Phytochemical composition and anti-inflammatory potential of Hermannia geniculata leaf extracts

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Research Article

Keywords:

Posted Date: June 29th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9197777/v1

Additional Declarations: No competing interests reported.

Abstract

Chronic inflammation contributes to the development of many non-communicable diseases; this causes high demand for safe plant-derived anti-inflammatory agents. *Hermannia geniculata* is a Basotho traditional medicine used to treat various ailments, and it remains poorly studied at the phytochemical level, especially its leaves. This study aimed to characterize the phytochemical composition of *H. geniculata* leaf extracts and evaluate their in vitro anti-inflammatory activity using a 5-lipoxygenase inhibition assay. Fresh leaves were collected from Phuthaditjhaba, South Africa, dried, powdered, and extracted using five solvents: acetone, ethanol, hexane, methanol, and distilled water. Qualitative and quantitative phytochemical analyses were performed to identify major classes of secondary metabolites. Total phenolic content (TPC) was determined using the Folin–Ciocalteu method. Anti-inflammatory activity was assessed using a 5-lipoxygenase (5-LOX) inhibition assay, with nordihydroguaiaretic acid (NDGA) as the positive control. Methanol produced the highest extraction yield (16.71 ± 2.43%) and the highest TPC (85.6 mg GAE/g), with ethanol showing comparable phenolic content (84.4 mg GAE/g). Qualitative screening revealed the presence of phenolics, flavonoids, alkaloids, tannins, glycosides, saponins, and phytosterols in multiple extracts, while anthraquinones were absent. All extracts exhibited weak 5-LOX inhibitory activity, with maximum inhibition of 18.13% at 0.40 mg/mL, compared with 84.93 ± 6.36% for the positive control. *H. geniculata* leaves contain diverse phytochemicals, with polar solvents demonstrating greater extraction efficiency. Although crude extracts showed limited 5-LOX inhibition, the phytochemical profile suggests potential for further investigation through extract fractionation and complementary anti-inflammatory assays to better evaluate the therapeutic potential of this species.

Introduction

Inflammation is a defence mechanism deployed by the body to recognize and remove harmful and foreign stimuli so the healing process can begin (Boaru, et al., 2024). However, long-term (chronic) inflammation which is characterised by sustained immune activation and progressive tissue damage ends up being implicated in the development of numerous diseases (Cao, 2023). Which in their long-term treatment using conventional remedies often results in significantly adverse effects. This, therefore, increases the interest in effective therapeutic remedies that are plant derived and safer (Nakadate, et al., 2025).

Dietary polyphenols derived from plants are increasingly regarded as relatively safe and effective agents for the prevention of chronic diseases (Li, et al., 2023). They are said to have potent antioxidant properties that combat oxidative stress, as well as anti-inflammatory effects that modulate gene expression (Shanmugam, 2025). Plant polyphenols modulate lipoxygenase-mediated arachidonic acid metabolism by inhibiting enzyme activity (Lončarić, et al., 2021). In this case, 5-lipoxygenase (5-LOX), a key enzyme responsible for leukotriene biosynthesis (Lončarić, et al., 2021), represents a validated therapeutic target in anti-inflammatory drug development.

There is a long standing history of the use of medicinal plant extracts to either prevent, alleviate or cure a wide range of disease, yet the mechanisms of how bioactive plant extracts work remain poorly understood because of lack of knowledge and scientific validation (Cao, 2023). Therefore, highlighting the need for medicinal plants with antioxidant properties for their ability to treat or prevent human pathogens (Sharifi-Rad, et al., 2020).

Hermannia geniculata is a species under the genus *Hermannia* (family Malvaceae), whose members are traditionally used across Southern Africa to treat a wide range of diseases and scientific investigation have shown promising pharmacological activities amongst members of the genus (Nhlapo, et al., 2025). *H. geniculata*, known as “Kgwagwa” by the Basotho is traditionally used for management of blood sugar, gastrointestinal pains, colic and heartburn (Nhlapo, et al., 2025) (Kazeem & Ashafa, 2015). However, the plant’s bioactive potential remains limited especially leaf extracts.

Relaying on roots for medicinal preparations poses a conservation challenge, as repeated uprooting threatens the long-term survival of a species (Chen, et al., 2016). Therefore, the aim of the study was to profile the phytochemical constituents of *Hermannia geniculata* leaf extracts and to evaluate their in vitro anti-inflammatory activity using a 5-lipoxygenase inhibition assay. This study seeks to provide scientific evidence that supports the traditional relevance of *H. geniculata* and to contribute to the identification of plant derived compounds with anti-inflammatory potential by correlating phytochemical composition with enzyme inhibitory potential.

Materials and Methods

Plant Material Collection and Authentication

Fresh leaves of *Hermannia geniculata* were obtained from a local herbalist in Phuthaditjhaba (Maluti-a-Phofung district, South Africa). Plant collection and use were authorised by the Department of Economic, Small Business Development, Tourism and Environmental Affairs (DESTEA) permit number 202504000019164. A voucher specimen (Mojamed/1/2016/Qhb) was prepared by Pheello Mojau, authenticated and deposited at the University of the Free State, Qwaqwa Campus Herbarium.

Preparation of Plant Material

Leaves were separated from stems, washed with running water, and oven-dried at 40°C for 24 h. The dried material was ground into a fine powder using a laboratory mill and stored in airtight containers protected from light until extraction.

Extraction Procedure

Powdered plant material (10 g) was extracted separately with 100 mL of acetone, ethanol, hexane, methanol, or distilled water using maceration with continuous shaking for 48 h. Extracts were filtered, and the solvents were removed by incubation at 30°C until dryness. The crude extracts were weighed to

determine extraction yield and stored for further analysis. Percentage yield was calculated relative to the initial dry plant mass.

Phytochemical Screening

Qualitative Analysis

Qualitative phytochemical screening for alkaloids, glycosides, steroids, triterpenoids, tannins, flavonoids, phenols, proteins, phytosterols, saponins, and anthraquinones was performed using standard procedures as previously described in the literature. The presence of each phytochemical class was determined based on characteristic colour changes or precipitate formation (Abubakar & Haque, 2020) (Banu & Cathrine, 2015).

Quantitative Analysis

Total phenolic content (TPC) was determined using the Folin–Ciocalteu method and expressed as gallic acid equivalents (mg GAE/g extract). Absorbance was measured at 765 nm (Hagerman, et al., 2000).

Total flavonoid content (TFC) was determined using an established methanolic extraction method, and results were expressed relative to extract dry weight (Krishnaiah, et al., 2009).

Total saponin and alkaloid contents were quantified gravimetrically following established protocols (Gracelin, et al., 2013). All experiments were performed in triplicate.

5-Lipoxygenase (5-LOX) Inhibition Assay

In vitro anti-inflammatory activity was evaluated using a 5-lipoxygenase (5-LOX) inhibition assay as previously described by (Njenga & Viljoen, 2006), with minor modifications. Linoleic acid was used as the substrate. The reaction mixture contained potassium phosphate buffer (0.1 M, pH 6.3), substrate, and 5-LOX enzyme. Extracts were tested at concentrations ranging from 0.02 to 0.4 mg/mL. The formation of conjugated dienes was monitored spectrophotometrically at 234 nm over 10 min. Nordihydroguaiaretic acid (NDGA) was used as a positive control, while DMSO/Tween 20 served as the negative control. Percentage inhibition was calculated relative to the control, and IC_{50} values were determined using GraphPad software. All assays were conducted in triplicate.

Statistical Analysis

Results are expressed as mean ± SEM. Statistical comparisons were performed using one-way ANOVA followed by Tukey's post hoc test. A value of $p < 0.05$ was considered statistically significant.

Results

Percentage Yield

The extraction yields of *H. geniculata* leaves differed significantly among solvents (Table 1). Methanol had the highest yield (16.71 ± 2.43%), which was significantly greater than hexane and acetone ($p < 0.05$). Aceton had the lowest yield (5.89 ± 1.19%). In all, polar solvents showed higher extraction efficiency than non-polar solvents.

Table 1
Percentage yield of extracts made from *Hermannia geniculata* leaves using different solvents

Solvents	**Percentage yield (%)**
Methanol	16.71 ± 2.43^{a}
Ethanol	11.25 ± 2.24^{ab}
Distilled water	9.43 ± 3.56^{ab}
Hexane	8.49 ± 3.85^{b}
Acetone	5.89 ± 1.19^{b}

Qualitative Phytochemical Analysis

Qualitative screening revealed presence of alkaloids, phytosterols, saponins and tannins in most extracts (Table 2). Methanol and ethanol extracts exhibited a broader range of phytochemicals in comparison to other solvents. Anthraquinones were absent in all extracts.

Table 2
A table summarising qualitative phytochemical analysis of leaves of Hermannia geniculata in different extracts

Qualitative Analysis Results	**Acetone**	**Hexane**	**Distilled water**	**Ethanol**	**Methanol**
Alkaloids	+	+	-	+	+
Steriods & Triterpenoids	-	+	+	+	++
Tannins	+	-	-	-	+
Gelatin test	+	+	+	+	++
Ferric chloride test					
Flavonoids	-	+	+	-	+
Phenols	-	-	+	-	+
Proteins	+	+	+	+	-
Glycosides	+	++	++	+	+
Phytosteriods	++	+	+	++	++
Saponnins	+	++	++	+	+
Anthraquinones	-	-	-	-	-

Quantitative Phytochemical Analysis

Quantitative analysis (Table 3) showed that methanol extract had the highest total phenolic content (8.56 ± 0.008 mg GAE/g), while ethanol had the highest flavonoid content (0.42 mg/g). Saponin levels were comparable across extracts (0.03–0.04 mg/g). Methanol yielded the highest alkaloid concentration (1.38 mg/g).

Table 3
Total phenolic content, flavonoid content, total saponins content and total alkaloids content of *H. geniculata* leaves extracts

Extracts	**Total phenolic content (mg GAE/g extract)**	**Total flavonoid content (mg/g)**	**Total saponins content**	**Total alkaloid content**
Acetone	74,4 ± 0,010	0,27	0,03	0,68
Hexane	30,3 ± 0,016	0,04	0,03	0,74
Water	10,9 ± 0,003	0,02	0,04	0,04
Ethanol	84,4 ± 0,018	0,42	0,03	0,50
Methanol	85,6 ± 0,008	0,23	0,03	1,38

Anti-Inflammatory (5-LOX)

All extracts demonstrated weak 5-lipoxygenase inhibition compared to the positive control (Table 9; Figs. 17–18). Acetone showed the highest inhibition (18.13% at 0,40mg/ml), followed by ethanol (11.74%). Hexane exhibited no inhibitory activity and produced negative values across concentrations. No clear dose-dependent trend was observed.

Table 9
Percentage inhibition of acetone, hexane, distilled water, ethanol and methanol extracts on the 5-lypoxygenase enzyme as the concentration decreases

Concentration(mg/ml)	Acetone	Hexane	Distilled water	Ethanol	Methanol
0,40	18,13 ± 9,33	-1,90 ± 1,96	10,88 ± 6,22	6,74 ± 3,11	11,74 ± 6,43
0,20	11,57 ± 5,54	-2,42 ± 4,34	6,87 ± 1,58	5,01 ± 3,45	8,81 ± 4,61
0,10	8,46 ± 3,37	-6,91 ± 0,3	5,56 ± 1,82	2,76 ± 3,45	7,60 ± 5,71
0,05	7,43 ± 1,30	-3,80 ± 4,67	5,35 ± 1,30	1,73 ± 3,17	6,22 ± 3,63
0,03	6,91 ± 3,45	-3,28 ± 5,09	5,35 ± 2,54	0,00 ± 2,37	4,15 ± 0,52
Positive control	84,93 ± 6,36				

Discussions

Extraction Yield

Methanol is usually associated with high extraction yield in comparison to other solvents (Dhawan & Gupta, 2017). This is because of methanol's high polar protic nature and high dielectric constant that allows it to solubilize polar secondary metabolites as well as some non-polar constituents, therefore allowing a wide range of extraction (Sharma & Cannoo, 2017). Other polar solvents include ethanol and water and together they are good at extracting hydrophilic compounds such as phenolics and flavonoids from natural materials. And reports show that plant products with phenolic compounds show antimicrobial activity, reduce inflammation, lower cancer incidence and diabetes (Nofita, et al., 2022). The lower yields observed with acetone and hexane further support the influence of solvent polarity, suggesting that dominant constituents of *H. geniculata* leaves are polar in nature.

Phytochemical Profile

Quantitative screening proved the presence of diverse secondary metabolites in *H. geniculata* leaves extracts. These wide variation in phytochemical distribution among solvents reflects the polarity-dependent extraction, as previously discussed. While presence of glycosides and phytosterols in all extracts and saponins in most shows that these compounds have a broad solubility range in the leaf matrix.

The total phenolic content found in this study aligns with reports from other medicinal plant species, where methanol consistently yields higher phenolic concentration that less polar solvent (Abubakar, et al., 2017) (Duniya, et al., 2018) (Dhawan & Gupta, 2017). Although water is highly polar, its comparatively lower phenolic yield may be due to limited solubility as a pure and sources indicate that alcohol-water mixture is significantly more effective (Osorio-Tobón, 2020).

The total flavonoid content was highest in the ethanol extract, marking difference from the phenolic trend in which methanol predominated. This suggests that the flavonoid fraction of *H. geniculata* may consist of moderately polar compounds more efficiently extracted by ethanol. Therefore, in this case, flavonoid and total phenolic distributions across solvents are not parallel. The same was also found by (Sharma, et al., 2021), whereby the methanolic extract of the leaves yielded maximum total flavonoid content of all tested parts yet it had no detectable total phenolic content.

Alkaloid content was highest in the methanol extract ad minimal in the aqueous extract. In this case, alkaloids dissolved better in organic solvent over in aqueous and this was the same case in study by (Pandey, et al., 2025) that showed alkaloids were present in high concentrations in methanol extract while completely absent in aqueous extract. However, the results can vary depending on the plant matric as *Datura metel* leaves revealed alkaloids presence in all tested solvents including distilled water (Dhawan & Gupta, 2017). Therefore, chemical structure of alkaloids allows them to be captured by both organic and aqueous extracts.

Anti-inflammatory Activity

The weak inhibitory activity observed in this study suggest that *H. geniculata* crude leaf extracts do not strongly modulate 5-lipoxygenase (5-LOX) pathway at the concentrations tested. And this does not exclude potential anti-inflammatory activity through other mechanisms. Effective LOX inhibition is significantly influenced by both the quantity of phenolic compounds and their specific structural classes as well as their chemical properties. For instance, the best LOX inhibition in *Cydonia oblonga* was achieved by methanol extract with TPC of 367 mg GAE/g which is substantially higher that the values recorded for *H. geniculata* in this study (Lončarić, et al., 2021).

A similar trend is noted in a study by (Adebayo, et al., 2016) that was evaluating 25 South African medicinal plant species that are traditional used to treat pain, confirmed that *Peltophorum africanum* and *Zanthoxylum capense* were the only species to demonstrate promising inhibitory activity against 15-lipoxygenase. Also, crude extracts often lack a clear dose-response relationship because of their nature as complex mixtures that contain multitude of different molecules (Khumalo, et al., 2022) (Azab, et al., 2016).

Future studies should therefore focus on fractionation of the crude extracts, particularly the acetone and methanol extracts that showed relatively higher activity, to isolate and evaluate specific bioactive compounds. Additional assays targeting antioxidant activity or other inflammatory pathways, such as

COX-2 inhibition or nitric oxide scavenging, may also provide a more comprehensive evaluation of the anti-inflammatory potential of *H. geniculata*.

Conclusion

In conclusion, the study provided phytochemical characterization of leaf extracts from *Hermannia geniculata* and a preliminary evaluation of their vitro anti0inflammatory potential. The results showed that polar solvents, particularly methanol and ethanol, produced the highest extraction yields and phenolic contents. Although the crude extracts displayed weak inhibition of the 5-lipoxygenase enzyme, this does not necessarily rule out anti-inflammatory potential. Further studies should focus on fractionating the extracts to isolate active compounds and applying additional anti-inflammatory assays to better understand the therapeutic potential of *H. geniculata* leaves. Importantly, the presence of bioactive phytochemicals in the leaves suggests that leaf harvesting could serve as a more sustainable alternative to the traditional use of roots which may raise conservation concerns.

Declarations

Ethical approval and consent to participate

Ethical approval number UFS-HSD2024/0319

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article

Competing interests

The authors declare that they have no competing interests

Funding

The study was funded by the Central Research Fund (CRF), Department of Plant Science, University of the Free State. South Africa for financial support. The funding does not have any baring to the data or outcomes generated from the study.

Author Contributions

Sylvia Modibedi and Pheello Mojau conceptualized, designed the study, collected and analysed data, and the writing. All authors curated and analysed data and the writing, revised, and the writing. All authors read and approved the final manuscript.

Acknowledgement

The authors are most grateful to the Central Research Fund of the Plant Sciences Department University, South Africa for financial support.

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Figures

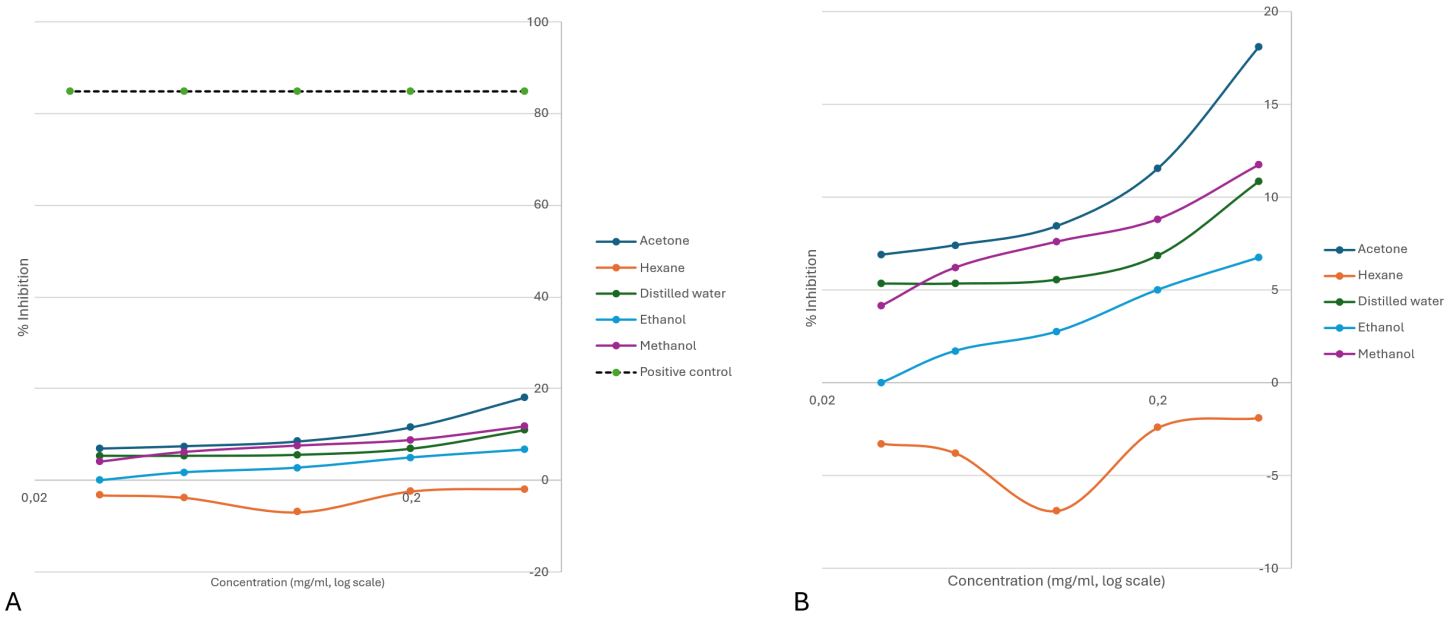

Figure 1

Figure 18. (a) Dose–response inhibition curves of H. geniculata extracts compared to positive control. (b) Extract-only dose–response curves (expanded scale to show low inhibition differences).