

In vitro analysis of antimicrobial, antioxidant, and anti-inflammatory effects of *Scabiosa columbaria* L. acetone extracts

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Abstract

Due to the rising adverse effects of synthetic drugs, there is growing interest in natural therapeutic alternatives. *Scabiosa columbaria* L., traditionally used for respiratory disorders, skin infections, STIs, and menstrual pain, was investigated to validate its medicinal potential. Whole-plant material was extracted with acetone and analyzed using colorimetric and chromatographic methods. Antimicrobial activity was assessed through the microdilution assay, while antioxidant activity was evaluated using ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. Cytotoxicity and anti-inflammatory activity were determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and nitric oxide (NO) assays, respectively. Liquid chromatography–mass spectrometry (LC–MS) identified iridoid glycosides, phenolic acids, flavonoids, and saponins. The extracts showed moderate phenolic (2.93 mg GAE/g) and flavonoid (1.64 mg QE/g) contents, strong reducing power (12.13 mg TE/g), and moderate antimicrobial activity, especially against *Neisseria gonorrhoeae* (MIC = 0.6 mg/ml). They also showed dose-dependent cytotoxicity and strong NO inhibition (>60%). The results support the therapeutic potential of *S. columbaria*, although moderate cytotoxicity highlights the need for further studies on isolated active compounds.

Keywords

Scabiosa columbaria L., anti-inflammatory activity, antimicrobial effects, antioxidant activity, bioactivity, phytochemical evaluation

Introduction

In response to the challenges associated with treating microbial infections, inflammatory disorders, and oxidative stress, synthetic treatment approaches are widely used. The production of synthetic agents such as non-steroidal anti-inflammatory drugs, antibiotics, and antioxidants through advanced scientific research over the years has transformed healthcare by offering targeted and rapid treatments for a wide range of diseases. However,

synthetic drugs pose challenges, including adverse side effects, antibiotic resistance, and high purchase prices in low-income communities (Atta et al. 2017; Bjarnason et al. 2018; Zhang et al. 2019; Davis and Choisy 2024). As a result, there is increasing emphasis on investigating the therapeutic properties of medicinal plants, with the goal of developing safer, effective, and sustainable treatment alternatives that can complement synthetic drugs.

Traditional medicine derived from plant-based remedies has been practiced for centuries and remains a

crucial component of healthcare in many developing countries (Mutombo et al. 2023). The integration of phytotherapy offers promising solutions for more effective disease treatment. Research into medicinal plants has revealed various bioactive compounds with therapeutic potential, some of which have been incorporated into modern treatments (James et al. 2018). The growing field of ethnopharmacology connects traditional medicinal knowledge with modern scientific developments, supporting the development of safer and more effective drugs (Anand et al. 2019). *Scabiosa columbaria* L., a plant from the family Caprifoliaceae, is used to treat respiratory disorders, skin infections, sexually transmitted infections, menstrual pain, heartburn, colic, and digestive issues. Widely distributed across Europe, Africa, and Asia, it has drawn attention for its antibacterial, antifungal, and antiprotozoal activities, as well as its phytochemical composition (Carlson et al. 2012; Maroyi 2019; Sagbo et al. 2020; Akar 2021; Ngobeni et al. 2024). Therefore, this plant is a valuable candidate for further scientific investigation to validate its traditional uses and assess its potential contribution to the development of novel therapeutic agents.

Studies on *S. columbaria* have shown that aqueous extracts from leaves and roots, as well as methanol–dichloromethane extracts, exhibit antimicrobial properties against sexually transmitted and other pathogens (Seleteng Kose et al. 2015; Maroyi 2019; van Vuuren and Naidoo 2010). Methanol extracts from the leaves and flowers have demonstrated strong antioxidant activity through various analytical methods. Phytochemical analysis using liquid chromatography–mass spectrometry (LC–MS) and gas chromatography–mass spectrometry (GC–MS) identified several bioactive compounds from the roots, leaves, and flowers (Sagbo et al. 2020; Akar 2021). Notably, bioactive compounds such as glycoside scabiosin, loganin, phthalic acid, and sweroside were isolated from the roots, while diisooctyl phthalate, dibutyl phthalate, and bis(ethylhexyl) phthalate were detected in the upper plant parts and roots (Watt and Brandwijk 1927; Horn et al. 2001; Roersch 2017). Despite these findings, limited research has been conducted on *S. columbaria* acetone extracts, leading to a gap in the scientific validation of its medicinal properties and bioactive compounds. This study evaluates the antimicrobial, anti-inflammatory, and antioxidant properties and further analyzes the phytochemical profile of the acetone extracts.

Materials and methods

Chemicals

This study used methanol and acetone (both high-performance liquid chromatography (HPLC) grade) and distilled water as solvents, all of which were procured from Lasec Group (South Africa). Mouse macrophages (RAW 264.7) were obtained from Cellonex (South Africa). Propidium iodide, Hoechst 33342, Dulbecco's Modi-

fied Eagle's Medium (DMEM), fetal bovine serum (FBS), 2,4,6-tripyridyl-S-triazine (TPTZ), and ferric chloride were purchased from Thermo Fisher Scientific. Lipopolysaccharide (LPS), Griess reagent, aminoguanidine, Folin–Ciocalteu reagent, sodium nitrite (NaNO_2), sodium hydroxide (NaOH), iodonitrotetrazolium salt (INT), aluminum chloride (AlCl_3), ferrous sulfate (FeSO_4), Mueller–Hinton agar and broth, 2,2-diphenyl-1-picrylhydrazyl (DPPH), melphalan, chloramphenicol, and amphotericin B were procured from Sigma-Aldrich. The microorganisms used were sourced from Lancet Laboratories (South Africa). Absorbance measurements were taken using a multiwell plate spectrophotometer (Spectra Max 340 microplate reader). Trolox, ascorbic acid, and the reagents required for preparing the FRAP reagent were obtained from Fisher Scientific. The PowerWave XS spectrophotometer was purchased from BioTek (Winoski, VT, USA).

Plant collection and extract preparation

Scabiosa columbaria L. whole-plant material (roots, stems, twigs, and flowers) was harvested from Thaba Nchu, located in the Free State Province of South Africa (29.2099°S, 26.8392°E) (Fig. 1). The plant was identified, and the dried plant specimen was deposited at the National Museum, Bloemfontein, South Africa Herbarium (voucher specimen no.: NMB 28715) by an accredited botanist. The whole-plant material was thoroughly cleaned with deionized water, air-dried at 25 °C, and then ground into a fine powder. Ground plant material (50 g) was soaked in absolute acetone and subjected to rotary shaking for 48 hours. Acetone was chosen as the solvent of extraction due to limited studies on *S. columbaria* acetone extracts and its ability to extract polar (phenolics, flavonoids, tannins) and nonpolar/semi-polar compounds (terpenoids, lipids) with significant pharmacological properties (Eloff 1998; Anokwuru et al. 2011; Kumar et al. 2023). The extracts were concentrated using a rotary evaporator and thereafter stored at 4 °C to maintain integrity.

Quantitative phytochemical analysis of extracts

Determination of total phenolic content (TPC)

To determine the total phenolic content (TPC) of the acetone extracts of *S. columbaria*, the Folin–Ciocalteu assay was used (Khan et al. 2018). In triplicate, the reaction mixture consisting of 100 μl of 1 mg/ml extract, 2 ml of 7.5% sodium carbonate, and 100 μl of 50% Folin–Ciocalteu reagent was prepared and left to incubate for 2 hours. Absorbance was measured at 720 nm, and TPC was determined based on a gallic acid calibration curve, expressed in milligrams of gallic acid equivalents (GAE) per gram of dried extract.

Determination of total flavonoid content (TFC)

To quantify the total flavonoid content (TFC) within *S. columbaria* acetone extracts, the aluminum chloride assay was used (Aryal et al. 2019). A 50 µl aliquot of the 1 mg/ml extract was first diluted with 1 ml of methanol. To this mixture, 4 ml of water and 0.3 ml of 1% NaNO₂ were added, followed by incubation for 5 minutes. Then, 0.3 ml of 10% AlCl₃ was introduced, and the solution was incubated for 10 minutes. An additional 2 ml of 1 mol/l NaOH was added to the mixture, and the final volume was brought up to 10 ml. Absorbance was measured at 415 nm using a UV–VIS spectrophotometer, and the results were expressed as quercetin equivalents per 100 g of extract.

Phytochemical analysis of the plant extracts

Bioactive compounds in the acetone extracts of *S. columbaria* were characterized through liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) using a Waters Synapt G2 system. The analysis was conducted using electrospray ionization (ESI) operated in both positive and negative ionization modes. A Waters BEH C18 column (2.1 × 100 mm) with a 2.5 µm particle size and a 130 Å pore diameter was used to achieve chromatographic separation. Initially, the mobile phase comprised a solvent mixture of 95% solvent A (0.1% formic acid in water) and 5% solvent B (0.1% formic acid in acetonitrile), maintained for 1 minute, followed by a gradual gradient increase to 100% solvent B for 11 minutes. This composition was held steady for 4 minutes before returning to 5% solvent B within 2 minutes. The system operated at a flow rate of 0.4 ml/min with an injection volume of 0.01 ml and a total run time of 15 minutes. Identification of compounds was achieved through HPLC with diode array detection coupled with ESI–LC/MS/MS.

Cytotoxicity analysis of the extracts

The cytotoxicity of *S. columbaria* acetone extracts was assessed using the MTT assay, a standard colorimetric method for evaluating cell viability (Ogbole et al. 2018). This technique measures mitochondrial reduction of MTT to insoluble formazan, which is solubilized in DMSO and quantified spectrophotometrically. RAW 264.7 cells (1 × 10⁵ cells/well) were seeded in 96-well plates and treated with extract concentrations ranging from 50 to 200 µg/ml for 24 hours. After 72 hours of incubation at 37 °C with 5% CO₂, MTT solution (0.5 mg/ml) was added and incubated for 2 hours. The solution was then replaced with 200 µl of DMSO to dissolve the formazan crystals. Absorbance was measured at 540 nm. The experiment was performed in triplicate, and both cell viability percentages and the 50% cytotoxic concentration (CTC₅₀) were calculated.

Antimicrobial activity evaluation of *S. columbaria* extracts

Preparation of bacterial/yeast cultures

The antimicrobial effects of the extracts were assessed against a range of bacterial and fungal strains. These microorganisms were maintained on Mueller–Hinton agar plates at 4 °C for preservation. Prior to exposure to the plant extracts, bacterial cultures were grown in Mueller–Hinton broth under shaking conditions at 100 revolutions per minute for 24 hours to ensure both viability and purity. The bacterial and fungal strain preparations were standardized to a 0.5 McFarland turbidity standard, corresponding to approximately 1–2 × 10⁸ CFU/ml for bacteria and 1–5 × 10⁶ CFU/ml for yeasts.

Determination of the minimum concentration required to inhibit microbial growth

Microbial growth inhibition of the crude extracts was assessed using the microdilution technique (Hemeg et al. 2020). Extracts were dissolved in 2% DMSO and diluted from 0.16 to 2.5 mg/ml. Each well of a 96-well plate contained 80 µl of bacterial suspension and 80 µl of extract. Controls included culture medium, bacteria, solvent (2% DMSO), and extract alone. Chloramphenicol (0.125 mg/ml) served as the positive control for bacteria, and amphotericin B (1–0.03 µg/ml) for fungi. After incubation, 40 µl of 4 mg/ml INT was added; a color change indicated microbial growth. The minimum inhibitory concentration (MIC) was defined as the lowest extract concentration preventing visible growth.

Assessment of antioxidant properties of acetone extracts

2,2-diphenyl-1-picrylhydrazyl (DPPH) technique

The DPPH assay was used to assess the antioxidant capacity of *S. columbaria* extracts (Ibrahim et al. 2017). A DPPH solution at a concentration of 0.1 mM was prepared in methanol. Then, 10 µl of the plant extract or methanol (as a negative control) was added to 175 µl of the DPPH solution, with final concentrations ranging from 6.25 to 200 µg/ml. After a 20-minute incubation period, the absorbance was recorded at 520 nm. Antioxidant capacity was quantified based on the IC₅₀ value, representing the concentration required to reduce DPPH radicals by 50%. Trolox and ascorbic acid were used as reference antioxidants, and Trolox equivalent values were derived from the IC₅₀ results.

Ferric reducing antioxidant power (FRAP) technique

The antioxidant capacity of *S. columbaria* acetone extracts was assessed using a modified FRAP assay, which mea-

sure ferric-to-ferrous ion reduction (Elisha et al. 2017). The FRAP reagent was formulated by combining TPTZ (10 mM), buffer (pH 3.6), ferric chloride (20 mM), and water. Ten microliters of either the extracts (concentrations ranging from 0 to 200 µg/ml) or Trolox/ascorbic acid solutions (0 to 12.5 µg/ml) were added to 200 µL of the FRAP reagent. The mixtures were then vortexed to ensure thorough mixing and incubated at 37 °C for 30 minutes. Absorbance was recorded at 593 nm. Experiments were performed in triplicate, and antioxidative power was expressed in micromolar ferrous ion equivalents, using FeSO₄ as a calibration standard.

Assessment of the anti-inflammatory properties of *S. columbaria* acetone extracts

Nitric oxide (NO) assay

The anti-inflammatory capacity of the extracts was evaluated by determining nitric oxide (NO) production in RAW 264.7 macrophages stimulated with LPS. The cells were grown in DMEM at 37 °C in a moist atmosphere containing 5% CO₂ (Atta et al. 2017). Macrophages were seeded into 96-well plates, allowed to adhere overnight, and then exposed to varying concentrations of the extracts (50–200 µg/ml) along with 500 ng/mL LPS to trigger inflammation. Aminoguanidine (AG) was used as a positive control. After an 18-hour incubation period, 50 µl of the culture supernatant was pipetted into a new plate and mixed with Griess reagent. Absorbance was read at 540 nm, and the percentage of NO production inhibition was determined in comparison with untreated control cells.

Selectivity index

The selectivity index (SI) was determined by dividing the cytotoxic concentration (CTC₅₀) by either the MIC or the half-maximal inhibitory concentration (IC₅₀). An SI value less than 1 indicates that the extract is more toxic to Vero cells than to microorganisms, implying low microbial selectivity. Conversely, an SI greater than 1 indicates that the extract is more active against microorganisms than it is toxic to Vero cells, suggesting a higher degree of microbial selectivity (Elisha et al. 2017).

Statistical analysis

The results were presented as mean ± standard deviation from three independent measurements. Correlation, regression, and one-way ANOVA were conducted using Microsoft Excel. Post hoc comparisons were carried out using Duncan's and Tukey–Kramer tests, with statistical significance determined at a threshold of $p < 0.05$.

Results

Quantitative and qualitative phytochemical analysis of *S. columbaria* acetone extracts

Quantitative phytochemical analysis was performed to determine the total phenolic and flavonoid contents in *S. columbaria* acetone extracts (Table 1). The total phenolic content (TPC) was calculated using a standard calibration curve ($y = 0.0029x - 0.0121$; $R^2 = 0.995$) and expressed as gallic acid equivalents (GAE) per gram of dry extract weight. The phenolic content of *S. columbaria* acetone extracts was estimated to be 2.93 mg GAE/g. The total flavonoid content (TFC) results were obtained using the linear regression formula ($y = 0.6961x - 0.0046$; $R^2 = 0.997$) and expressed as quercetin equivalents (QE) per gram of extract weight. The TFC of the extracts was estimated to be 1.64 mg QE/g.

Table 1. Antioxidant capacity, total flavonoid, and phenolic contents of acetone extracts of *Scabiosa columbaria*.

Extracts/ positive control	FRAP (mg TE/g)	DPPH IC ₅₀ (µg/ml)	TFC (mg QE/g)	TPC (mg GAE/g)
Acetone	12.13 ± 0.39 ^a	76.75 ± 1.95 ^a	2.29 ± 0.03	2.93 ± 0.01
Ascorbic acid	0.36 ± 0.01 ^c	6.57 ± 0.98 ^b	ND	ND
Trolox	1.00 ± 0.02 ^b	6.16 ± 0.43 ^b	ND	ND
P value	0.00	0.00	ND	ND

Mean values within the same column that have different superscript letters indicate statistically significant differences. D = not determined; FRAP = ferric reducing antioxidant power; DPPH = 2,2-diphenyl-1-picrylhydrazyl; TPC = total phenolic content; TFC = total flavonoid content.

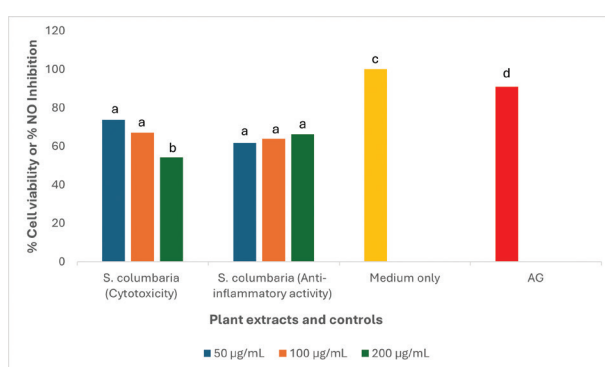
LC–MS/MS analysis of *S. columbaria* acetone extracts identified diverse bioactive compounds with notable pharmacological properties. Table 2 presents the peak numbers, proposed compounds, retention times (RT), ionization modes, molecular weights, and formulas of the major constituents. The detected compound classes include fatty acids, phenolic acids, iridoid and flavonol glycosides, flavones, polyphenolic quinones, triterpenoid saponins, and dihydroflavonols. These compounds are associated with antimicrobial, anti-inflammatory, antioxidant, and cytotoxic activities.

Cytotoxicity and anti-inflammatory analysis of *S. columbaria* acetone extracts

The cytotoxicity results of *S. columbaria* acetone extracts showed a concentration-dependent decline in Vero cell viability (Fig. 1). At 50 µg/ml, viability remained high (~74%), indicating low cytotoxicity. Increasing concentrations to 100 and 200 µg/ml reduced viability to ~68% and 55%, respectively, suggesting moderate cytotoxicity at higher doses but general safety at lower concentrations (vi-

Table 2. Pharmacologically significant compounds identified in *Scabiosa columbaria* acetone extracts by LC–MS/MS analysis in negative and positive ionization modes.

Class	Peak	Proposed compound	m/z	Retention time	Mode	Molecular formular	References
Phenolic acids	1	Eudesmic acid	213.076	3.82	+	C ₁₀ H ₁₂ O ₅	Bjarnason et al. 2018
Coumarins	6	Xanthoxylin	197.119	5.38	+	C ₁₀ H ₁₂ O ₄	Bjarnason et al. 2018
Flavonoids	11	Eupatilin (Flavone)	345.099	7.80	+	C ₁₈ H ₁₆ O ₇	James et al. 2018
	4-	Guaijaverin (Flavonol glycoside)	433.133	4.57	-	C ₂₀ H ₁₈ O ₁₁	Maroyi. 2019
Iridoid glycosides	2+	Sweroside	359.135	4.09	+	C ₁₆ H ₂₂ O ₉	Zhang et al. 2019
	1-	Loganin	389.108	3.66	-	C ₁₇ H ₂₆ O ₁₀	Carlson et al. 2012
	3-	Secoxyloganin	403.123	4.18	-	C ₁₇ H ₂₄ O ₁₁	Mutombo et al. 2023
	6-	Laciniatoside V derivative	583.207	5.63	-	C ₁₆ H ₂₀ O ₉	Sagbo et al. 2020
	7-	Laciniatoside V	585.222	6.32	-	C ₂₇ H ₃₈ O ₁₄	Sagbo et al. 2020
Polyphenolic Quinones	14	Miltipolone isomer	301.217	10.17	+	C ₁₉ H ₁₄ O ₃	Anand et al. 2019
Fatty Acids	4+	Dihydroxy-triacetoxy-tetratriacontanoic acid	759.273	4.56	+	C ₄₀ H ₇₄ O ₁₀ Na ₂	Mutombo et al. 2023
	9-	Vernolic acid	295.227	10.11	-	C ₁₈ H ₃₂ O ₃	James et al. 2018
	10-	Vernolic acid	295.226	10.48	-	C ₁₇ H ₂₆ O ₄	James et al. 2018

**Figure 1.** Vero cell viability (cytotoxicity) and nitric oxide percentage inhibition (anti-inflammatory activity) following treatment with the positive control and various concentrations of *S. columbaria* acetone extracts. Means from the same test (cytotoxicity or anti-inflammatory) with different letters differ significantly; identical letters indicate no significant difference. AG = aminoguanidine (positive control).

ability > 50%). The anti-inflammatory activity of *S. columbaria* was assessed by the extract's ability to suppress nitric oxide (NO) production. Extracts reduced NO production by over 60% at concentrations of 50–200 µg/ml, indicating strong anti-inflammatory potential. The cytotoxicity assay showed significant differences among treatments ($p < 0.05$), whereas the anti-inflammatory assay revealed no significant differences ($p < 0.05$). As indicated by the lettering system, extracts sharing the same letter did not differ statistically, whereas those with different letters were significantly different, suggesting comparable activity.

Antimicrobial activity analysis of *S. columbaria* acetone extracts

Table 3 presents the antimicrobial activity of *S. columbaria* acetone extracts against different microorganisms, as indicated by their MIC values. The extracts demonstrated inhibitory effects against *Candida albicans*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*,

Table 3. Minimum inhibitory concentrations of acetone extracts of *Scabiosa columbaria* against various microorganisms.

Microbial species	ATCC #	Gram (+ or -)	MIC (mg/ml)	CHPL (mg/ml)	AMPT B (mg/ml)
<i>Candida albicans</i>	90028	-	1.25	ND	<0.1
<i>Candida krusei</i>	6258	-	-	ND	<0.1
<i>Candida parapsilosis</i>	22019	-	-	ND	<0.1
<i>Bacillus cereus</i>	13061	+	-	<0.1	ND
<i>Streptococcus pyogenes</i>	8668	+	1.25	<0.1	ND
<i>Clostridium perfringens</i>	13124	+	-	<0.1	ND
<i>Enterococcus faecalis</i>	29212	+	-	<0.1	ND
<i>Escherichia coli</i>	13762	-	1.25	<0.1	ND
<i>Neisseria gonorrhoeae</i>	19424	-	0.6	<0.1	ND
<i>Pseudomonas aeruginosa</i>	27853	-	-	<0.1	ND
<i>Streptococcus pneumoniae</i>	15909	+	1.25	<0.1	ND
<i>Staphylococcus aureus</i>	11632	+	1.25	<0.1	ND
<i>Staphylococcus epidermidis</i>	12228	+	-	<0.1	ND

and *Escherichia coli* at an MIC of 1.25 mg/ml, except for *Neisseria gonorrhoeae*, which was inhibited at 0.6 mg/ml. The extracts showed no inhibitory effects against *Candida krusei*, *Candida parapsilosis*, *Bacillus cereus*, *Clostridium perfringens*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis*, as indicated by the absence of recorded MIC values.

Antioxidant activity of *S. columbaria* using DPPH and FRAP

The antioxidant activity of *S. columbaria* acetone extracts was evaluated using DPPH and FRAP assays, with results shown in Table 1. The FRAP results showed strong reducing power (12.13 ± 0.39 mg TE/g), surpassing the standard antioxidants ascorbic acid (0.36 ± 0.01 mg TE/g) and Trolox (1.00 ± 0.02 mg TE/g), suggesting the presence

of potent electron-donating compounds such as phenolics and flavonoids. In contrast, the DPPH assay showed a higher IC_{50} value ($76.75 \pm 1.95 \mu\text{g/ml}$) than ascorbic acid ($6.57 \mu\text{g/ml}$) and Trolox ($6.16 \mu\text{g/ml}$), indicating weaker radical-scavenging ability. Overall, the extract exhibited strong reducing capacity but lower free radical scavenging compared with standard antioxidants.

Selectivity index

The selectivity index (SI) of *S. columbaria* acetone extracts suggests limited therapeutic safety, with several values below the acceptable threshold of 1 (Table 4). Antimicrobial SI values ranged from 0.09 to 0.18, indicating higher cytotoxicity to host cells than antimicrobial efficacy. The anti-inflammatory activity showed an SI of 1.02, and the DPPH antioxidant assay yielded an SI of 1.41. According to Elisha et al. (2017), the antioxidant and anti-inflammatory effects demonstrated in this study are not attributed to the cytotoxicity of the extracts.

Table 4. Selectivity index (SI) of *Scabiosa columbaria* acetone extracts based on antimicrobial, anti-inflammatory, and antioxidant activities.

Microorganism/Assay	MIC ($\mu\text{g/ml}$)	Selectivity index = $\frac{CTC_{50}}{IC_{50} \text{ Or MIC}}$
<i>Candida albicans</i>	1250	0.09
<i>Escherichia coli</i>	1250	0.09
<i>Neisseria gonorrhoeae</i>	600	0.18
<i>Staphylococcus aureus</i>	1250	0.09
<i>Streptococcus pneumoniae</i>	1250	0.09
<i>Streptococcus pyogenes</i>	1250	0.09
Anti-inflammatory activity ($IC_{50} \mu\text{g/ml}$)	106.08	1.022
DPPH ($IC_{50} \mu\text{g/ml}$)	76.75	1.41
Cytotoxicity $CTC_{50} (\mu\text{g/ml})$	108.43	ND

MIC = minimum inhibitory concentration.

Discussion

The quantitative analysis of flavonoid and phenolic contents in *S. columbaria* acetone extracts revealed a low to moderate abundance of these compounds. This group of compounds is widely known for its broad range of biological effects, such as antimicrobial, antioxidant, anti-inflammatory, and various other activities (Chen et al. 2023). Earlier investigations have focused on the phytochemical quantification of methanol extracts of *S. columbaria* flowers and leaves, identifying various phenolic constituents such as 4-hydroxybenzoic, gallic, chlorogenic, and caffeic acids, 4-hydroxybenzaldehyde, and catechin in differing concentrations using LC–MS, which are likely responsible for the bioactivities previously attributed to the plant. The previously reported bioactivities of *S. columbaria* extracts include antimicrobial (Maroyi 2019), antioxidant (Akar 2021), anti-inflammatory (Ezeofor 2023), and many other properties. Given the relatively low to moderate levels detected in the acetone extracts, a more sensitive and com-

prehensive phytochemical analysis was conducted using LC–MS to accurately profile and quantify the full range of compounds present in *S. columbaria* acetone extracts. The LC–MS qualitative analysis identified a wide variety of phytochemicals classified as iridoid glycosides, phenolic acids, fatty acids, flavonoids, triterpenoid saponins, and polyphenolic quinones, which contribute to the plant's potential therapeutic properties. Eudesmic acid and xanthoxylin, which are types of phenolic acids known to demonstrate potent antioxidant and anti-inflammatory properties (Yuan et al. 2022), and iridoid glycosides such as sweroside, secoxyloganin, and loganin, along with their derivatives, enhance antimicrobial (Kilinc et al. 2023) and anti-inflammatory activities (Pferschy-Wenzig et al. 2022). Fatty acids, including vernolic acid and dihydroxy-triacontoxy-tetratriacontanoic acid, provide additional antioxidant and anti-inflammatory benefits (Mascherpa et al. 2012; Pferschy-Wenzig et al. 2022). Flavonoids such as eupatilin, guaijaverin, and 7-methyltaxifolin, along with their glycoside derivatives, contribute to antioxidant, anti-inflammatory, and potential anticancer activities (Jaiswal et al. 2014; Zhang et al. 2021). Triterpenoid saponins such as laciniatoside V and its derivatives exhibit antimicrobial and anti-inflammatory properties (Maroyi 2019), while polyphenolic quinones such as multipolone isomer add further antioxidant potential (Otang-Mbeng and Sagbo 2020). Previous studies have also reported the presence of sweroside and loganin from the roots of *S. columbaria* (Horn 2001). The traditional application of *S. columbaria* in treating various ailments may be linked to the presence of these phytochemicals because of their known wide range of therapeutic properties. Cytotoxicity studies are crucial in evaluating the safety profile of plant extracts, as they help determine whether compounds extracted from plants have harmful effects on normal human cells, a critical consideration in the production of potential therapeutic agents (Anywar et al. 2022). The present study assessed the cytotoxic effects of *S. columbaria* acetone extracts at various concentrations using RAW 264.7 mouse macrophage cells. The results revealed a concentration-dependent decrease in cell viability following treatment with *S. columbaria* acetone extracts. However, the extracts were considered non-significantly toxic, as cell viability remained above 50% at the highest concentration tested. In this study, compounds such as sweroside (Han et al. 2017), eupatilin (Razak et al. 2019), and xanthoxylin (de Carvalho et al. 2018) were detected and have previously shown cytotoxic effects against leukemic, breast cancer, and HepG2 human hepatocellular carcinoma cells, respectively. Consequently, these compounds may have contributed to the viability reduction observed in RAW 264.7 mouse macrophage cells. In contrast, previous findings revealed that methanol extracts from *S. columbaria* leaves did not exhibit significant toxicity toward human dermal fibroblast cells (Otang-Mbeng and Sagbo 2020). These results underscore the role of specific bioactive compounds in the observed cytotoxicity. The different cytotoxicity results compared with previous studies suggest

that both the choice of solvent type and cell line significantly influence toxicity outcomes. Therefore, it is important to exercise caution when using *S. columbaria* for therapeutic purposes, as improper use may lead to toxic effects, especially at higher doses. The *S. columbaria* acetone extracts' antimicrobial effects were assessed against gram-negative and positive bacterial and yeast species. The antimicrobial effects of the plant extracts were considered significant if the MIC value was ≤ 0.1 mg/ml, moderate if $0.1 < \text{MIC} \leq 0.625$ mg/ml, and weak if $\text{MIC} > 0.625$ mg/ml (Zouine et al. 2024). The extracts demonstrated moderate efficacy against *N. gonorrhoeae* and weak activity against *S. pneumoniae*, *C. albicans*, *S. aureus*, *S. pyogenes*, and *E. coli* according to these criteria. Similar results were observed in a previous study where *S. columbaria* methanol and dichloromethane leaf extracts demonstrated antibacterial activity against sexually transmitted pathogens such as *Ureaplasma urealyticum*, *Gardnerella vaginalis*, *Neisseria gonorrhoeae*, and *Oligella ureolytica* at MICs ranging from 2.0–16.0 mg/ml, which can be regarded as weak activity using the current study's criteria (van Vuuren and Naidoo 2010). Additionally, aqueous root extracts showed weak activity (MIC: 1.3–8.0 mg/ml) against various bacteria, including *Mycobacterium fortuitum*, *Citrobacter freundii*, *Enterobacter homaendis*, *Klebsiella pneumoniae*, *Mycobacterium smegmatis*, *Moraxella catarrhalis*, and *S. aureus*. Overall, considering the challenges and rising drug resistance associated with treating gonorrheal infections, these results highlight the capacity of *S. columbaria* as an antimicrobial agent against *N. gonorrhoeae* and underscore the need for further studies on the efficacy of its active compounds against resistant strains. However, the selectivity index values below 1 imply that the extracts exhibit greater toxicity toward Vero cells than toward microorganisms, raising concerns regarding their safety as antimicrobial agents. Nonetheless, the toxicity toward mammalian cells does not necessarily render the extracts pharmacologically irrelevant. Further tests must be conducted on isolated compounds to distinguish the biologically active from the cytotoxic components. Additionally, it is possible to separate an active non-toxic compound from a toxic crude plant extract (Elisha et al. 2017). Therefore, purification and isolation of these compounds are necessary to enhance selectivity and reduce cytotoxicity for potential therapeutic applications. The nitric oxide (NO) method was utilized to assess the anti-inflammatory effects of *S. columbaria* acetone extracts. NO, a reactive free radical, which plays a key role in innate immune defense by damaging microbial proteins and genetic material (Roberts et al. 2024). Since microbial infections often lead to inflammation, the extract's potential to alleviate inflammation was evaluated. The acetone extracts significantly suppressed NO production by over 60% in LPS-stimulated RAW 264.7 macrophage cells. Similar findings were previously reported for ethanol root extracts of *S. columbaria*, which showed inhibition of NO in the same macrophage model. Additionally, these ethanol extracts contained various triterpenoids, including ursolic acid, hederagenin,

and 24-nor-2 α ,3 β -dihydroxyolean-4(23),12-ene. Among these, the isolated 24-nor-2 α ,3 β -dihydroxyolean-4(23),12-ene showed strong NO inhibitory activity (Ezeofor 2023). Therefore, this study further supports and validates the use of *S. columbaria* in managing inflammatory disorders. Microbial infections are often linked to inflammatory responses that trigger overproduction of reactive oxygen species (ROS), leading to oxidative stress and tissue damage (Kayesh et al. 2025). Assessing the antioxidant potential of medicinal plants is therefore crucial. In this study, the antioxidant activity of *S. columbaria* acetone extracts was evaluated using DPPH and FRAP assays. The FRAP results showed strong ferric-reducing power, indicating effective electron-donating capacity compared with Trolox and ascorbic acid, suggesting notable antioxidant potential. In contrast, the DPPH assay showed weak antioxidant activity, as indicated by high IC_{50} values in this study. The FRAP findings align with a previous study where *S. columbaria* methanol extracts exhibited strong reducing activity using the FRAP method (Otang-Mbeng and Sagbo 2020). This discrepancy between FRAP and DPPH results may be attributed to differences in assay mechanisms. FRAP measures the ability of compounds to reduce Fe^{3+} to Fe^{2+} under acidic conditions, a process favored by hydrophilic antioxidants with strong reducing power. In contrast, DPPH assesses the ability to donate hydrogen atoms or electrons to a stable free radical in an organic medium, which can be less responsive to certain hydrophilic or polar compounds, particularly if they have poor solubility in the assay solvent or react slowly with the DPPH radical. This suggests that the extracts may be rich in compounds with strong reducing potential but limited radical-scavenging efficiency in lipophilic environments (Nwachukwu et al. 2021). Notably, previous studies also reported strong antioxidant effects from methanol flower and leaf extracts of *S. columbaria* using DPPH, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), FRAP, and cupric reducing antioxidant capacity (CUPRAC) methods (Akar 2021). The current findings further support *S. columbaria* as a promising source of natural antioxidants for managing oxidative stress. The selectivity index comparing cytotoxicity with antioxidant and anti-inflammatory activities of *Scabiosa columbaria* acetone extracts revealed that the observed effects were not a result of cytotoxicity. This suggests that the extracts may offer a safe and effective source for the development of anti-inflammatory and antioxidant agents.

Conclusion

This study highlights the therapeutic potential of *Scabiosa columbaria* acetone extracts, which contain bioactive compounds such as flavonoids, iridoid glycosides, phenolic acids, triterpenoid saponins, and polyphenolic quinones. These compounds likely contribute to the antimicrobial, antioxidant, and anti-inflammatory effects of the extracts. The findings further demonstrated strong

anti-inflammatory effects of *S. columbaria*, validating its traditional use in the treatment of inflammatory ailments such as menstrual pain, colic, and related conditions. Notable ferric-reducing capacity (FRAP assay) and antibacterial activity against *Neisseria gonorrhoeae* suggest potential applications in managing oxidative stress and gonorrheal infections, thereby highlighting the plant's value as a promising candidate for the development of novel antioxidant and antimicrobial agents. However, weak radical-scavenging activity based on the DPPH method and the low selectivity index from cytotoxicity and antimicrobial results indicate the need for further research to optimize extraction, isolate active compounds, and evaluate their pharmacological properties. Future studies should also focus on *in vivo* validation, toxicity profiling, and assessment of synergistic effects with existing therapies. Overall, *S. columbaria* shows considerable promise as a source of alternative treatments for infections, inflammation, and oxidative stress.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

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- The authors declared that no experiments on humans or human tissues were performed for the present study.
- The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.
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- ### Data availability
- All data supporting the findings of this study are included within the main text and the supplementary files.
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Supplementary material 1

Supplementary figures

Authors: Brian Ngobeni

Data type: docx

Explanation note: **fig. 1:** LCMS chromatogram of *S. columbaria* acetone extracts analysis in negative mode. **fig. 1:** LCMS chromatogram of *S. columbaria* acetone extracts analysis in positive mode.

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