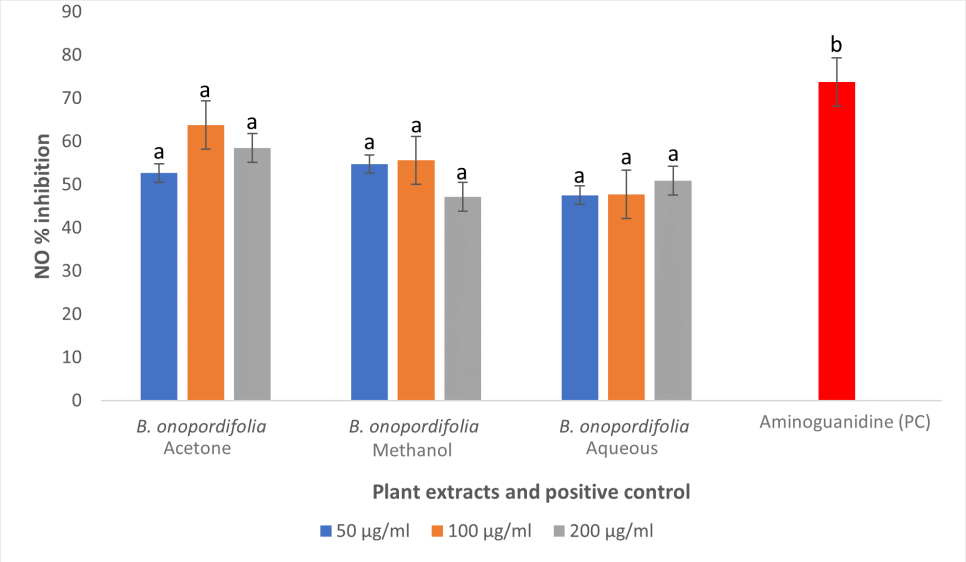

Figure 3. The percentage inhibition of nitric oxide by *Berkheya onopordifolia* extracts. a: Insignificantly different from each other and b significantly variant to the positive control ($P < 0.05$).

findings of the current study, despite the differences in assay methods employed (7). The antimicrobial effectivity of *B. onopordifolia* extracts was assessed against gram-negative, gram-positive bacterial and yeast species. The antimicrobial efficacy of the plant extracts is recognized as significant when the MIC value is 0.1 mg/mL or lower, moderate when 0.1 < MIC ≤ 0.625 mg/mL and weak when MIC > 0.625 mg/mL (37). Based on this criteria, *B. onopordifolia* acetone and methanol extracts presented moderate to poor antimicrobial potency against the microbial species used. Extracts from *B. setifera* flowers, leaves, roots, and stems have shown antibacterial and antifungal properties, and comparable results were observed with *Berkheya bergiana* leaf extracts, which demonstrated antimicrobial effects against different bacterial strains at MICs ranging between 0.07 to 5.0 mg/mL (7,38). Therefore, the antimicrobial efficacy observed in the present study necessitates additional exploration using isolated compounds to identify potential leads for discovering new drugs using *B. onopordifolia*. Using the selective index results from this study, the antimicrobial effects indicated by the extracts against the chosen microbial species may have been influenced by the plant's cytotoxicity effects. Plant extracts that show greater toxicity to human cells compared to bacterial or fungal cells are usually considered to have low therapeutic value. However, using one or few cell lines may result lead in inaccurate conclusions regarding the cytotoxicity and efficacy of a plant extract. Before drawing any strong conclusions, the toxic effects must also be assessed through in vivo studies (39). Therefore, dismissing an extract because it showed cytotoxic effects on a single cell line may be counterproductive. The antibacterial compound may or may not be the cytotoxic substance. It is scientifically possible to separate a potent and non-toxic novel metabolite from plant extracts that initially showed cytotoxicity (40). It is therefore recommended that antimicrobial compounds be isolated from the extracts showing promising activity in this study to assess whether their antimicrobial effects were influenced by toxicity or resulted from other factors. NO plays a vital role in the immune system's defence against microbial invasions. Since microbial infections are usually associated to inflammation, the assessment of the ability of *B. onopordifolia* as a NO production inhibitor is beneficial for the treatment of the inflammation. The ability of *B. onopordifolia* acetone extracts to inhibit NO production is crucial in the alleviation of inflammation caused by microbial infections. Similar results were noted when *B. setifera* was also reported to have anti-inflammatory properties using the 15-LOX anti-inflammatory assay (41). However, further investigation using other inflammatory mediators and methods is required to evaluate *B. onopordifolia* anti-inflammatory properties extensively. Oxidative stress, inflammation, and microbial infections are closely intertwined, with each factor influencing and amplifying the effects of the other. Managing oxidative stress caused by inflammation and microbial infections using antioxidants is crucial for supporting overall health and minimizing the risk of developing chronic illnesses (42,43). The weak antioxidant capacity observed in both *B. onopordifolia* methanol and aqueous extracts, as assessed by the FRAP and DPPH methods, could be attributed to several factors. The composition of these extracts may lack significant concentrations of antioxidant metabolites compared to extracts obtained using other solvents such as acetone since the extracts showed moderate antioxidant capacity as compared to the positive controls. Previous studies have identified acetone as the most effective solvent for extracting compounds with antioxidant properties, which may have contributed to the acetone extracts moderate antioxidant capacity observed in the current study (44).

Conclusion

This study scientifically validates the traditional medicinal use of *B. onopordifolia*. The in vitro analysis of its methanol and acetone extracts showed antimicrobial, anti-inflammatory, and moderate antioxidant activities. However, acetone extracts exhibited significant bioactivity despite the toxic effects, poor selectivity, and weak relationship between phenolic content and antioxidant activity. The presence of alkaloids, glycosides, flavonoids, fatty acids, diterpenes, steroids, and dicarboxylic acids likely contributes to its therapeutic potential. As the first report on *B. onopordifolia* pharmacological properties, further research is needed on isolated bioactive compounds to explore their potential therapeutic applications creating an avenue for the development of novel therapeutic agents.

Acknowledgements

The authors wish to express gratitude to the Central University of Technology for supplying resources and facilities that supported this project.

Authors' contribution

Conceptualization: Brian Ngobeni and Idah Manduna.
Data curation: Brian Ngobeni.
Formal analysis: Brian Ngobeni.
Investigation: Brian Ngobeni.
Methodology: Brian Ngobeni.
Resources: Idah Manduna.
Validation: Idah Manduna.
Writing-original draft: Brian Ngobeni.
Writing-review & editing: Brian Ngobeni and Idah Manduna.

Conflict of interests

The authors state that there are no conflicts of interest to disclose.

Ethical considerations

The authors affirm that ethical concerns, including copyright, plagiarism, data fabrication, duplicate publication, and redundancies, have been addressed. This study did not involve human or animal subjects.

Funding/Support

This work was supported financially by the National Research Foundation (NRF) under grant number BAAP2204092865.

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