



Determination of the correlative association between phytochemical content and nitrite inhibition in selected medicinal plants used traditionally to treat respiratory infections

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ABSTRACT

Introduction: Several medicinal plants, including *Artemisia afra* Jacq. Ex Willd, *Lippia javanica* (Burm.f.) Spreng, *Tetradenia riparia* (Hochst.) Codd, and *Tulbaghia violacea* Harv have been used in treating illnesses such as respiratory infections and their associated inflammatory dysregulation. The study aimed to evaluate the quantitative phytochemical content and the nitrite inhibition strength of the selected medicinal plant extracts.

Methods: A cold-maceration aqueous extraction procedure was employed for phytoconstituent extraction from dried plant material. The total phenolic content (TPC), along with total flavonoid content (TFC), total tannin content (TTC), and total saponin content (TSC), was quantitatively analyzed in the plant extracts. Cytotoxicity evaluation was done using the MTT assay to measure the minimum lethal concentrations against RAW 264.7 macrophage cell lines, and inhibition of nitrite production was assessed using a RAW macrophage cell-based assay

Results: All plant extracts contained measurable levels of the tested phytochemicals, with *A. afra* roots showing the highest phenolic and flavonoid content at 48.54 ± 1.33 mg GAE (gallic acid equivalent)/g and 39.76 ± 1.56 mg QE (quercetin equivalents)/g, respectively. The extracts exhibited relatively low cytotoxicity against RAW macrophage cells, with LC_{50} values ranging between 64 ± 2.2 and 1443 ± 86.2 μ g/mL. The most nitrite inhibition was observed in *T. riparia* leaf extracts, with 86% inhibition at 12.5 μ g/mL. Correlation analysis further showed that extracts with higher phenolic content exhibited stronger nitrite inhibition properties.

Conclusion: Overall, all extracts demonstrated nitrite inhibition activity, showing the possible medicinal uses of these plants against respiratory infections with their associated inflammation.

Implication for health policy/practice/research/medical education:

The selected medicinal plants *A. afra*, *L. javanica*, *T. riparia*, and *T. violacea* exhibit nitrite inhibitory properties, which may help reduce chronic inflammation. Their nitrite inhibition properties are likely linked to phenols, demonstrating the extracts' potential for multiple phytochemical-based actions. Therefore, the results support the anti-inflammatory usage of the medicinal plants based on their possession of phenol compounds that showed nitrite inhibition activity. The inflammatory properties would thus in turn support their usage in alleviation of symptoms associated with respiratory infections.

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Introduction

Studying medicinal plants is of considerable importance due to their widespread use among the general public, their role in preserving traditional knowledge, and the need to disseminate evidence-based information (1). Despite advances in modern medicine and therapeutic

strategies, respiratory infections and their associated inflammatory complications remain a major health challenge, playing a major role in incidence, morbidity, and mortality seen worldwide (2,3). Furthermore, many steroidal anti-inflammatory drugs currently available are associated with adverse effects, highlighting the need

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for safer alternatives. Plant-derived products present a promising source of novel anti-inflammatory agents. The efficiency of extracting these compounds is strongly influenced by variables, including the specific plant part or tissue utilized (4).

Phytochemicals play a critical role in combating life-threatening diseases, including those linked to inflammation (5). Inflammation is a biological defence mechanism that protects cells against infectious pathogens, physical agents, and immune dysfunction (6). While acute inflammation is a protective output of the innate immune system, prolonged inflammation can result in tissue damage and facilitate the spread of infection (7). *In vitro* assays provide valuable tools for elucidating the mechanisms of anti-inflammatory agents and their therapeutic potential (8).

Innate immune cells, particularly macrophages, play a pivotal role in inflammation by synthesizing and releasing cytokines and other mediators such as nitric oxide species (9–11). These pro-inflammatory mediators contribute to pathogen-induced tissue damage. Inducible nitric oxide synthase (iNOS) is relevant in macrophages, where it produces high concentrations of nitric oxide species in response to bacterial toxins (12). Lipopolysaccharides (LPS), structural components of gram-negative bacteria, act as potent toxins that activate macrophages during inflammation (13).

Several phytochemical groups have been shown to possess bioactive properties against viral and inflammatory conditions (14–17). In Southern Africa, medicinal plants such as *Artemisia afra*, *Lippia javanica*, *Tetradenia riparia*, and *Tulbaghia violacea* have been used for centuries to treat colds, coughs, chest pain, sore throat, asthma, fever, and inflammatory conditions (18–25). These plants are rich in secondary metabolites that exhibit strong anti-inflammatory potential (26). Although phytochemical screening has been conducted for many of these species, results pertaining to quantifying specific phytochemical groups in some plant parts are still lacking, which is essential for the selected plants to better understand their bioactive roles in inflammation and disease (20,21,27–29). Phytochemicals such as phenols, flavonoids, tannins, and saponins are particularly noteworthy for their therapeutic and anti-inflammatory properties (14,30,31).

As such, several medicinal plants have been shown to contain groups of phytochemical compounds, including phenolics, flavonoids, saponins and tannins (23,25,32). In addition, most of those medicinal plants have shown anti-inflammatory activity through nitrite-inhibition properties (33,34). However, information on the determination of the correlative association between the phytochemical content and nitrite inhibition remains scanty. Therefore, the current study seeks to contribute knowledge in this regard by determining the association between phytochemical content and nitrite inhibition strength in selected medicinal plants traditionally used to

treat respiratory infections.

Methods

Mechanical equipment used

Microplate reader (Multiskan Sky High, Thermo Fisher Scientific, Centurion, Gauteng, South Africa), Orbital shaker (OrbiShake platform shaker, Labotec, Midrand, Gauteng, South Africa), Grinder (PX-MFC 90 D, Lucerne, Switzerland), Freeze dryer (Surface Freeze Dryer, LYO60V-1/LYO80V-1 Series, Bics, Johannesburg, Gauteng, South Africa), Cell culture CO₂ incubator (EC160 incubator, NUVE, Ankara, Turkey) were used in this study.

Plant selection, identification and voucher specimen preparation

A desktop study was undertaken to identify medicinal plants that met the inclusion criteria. The selection focused on species with documented ethnobotanical use against respiratory viral infections and inflammation, particularly those classified as shrubs or smaller plants, as these allow for easier study of different plant parts. Preference was given to species naturally occurring in South Africa, especially within KwaZulu-Natal, and those extensively reported in scientific literature. Exclusion criteria eliminated trees and endangered species to promote sustainable, long-term utilization of the selected plants. This consideration was essential, as favorable results could lead to increased demand and usage, making sustainability a critical factor in the study design. Medicinal plants were all collected in South Africa, at the following locations in KwaZulu Natal: *Artemisia afra* Jacq. Ex Willd and *L. javanica* (Burm.f.) Spreng were collected at 28°28'47.3"S 29°45'19.0"E, *T. riparia* (Hochst.) Codd at 29°47'47.0"S 30°53'49.6"E, and *T. violacea* Harv. at 28°35'40.5"S 29°47'47.4"E conditions. The plants were then pressed and deposited at a herbarium, where they were identified by a specialist at SANBI. The plants were then stored with voucher numbers *A. afra* (PRE2024-0001), *L. javanica* (PRE2024-0002), *T. violacea* (PRE2024-0003) and *T. riparia* (PRE2024-0004).

Plant material preparation and extraction

Artemisia afra (A.A) stems and roots, *L. javanica* (L.J) leaves and roots, *T. riparia* (T.R) leaves and stems, and *T. violacea* (T.V) stems and roots were utilized for the project. The following abbreviations for plant parts were used: L-Leaves, S-Stems, R-Roots. The plant material was ground into a fine powder. An aqueous solvent (distilled water) was used for the extraction of the medicinal plants at 1g/10 mL on an orbital shaker using cold maceration serially for 24 hours x 3. The extracted plant materials were filtered after each 24-hour period using filter paper and then dried in a freeze dryer until a constant dried weight was achieved. The prepared dry plant extracts were then placed in a sealable container and kept in the dark until further evaluation.

Determination of phytochemical concentrations

Measurement of total phenolic concentrations

The phenolic content in the extracts was assessed using the Folin-Ciocalteu assay, following the procedure of (35) with slight adjustments. In brief, 1 mL of plant extract (1 mg/mL) was combined with 2.5 mL of 10% Folin-Ciocalteu reagent and 2 mL of 2% sodium carbonate. After incubation at room temperature for 15 minutes, absorbance was taken at 765 nm. Gallic acid served as the reference standard, with solutions (30–100 µg/mL) prepared in 99% dimethyl sulfoxide (DMSO). Results were expressed as milligrams of gallic acid equivalents (GAE) per gram of extract, calculated from the calibration curve (35).

Measurement of total flavonoid concentrations

Flavonoid levels were quantified by the aluminum chloride colorimetric method adapted from (36), with slight modifications. Quercetin standards (0.3–1 mg/mL) were formulated in 99% DMSO. A 50 µL portion of extract (1 mg/mL) or standard was mixed with 10 µL of 10% aluminum chloride, followed by 150 µL of 99% ethanol and 10 µL of 1 M potassium acetate in a 96-well plate. Ethanol (99%) was used as the blank. Samples were incubated for 40 minutes at room temperature in the dark, and absorbance was taken at 415 nm. Flavonoid content was expressed as milligrams of quercetin equivalents (QE) per gram of extract, based on the calibration curve (36).

Measurement of total tannin concentrations

Tannin concentration in aqueous extracts was determined using a modified version of (35). Extracts were treated with 0.1 mL of diluted Folin-Ciocalteu reagent (1:10) and 1 mL of 7.5% sodium carbonate. After thorough mixing and incubation at room temperature for 30 minutes, absorbance was measured at 700 nm. Tannic acid standards (0.3–1 mg/mL) were prepared in distilled water. Results were expressed as milligrams of tannic acid equivalents (TAE) per gram of extract, calculated from the calibration curve (35).

Measurement of total saponin concentrations

Saponin concentration in aqueous extracts was measured using the vanillin-sulfuric acid colorimetric assay described by (37), with slight modifications. A 50 µL aliquot of plant extract was diluted with 250 µL distilled water, then mixed with 250 µL of vanillin-sulfuric acid reagent (8% vanillin in 99% ethanol plus 5 mL of 70% sulfuric acid). The mixture was incubated at 60 °C for 20 minutes in a water bath, cooled to room temperature, and the absorbance was read at 544 nm. Diosgenin standards (0.3–1 mg/mL in 99% ethanol) were used, and saponin levels were expressed as milligrams of diosgenin equivalents (DE) per gram of extract, based on the calibration curve (37).

Nitrite inhibition

Cell line preparation

RAW 264.7 macrophage cells (Sep-Scie, South Africa) were used for the project. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (10,000 units/mL penicillin G and 10 mg/mL streptomycin). Cultures were maintained at 37 °C with 5% CO₂. The medium was timeously refreshed as cell density increased until cultures reached 70–80% confluency.

In vitro cytotoxicity assay

Cytotoxic effects of the plant extracts were assessed using the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) colorimetric assay on RAW 264.7 cells, following (38). This assay relies on mitochondrial succinate dehydrogenase activity, which reduces MTT to insoluble formazan crystals. The quantity of formazan formed correlates directly with cell viability, as only metabolically active cells can perform this reduction. RAW 264.7 cells were grown to 70–80% confluency in DMEM (100 mL) supplemented with FBS (10 mL). Cells were washed, trypsinized, and seeded into the 96-well plate at a density of 1.0×10^5 cells/mL, then incubated for 24 hours at 37 °C with 5% CO₂.

Cells were treated to decreasing concentrations of solvent extract fractions (100–12.5 µg/mL). Hydrogen peroxide (H₂O₂) functioned as the positive control, whereas dimethyl sulfoxide (DMSO) acted as the negative control. Treatments were conducted in triplicate, and the cells were incubated for 24 hours in the incubator. After incubation, 20 µL of MTT solution (5 mg/mL) was added to each well, and the plates were incubated for an additional 2–4 hours until purple formazan crystals were visible under a microscope. The MTT-containing medium (190 µL) was removed, and 100 µL of DMSO was added to dissolve the crystals. Plates were shaken for 5 minutes, and absorbance was measured at 560 nm using a microplate reader. Cell mortality (%) was calculated using the formula:

$$\text{Cell mortality (\%)} = (\text{mean OD} \div \text{control OD}) \times 100$$

The lethal concentration (LC₅₀), representing the concentration at which 50% of RAW 264.7 cells were non-viable, was determined by regression analysis. OD is the Optical Density representing the absorbance reading from the microplate reader at 560 nm.

In this study, the LC₅₀ values of RAW 264.7 macrophage cells were grouped into clusters using alphabetical denotation (A–D) based on the k-means test. These letters serve as categorical markers that distinguish statistically significant groupings according to natural breaks and spacing in the data. Cluster A represents extracts with the lowest LC₅₀ values, indicating higher cytotoxicity and greater sensitivity of the cells to those treatments.

Cluster B corresponds to extracts with moderately low LC_{50} values, reflecting intermediate cytotoxicity. Cluster C denotes extracts with moderately high LC_{50} values, suggesting lower cytotoxicity compared to clusters A and B. Finally, Cluster D includes extracts with the highest LC_{50} values, signifying minimal cytotoxicity and greater cell tolerance. Thus, the alphabetical denotation provides a clear and systematic way to interpret relative cytotoxicity levels across the different plant extracts.

LPS-induced intracellular nitrite inhibition

The assessment of nitrite species was performed using the RAW 264.7 macrophage cells following the protocol described by More and Makola (39) with minor modifications. Quercetin was included as a reference inhibitor of nitric oxide generation (40). Cells were seeded in a 96-well plate and incubated for 24 hours at 37 °C in a humidified atmosphere containing 5% CO_2 . After incubation, cells were treated with plant extract (12.5 µg/mL) at non-toxic concentrations, in the absence or presence of 1 µg/mL LPS. Quercetin served as the positive control. Nitrite accumulation in the culture medium was quantified using the Griess reaction. Briefly, 100 µL of Griess reagent (1% sulfanilamide and 0.1% naphthylendiamine in 5% phosphoric acid) was added to 100 µL of cell culture supernatant. The mixture was incubated at room temperature for 10 minutes, and absorbance was recorded at 550 nm using a microplate reader. Nitrite concentrations in samples and controls were calculated using the following equation:

$$\text{Nitrite}(\mu\text{M}) = \left[\frac{\text{CSM} - (y - \text{intercept})}{\text{Slope}} \right] \times 2 \times \text{Sample dilution}$$

where CSM represents the corrected sample measurement. The lower limit of detection (LOD) for the nitrite assay was 0.62 µM.

Data presentation

Each assay was done in triplicate. In tables, the results

are found as mean values $\pm$ standard deviation. Total phytochemical content in the extracts, was quantified by regression analysis using a known compound as the standard for each phytochemical group.

Statistical analysis

Regression analysis was done to determine the LC_{50} values of plant extracts. Data were analyzed using a one-way ANOVA, with statistical significance defined at $P < 0.05$. The LC_{50} and p values were calculated using AAT Bioquest as the analytical tool. Quantitative data were grouped into four clusters (A, B, C, D) using K-means clustering. Relationships between total phytochemical content and nitrite inhibition were assessed through Spearman's rank correlation. Microsoft Copilot (2024) was used for conducting the K-means clustering and also the Spearman's rank correlation analyses.

Results

Quantitative phytochemical analysis

The quantitative phenolic, flavonoid, tannin, and saponin concentrations of extracts are presented in Table 1. Calibration curves for the respective standards are shown in Figure 1. The greatest values of quantitative phenolic and flavonoid contents were seen in *A. afra* root extracts, with values of 48.54 ± 1.33 mg GAE/g and 39.76 ± 1.56 mg QE/g, respectively. The greatest total tannin concentration was observed in *A. afra* stem extracts at 1338.67 ± 31.77 mg TAE/g, followed by the *A. afra* root extracts at 1212.00 ± 25.06 mg TAE/g. The extract with the highest total saponin content (TSC) was *T. violacea*, at 1426.67 ± 34.03 mg DE/g.

Cytotoxicity results

Before assessing the anti-inflammatory bioactivity of the plant extracts, their cytotoxic effects on RAW 264.7 macrophages were determined using the MTT assay. RAW macrophages were treated with extract concentrations ranging from 12.5 to 100 µg/mL. The lethal concentration

Table 1. Total phenolic, flavonoid, tannin, and saponin contents of plant extracts

Plant extract	TPC (mg GAE/g)	TFC (mg QE/g)	TTC (mg TAE/g)	TSC (mg DE/g)
A.A S	37.05 ± 0.81^B	6.92 ± 0.17^C	1338.67 ± 31.77^A	414.47 ± 8.36^C
A.A R	48.54 ± 1.33^A	39.76 ± 1.56^A	1212.00 ± 25.06^A	670.17 ± 31.78^B
L.J R	17.40 ± 1.02^C	22.11 ± 1.02^B	1201.33 ± 51.39^A	1407.67 ± 43.66^A
L.J L	14.20 ± 0.46^C	8.86 ± 0.32^C	598.67 ± 20.03^C	433.03 ± 17.18^C
T.R L	13.64 ± 0.40^C	5.85 ± 0.29^C	976.67 ± 30.55^B	425.00 ± 17.32^C
T.R S	4.93 ± 0.18^C	23.98 ± 0.65^B	867.33 ± 27.23^B	681.67 ± 28.87^B
T.V R	6.87 ± 0.11^C	17.56 ± 0.71^B	1000.07 ± 29.46^B	1426.67 ± 34.03^A
T.V S	6.53 ± 0.07^C	12.66 ± 0.54^C	944.67 ± 30.55^B	640.00 ± 25.00^B

Aqueous plant extracts of *A. afra* (A.A), *L. javanica* (L.J), *T. riparia* (T.R), and *T. violacea* (T.V) were obtained from different plant parts: leaves (L), stems (S), and roots (R). The quantitative phytochemical content of each extract was categorized into clusters (A-D) based on K-means analysis. Cluster assignment was determined according to natural breaks and spacing observed per individual column. The plant extracts that differed significantly $p < 0.05$ in terms of the phytochemical content in that column were designated different denotations. TPC: Total phenolic content; TFC: Total flavonoid content; TTC: Total tannin content; TSC: Total saponin content; QE: Quercetin equivalent; TAE: Tannic acid equivalent.

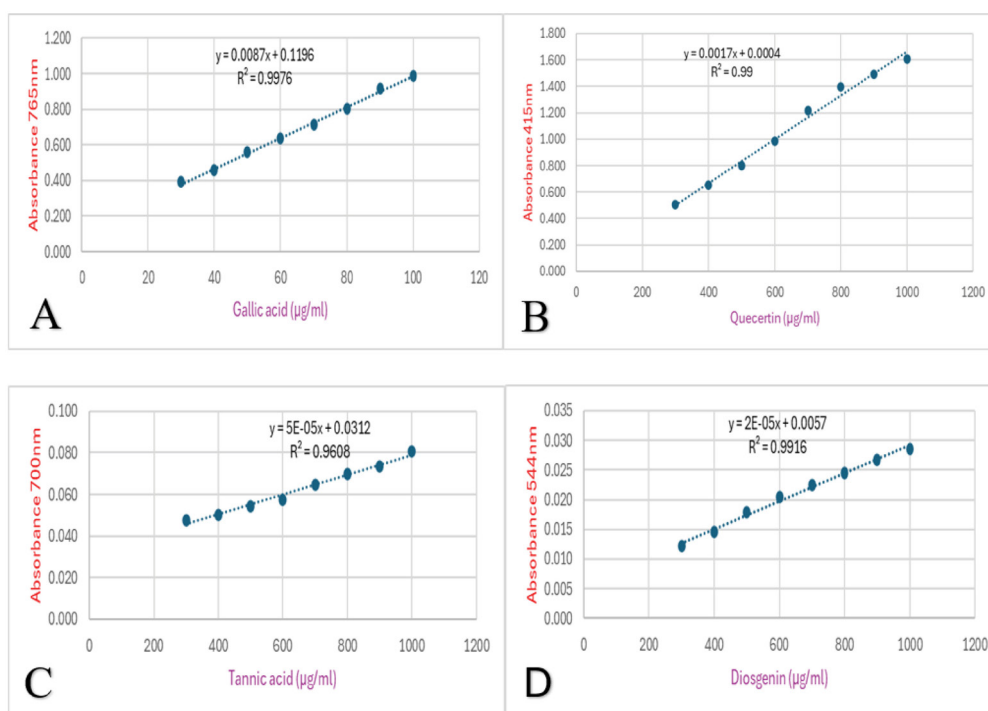


Figure 1. Standard calibration curves for quantitative evaluations of phenolic, flavonoid, tannin, and saponin contents of the extracts. **A.** Gallic acid calibration curve for total phenol evaluation. **B.** Quercetin calibration curve for total flavonoid evaluation. **C.** Tannic acid calibration curve for total tannin evaluation. **D.** Diosgenin calibration curve for total saponin evaluation.

(LC_{50}) that reduced cell viability by 50% was determined (Table 2). All extracts demonstrated varying degrees of non-toxic behavior, showing LC_{50} values ranging from 64 to 1443 $\mu\text{g/mL}$. Importantly, all LC_{50} values exceeded 64 $\mu\text{g/mL}$, indicating that the extracts were generally safe at the tested concentrations, including the positive controls quercetin and disprin. The lowest viability (30.5%) was recorded in *L. javanica* root extract versus the highest concentration tested (Figure 2).

Table 2. Lethal concentration (LC_{50}) values of the plant extracts and positive control quercetin for the determination of their cytotoxic effects

Plant extract/Sample	Raw macrophage cells
	LC_{50} ($\mu\text{g/mL}$)
<i>Artemisia afra</i> stems	152.7 $\pm$ 5.8 ^D
<i>Artemisia afra</i> roots	422.7 $\pm$ 15.5 ^C
<i>Lippia javanica</i> roots	64.0 $\pm$ 2.2 ^D
<i>Lippia javanica</i> leaves	560.5 $\pm$ 21.1 ^C
<i>Tetradenia riparia</i> leaves	784.9 $\pm$ 75.4 ^B
<i>Tetradenia riparia</i> stems	287.3 $\pm$ 10.2 ^D
<i>Tulbaghia violacea</i> roots	1443.1 $\pm$ 86.2 ^A
<i>Tulbaghia violacea</i> stems	1020.7 $\pm$ 47.6 ^B
Quercetin	479.9 $\pm$ 20.16 ^C

The LC_{50} values for RAW 264.7 macrophages were grouped into clusters (A–D) based on K-means analysis. Cluster assignment was determined according to natural breaks and spacing observed per individual column. The plant extracts/samples that differed significantly $P < 0.05$ in terms of the LC_{50} in that column were designated different denotations.

Measurement of LPS-induced intracellular nitrite inhibition

Plant extract concentrations showing 80-90% RAW macrophage cell viability were defined as the maximal non-cytotoxic concentrations (MNTCs). These MNTCs were subsequently tested for nitrite inhibition. Nitrite production inhibition was evaluated using the MNTC (12.5 $\mu\text{g/mL}$) of the extracts. The nitrogen species experiment was done for the evaluation of the inhibitory effects of plant extracts on nitrite production in RAW 264.7 macrophages. Macrophages were pre-treated with extracts for 24 hours, then stimulated with LPS at 1 $\mu\text{g/mL}$. LPS exposure significantly increased nitrite production when evaluated against unstimulated controls. Treatment with plant extracts (12.5 $\mu\text{g/mL}$) markedly reduced LPS-induced nitrite levels, except for the *T. riparia* stem extract, which did not show significant inhibition (Figure 3). At 12.5 $\mu\text{g/mL}$, nitrite inhibition values varied across the samples. *T. riparia* leaves (86.12 $\pm$ 0.55%), *L. javanica* roots (81.89 $\pm$ 1.76%), and *L. javanica* leaves (80.95 $\pm$ 1.91%) showed the highest inhibition percentages, while *T. riparia* stem (3.47 $\pm$ 0.10%) represented the lowest inhibition. Overall, inhibition values ranged from 3.47% to 86.12%, with most samples clustering in groups A and B showing good nitrite inhibition (Table 3).

Discussion

Phytomedicines have the ability to inhibit molecular targets that regulate the synthesis of pro-inflammatory mediators

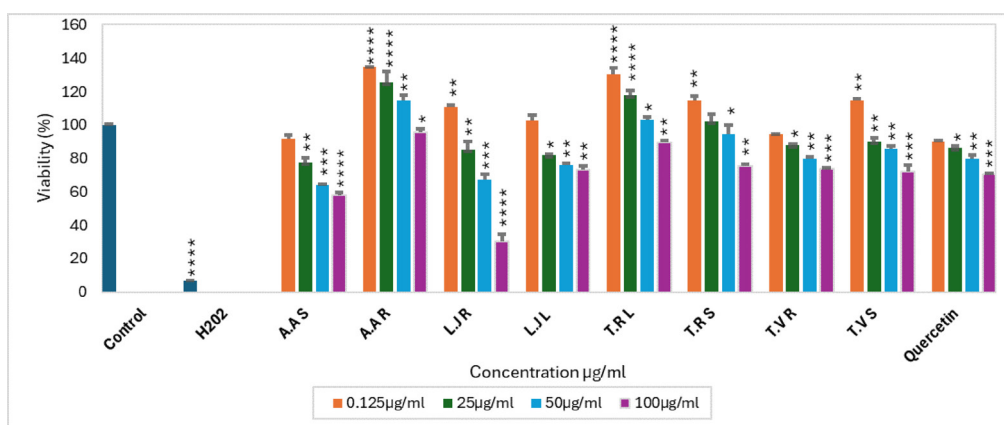


Figure 2. Cell viability of RAW 264.7 macrophages treated with aqueous extracts of *Artemisia afra* (A.A), *Lippia javanica* (L.J), *Tetradenia riparia* (T.R), and *Tulbaghia violacea* (T.V) obtained from different plant parts: leaves (L), stems (S), and roots (R), after 24 hours of incubation. The error bar found in each bar is the standard deviation of the means (n=3). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$, compared to the control group.

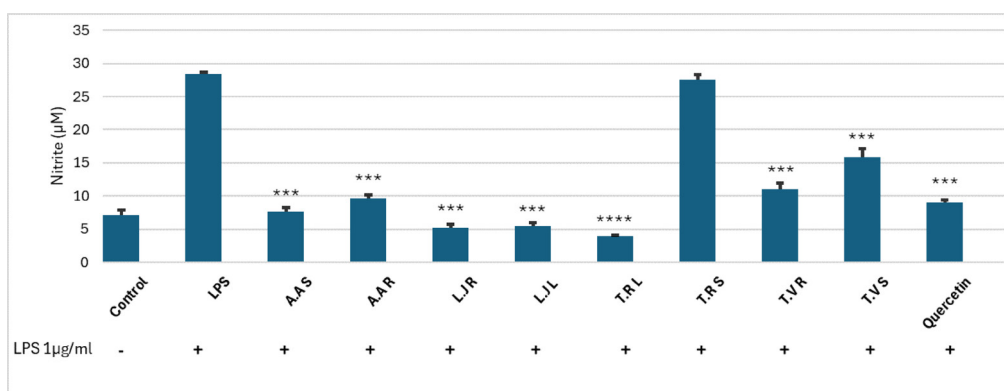


Figure 3. Nitrite concentration in RAW 264.7 macrophage cells treated using plant extracts and quercetin. Extracts were obtained from *Artemisia afra* (A.A), *Lippia javanica* (L.J), *Tetradenia riparia* (T.R), and *Tulbaghia violacea* (T.V) from different plant parts: leaves (L), stems (S), and roots (R). Cells were stimulated with lipopolysaccharides (LPS). The error bar found in each bar is the standard deviation of the means (n=3). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$, compared to the LPS group.

in inflammatory processes. Some causative organisms and factors that initiate and promote inflammation can be removed or neutralized, thereby suppressing the expression of pro-inflammatory agents by medicinal plant extracts (41). Although phytochemical screening studies have been conducted for most of the plants used in this study, it was important to quantitatively measure different phytochemical groups such as phenolic content, flavonoid content, tannin content, and saponin content. These quantitative measures are crucial for understanding their bioactive roles when testing for anti-inflammatory properties via nitrite inhibition and their high activity against certain diseases (20,21,23,25,27-29,32).

The *Artemisia afra* root extract showed the highest content for phenolics at 48.54 ± 1.33 mg GAE/g. The same *A. afra* root extract also exhibited the highest amount of flavonoids at 39.76 ± 1.56 mg QE/g. A study by Kane et al (25) confirmed *A. afra* to have a high concentration of phenols and flavonoids in ethanolic extracts of *A. afra* collected from Burundi, having phenol concentrations

of 606.9449 mg GAE/g and flavonoid concentrations of 242.4745 mg Rutin/g. The most tannin content was seen in the stem extract of *A. afra* at 1338.67 ± 31.77 mg TAE/g. This finding is supported by Kane et al (25), who revealed *A. afra* to possess tannins, phenols, and flavonoids. The highest saponin content was seen in the *T. violacea* root extract at 1426.67 ± 34.03 mg DE/g. The high saponin content is supported by other studies of *T. violacea* where phytochemical screening analysis has shown that the species contains a wide variety of pharmacologically active groups, including saponins (30).

Phytochemicals such as phenols, flavonoids, tannins, and saponins play crucial roles in exhibiting therapeutic properties, particularly anti-inflammatory activities (14,30,31). The results of the current study revealed a consistent trend in saponin content: roots of medicinal plants contained higher saponin levels compared to stems, and stems contained higher levels compared to leaves. This observation was consistent across all four plants tested (*A. afra*, *L. javanica*, *T. riparia*, and *T. violacea*).

Table 3. Nitrite inhibition of the extracts and quercetin as the positive control at 12.5 µg/mL, evaluated for their lipopolysaccharides (LPS)-induced nitrite reduction in Raw 264.7 macrophage cells

Plant extract/Sample	LPS-induced nitrite inhibition (%)
	Mean ±SD
<i>Artemisia afra</i> stems	73.19±1.97 ^B
<i>Artemisia afra</i> roots	65.93±1.64 ^B
<i>Lippia javanica</i> roots	81.89±1.76 ^A
<i>Lippia javanica</i> leaves	80.95±1.91 ^A
<i>Tetradenia riparia</i> leaves	86.12±0.55 ^A
<i>Tetradenia riparia</i> stems	3.47±0.10 ^D
<i>Tulbaghia violacea</i> roots	61.19±3.28 ^B
<i>Tulbaghia violacea</i> stems	44.16±0.94 ^C
Quercetin	68.14±1.09 ^B

LPS-induced nitrite inhibition (%) was grouped into clusters (A-D) based on K-means analysis. The clustering assignment was determined according to natural breaks and spacing observed per individual column. The plant extracts/samples that differed significantly $P < 0.05$ in terms of the LPS-induced nitrite inhibition (%) in that column were designated different denotations.

Characterization techniques using mass spectrometry analyses of different plant parts have shown that phytochemicals vary in composition depending on the plant part (42). It is therefore expected that different plant parts will contain varying amounts of phytochemicals, resulting in differences in bioactivity.

Extracts of *A. afra* roots and *T. riparia* leaves demonstrated significantly higher cell viability than the controls at doses from 12.5 to 50 µg/mL. A comparable proliferative response was observed with *L. javanica* root extracts, though limited to 12.5 µg/mL. These findings align with previous reports indicating that medicinal plant extracts frequently enhance cell viability relative to untreated controls, suggesting protective or proliferative effects at low concentrations rather than cytotoxicity (43). Similarly, Khumalo et al (44) reported that ethanol and aqueous extracts tested in RAW 264.7 macrophages at 1.25 mg/mL to 2.5 mg/mL exhibited no toxicity in the MTS assay, with several extracts promoting proliferation, such as aqueous extracts of *H. procumbens*, which produced the highest viability, reaching 164% (45). It also demonstrated that 70% ethanol extracts of certain bryophytes increased cell viability more than that of the control in MTT assays. Comparable effects were noted with *T. violacea* stem extracts, though only at 12.5 µg/mL. Supporting evidence from Madike et al (46) indicates that crude aqueous extracts of *Tulbaghia violacea* leaves and roots are non-toxic at low concentrations to macrophages, where they promote proliferation, whereas higher concentrations tend to be cytotoxic. Fruit-derived phytochemicals reinforce this trend; a study showed that poncirin exhibited no significant toxicity up to 100 µg/mL, whereas naringin significantly enhanced cell viability at 20 and 40 µg/mL, higher than that of the control, as demonstrated by Lamichhane et al (47). Likewise, M.

domestica leaf extracts consistently increased cell viability across concentrations of 25–250 µg/mL, with water extracts supporting greater fibroblast viability compared to ethanol extracts, as reported by Zagórska-Dziok et al (48). Collectively, these findings highlight that medicinal plant extracts, when applied at non-toxic concentrations, can exert protective or proliferative effects on immune cells, underscoring their therapeutic potential.

Macrophages play a significant role in the body's immune response and in pathways that drive inflammation, such as the release of nitric oxide species (49). Nitric oxide species function as pro-inflammatory mediators that participate in diverse physiological processes, where their synthesis is crucial for host defense; nevertheless, excessive production results in tissue injury and triggers additional pro-inflammatory pathways linked to both acute and chronic inflammation (50). By setting the threshold at 80% cell viability, the study ensured that macrophages remained metabolically active and healthy, thereby confirming that any observed reduction in inflammation (e.g., nitric oxide species reduction) reflected a genuine pharmacological effect rather than cell death. Establishing 80% viability as the MNTC is a standard methodological practice to validate that anti-inflammatory outcomes are not confounded by cytotoxicity (51–53). Extracts were therefore tested at very low, non-toxic concentrations to reliably evaluate their anti-inflammatory potential in the cellular model (47).

The nitrite release inhibition bioassay was done to investigate the extracts ability to act against inflammatory activity, and *A. afra* showed strong nitrite inhibition with the stem extract at 73.19% compared to the root extract at 65.93%. The decrease in nitrite production induced by the plant extracts would be beneficial in disease conditions, as it could provide symptomatic relief from fever caused by infection and reduce inflammation in different tissues. Compounds with the ability to control nitric oxide species through properties such as nitrite inhibition have potential anti-inflammatory effects, as overproduction can lead to pathogenesis of many disorders (54). The *T. riparia* leaf extract showed higher nitrite inhibition activity at 86.12%. These values were superior to those observed for the positive control quercetin at 68.14%. This indicates that extracts of medicinal plants are indeed effective anti-inflammatory agents.

Further analysis of other mechanisms for the extracts is still needed, as some studies have shown that the positive control quercetin directly influences nitric oxide species inhibition and anti-inflammation via multiple pathways. The inhibitory effects of quercetin are linked to the downregulation of signaling molecules, including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), p38, Akt, Src, Janus kinase-1 (JAK1), Tyk2, signal transducer and activator of transcription-1 (STAT1), and nuclear factor kappa B (NF-

κB). Quercetin also exhibits antioxidant properties by scavenging free radicals and inhibiting serine or threonine and tyrosine phosphatase functions. Furthermore, quercetin has been shown to inhibit activation of the NLRP3 inflammasome, providing additional mechanistic insight into its anti-inflammatory potential (40,55).

Correlation analysis of phytochemical content and nitrite inhibition strength of the plants was carried out using Spearman's rank correlation. A moderate positive correlation ($\rho = 0.55$) was seen between total phenolic content (TPC) and nitrite inhibition, indicating that samples with higher phenolic content generally exhibited stronger nitrite inhibition activity. While a weak positive relationship was found between total tannin content (TTC) and nitrite inhibition ($\rho = 0.21$), suggesting only a slight association between higher tannin levels and increased activity. For total flavonoid content (TFC), a moderate negative correlation ($\rho = -0.5238$) was observed, while TSC also had a correlation of $\rho = -0.3810$. These results imply that extracts with high flavonoid and saponin content may not necessarily correspond to high nitrite inhibition. Overall, the findings highlight phenolics as potential key contributors to nitrite inhibition, while tannins, flavonoids, and saponins appear to play more complex or context-dependent roles.

Further work involving the isolation and characterization of specific compounds, particularly those from phytochemical groups with stronger correlations, will be a key factor in finding bioactive molecules responsible for nitrite-inhibiting properties. Plant-derived compounds like quercetin, a compound broadly distributed in plants such as seeds, flowers, stems, and leaves, have been widely investigated for their inhibitory effects in pathological processes. Studies of *in vitro* and *in vivo* assessments demonstrate quercetin as a compound that contributes to disease management by modulating inflammatory signaling pathways. Notably, it suppresses nitric oxide species production in LPS-stimulated cells (56). Such plant-derived compounds should be isolated using models that demonstrate favourable nitrite inhibition.

Conclusion

The selected medicinal plants *A. afra*, *L. javanica*, *T. riparia* and *T. violacea* have been shown to contain bioactive compounds with the ability to control excessive nitric oxide species, which is linked to chronic inflammation as one of the major complications of viral respiratory infections through nitrite inhibition. The exhibited nitrite inhibition activity could be due to the possession of the determined phytochemicals, whose correlative association was shown in this study. The demonstrated positive correlation with the nitrite inhibition activity appeared stronger with the phenols. This study has therefore, demonstrated a positive correlative association between phytochemical content and nitrite inhibition in selected medicinal plants used traditionally for treatment of respiratory infections. This

study focused on evaluating nitrite inhibition in RAW 264.7 macrophage cells as a potential anti-inflammatory mechanism of extracts from selected medicinal plants. It is, therefore, recommended for future studies to investigate the association of phytochemical content with other inflammatory mechanisms such as iNOS protein expression, mRNA levels, or the downstream NF-κB pathway.

Availability of data and materials

All data generated or analysed during this study have been included in this published article.

Authors' contribution

Conceptualization: Sithembiso Lethukuthula Ndwandwe.

Data curation: Sithembiso Lethukuthula Ndwandwe.

Formal analysis: Sithembiso Lethukuthula Ndwandwe.

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Project administration: Vuyisile Samuel Thibane.

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Supervision: Sechene Stanley Gololo, Vuyisile Samuel Thibane.

Validation: Sechene Stanley Gololo.

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Conflict of interests

The authors declare that they have no conflicting interests

Declaration of AI-assisted tools in the writing procedure

The Spearman's correlation analysis was done by Microsoft Copilot (2024). Upon generating the results, the author reviewed and analyzed the results and takes ultimate responsibility for the content of this publication.

Ethical considerations

This study was approved by the Sefako Makgatho University Research Ethics Committee (SMUREC), under protocol number SMUREC/S/498/2023:PG.

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References

1. Obute GC. Ethnomedicinal plant resources of South eastern Nigeria. *Ethnobot Leaflet*. 2005;1:5.
2. Okeke IN, Laxminarayan R, Bhutta ZA, Duse AG, Jenkins P, O'Brien TF, Pablos-Mendez A, Klugman KP. Antimicrobial resistance in developing countries. Part I: recent trends and current status. *Lancet Infect Dis*. 2005;5(8):481–93. doi:10.1016/S1473-3099(05)70189-4
3. Furman D, Campisi J, Verdin E, Carrera-Bastos P, Targ S, Franceschi C, et al. Chronic inflammation in the etiology of

- disease across the life span. *Nat Med*. 2019;25(12):1822–32. doi:10.1038/s41591-019-0675-0
4. Altemimi A, Lakhssassi N, Baharlouei A, Watson D, Lightfoot D. Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. *Plants*. 2017;6(4):42. doi:10.3390/plants6040042
 5. Oguntibeju O. Medicinal plants with anti-inflammatory activities from selected countries and regions of Africa. *J Inflamm Res*. 2018;11:307–17. doi:10.2147/JIR.S167789
 6. Dharmadeva S, Galgamuwa LS, Prasadine C, Kumarasinghe N. In vitro anti-inflammatory activity of *Ficus racemosa* L. bark using albumin denaturation method. *AYU*. 2018;39:239–42. doi:10.4103/ayu.AYU_27_18
 7. Gessner DK, Ringseis R, Eder K. Potential of plant polyphenols to combat oxidative stress and inflammatory processes in farm animals. *J Anim Physiol Anim Nutr (Berl)*. 2017;101(4):605–28. doi:10.1111/jpn.12579
 8. Sarveswaran R, Jayasuriya WJAB, Suresh TS. In-vitro assays to investigate the anti-inflammatory activity of herbal extracts: A review. *World J Pharm Res*. 2017;6:1–12. doi:10.20959/wjpr201717-10058
 9. Anyasor GN, Okanlawon AA, Ogunbiyi B. Evaluation of anti-inflammatory activity of *Justicia secunda* Vahl leaf extract using in vitro and in vivo inflammation models. *Clin Phytosci*. 2019;5(1):49. doi:10.1186/s40816-019-0137-8
 10. Adesso S, Autore G, Popolo A, Marzocco S, Quaroni A, Severino L. The food contaminants nivalenol and deoxynivalenol induce inflammation in intestinal epithelial cells by regulating reactive oxygen species release. *Nutrients*. 2017;9:1343. doi:10.3390/nu9121343
 11. Ghuman S, Ncube B, Finnie JE, McGaw LJ, Njoya EM, Coopooosamy RM, et al. Antioxidant, anti-inflammatory and wound healing properties of medicinal plant extracts used to treat wounds and dermatological disorders. *S Afr J Bot*. 2019;126:232–40. doi:10.1016/j.sajb.2019.07.013
 12. Amirghofran Z, Malek-Hosseini S, Golmoghaddam H, Kalantar F, Shabani M. Inhibition of nitric oxide production and proinflammatory cytokines by several medicinal plants. *Iran J Immunol*. 2011;8(3):159–69.
 13. Pepe G, Sommella E, Manfra M, De Nisco M, Tenore GC, Scopa A, et al. Evaluation of anti-inflammatory activity and fast UHPLC–DAD–IT–TOF profiling of polyphenolic compounds extracted from green lettuce (*Lactuca sativa* L.; var. Maravilla de Verano). *Food Chem*. 2015;167:153–61. doi:10.1016/j.foodchem.2014.06.105
 14. Afolayan AJ, Grierson DS, Mbeng WO. Medicinal plants for skin disorders in Eastern Cape. *J Ethnopharmacol*. 2014;153(1):220–32. doi:10.1016/j.jep.2014.02.023
 15. Benarba B, Pandiella A. Medicinal Plants as Sources of Active Molecules Against COVID-19. *Front Pharmacol*. 2020;11. doi:10.3389/fphar.2020.01189
 16. Grigore A, Cord D, Tanase C, Albulescu R. Herbal medicine, a reliable support in COVID therapy. *J Immunoassay Immunochem*. 2020;41(6):976–99. doi:10.1080/15321819.2020.1862867
 17. Agrawal PK, Agrawal C, Blunden G. Quercetin: antiviral significance and possible COVID-19 integrative considerations. *Nat Prod Commun*. 2020;15(12). doi:10.1177/1934578X20976293
 18. van Wyk BE. A broad review of commercially important southern African medicinal plants. *J Ethnopharmacol*. 2008;119(3):342–55. doi:10.1016/j.jep.2008.05.029
 19. Shikanga EA, Combrinck S, Regnier T. South African *Lippia* herbal infusions: Total phenolic content, antioxidant and antibacterial activities. *S Afr J Bot*. 2010;76(3):567–71. doi:10.1016/J.SAJB.2010.04.010
 20. Ncube B, Ngunge V, Finnie J, Van Staden J. A comparative study of the antimicrobial and phytochemical properties between outdoor grown and micropropagated *Tulbaghia violacea* Harv. plants. *J Ethnopharmacol*. 2011;134(3):775–80. doi:10.1016/j.jep.2011.01.039
 21. Okem A, Finnie JE, Van Staden J. Pharmacological, genotoxic and phytochemical properties of selected South African medicinal plants used in treating stomach-related ailments. *J Ethnopharmacol*. 2012;139:712–20. doi:10.1016/j.jep.2011.11.034
 22. Ncube B, Finnie JE, Van Staden J. Quality from the field: The impact of environmental factors as quality determinants in medicinal plants. *S Afr J Bot*. 2012;82:11–20. doi:10.1016/j.sajb.2012.05.009
 23. Maroyi A. *Lippia javanica* (Burm.f.) Spreng.: Traditional and Commercial Uses and Phytochemical and Pharmacological Significance in the African and Indian Subcontinent. *Evid Based Complement Altern Med*. 2017;2017(1). doi:10.1155/2017/6746071
 24. du Toit A, van der Kooy F. *Artemisia afra*, a controversial herbal remedy or a treasure trove of new drugs? *J Ethnopharmacol*. 2019;244:112127. doi:10.1016/j.jep.2019.112127
 25. Kane N, Kyama M, Nganga J, Hassanali A, Diallo M, Kimani F. Comparison of phytochemical profiles and antimalarial activities of *Artemisia afra* plant collected from five countries in Africa. *S Afr J Bot*. 2019;125:126–33. doi:10.1016/j.sajb.2019.07.001
 26. Salehi B, Kumar N, Şener B, Sharifi-Rad M, Kılıç M, Mahady G, et al. Medicinal Plants Used in the Treatment of Human Immunodeficiency Virus. *Int J Mol Sci*. 2018;19(5):1459. doi:10.3390/ijms19051459
 27. Chepngetich J, Ngule C, Jepkorir M, Mwangangi R, Njuguna D, Ndung'u J, et al. Total Phenolic Content and in vitro Antiproliferative Activity of *Tragia brevipes* (Pax) and *Tetradenia riparia* (Hochst) Leaves Extract. *European J Med Plants*. 2018;22(4):1–10. doi:10.9734/EJMP/2018/40058
 28. Osunsanmi FO, Zharare GE, Opoku AR. Antioxidant potential of *Lippia javanica*. *Pharmacogn J*. 2019;11(4):803–7. doi:10.5530/pj.2019.11.128
 29. Wafula MC, Mutisya MD, Malombe I. Non-volatile Chemical Composition and Botanical Extracts from *Lippia javanica* (Burm. F) Spreng. In Control of Cowpea Aphids. *J Appl Sci*. 2019;19(4):325–30. doi:10.3923/jas.2019.325.330
 30. Zakaryan H, Arabyan E, Oo A, Zandi KK. Flavonoids: Promising natural compounds against viral infections. *Arch Virol*. 2017;162(9):2539–51. doi:10.1007/s00705-017-3417-y
 31. Bhebhe M, Fuller TN, Chipurura B, Muchuweti M. Effect of Solvent Type on Total Phenolic Content and Free Radical Scavenging Activity of Black Tea and Herbal Infusions. *Food Anal Methods*. 2016;9(4):1060–7. doi:10.1007/s12161-015-0270-z
 32. Mutuku N, Ndiku M, Francis R, Ngule E. Preliminary phytochemical and antibacterial screening of fresh *Tetradenia riparia* leaves. *Int J Bioassays*. 2016;3:3413–8. doi:10.2174/2210315509666181214154345
 33. Rali S, Mshengu B, Van De Venter M, Maharaj VJ. In vitro nitric oxide inhibition of selected south African medicinal plants: A bio-guided purification of anti-inflammatory

- compounds from *Conyza scabrida*. Fitoterapia. 2025;184:106651. doi:10.1016/j.fitote.2025.106651
34. Adebayo SA, Dzoyem JP, Shai LJ, Eloff JN. The anti-inflammatory and antioxidant activity of 25 plant species used traditionally to treat pain in southern African. BMC Complement Altern Med. 2015;15(1):159. doi:10.1186/s12906-015-0669-5
 35. Vala M, Maitreya B. Phytochemical analysis and total tannin content (TTC) of *Delonix regia* (Bojer ex. Hook) Raf. bark by using different solvents collected from Saurashtra region. Int Assoc Biol Comput Dig. 2022;1(2):144–8. doi:10.56588/iabcd.v1i2.59
 36. Sembiring EN, Elya B, Sauriasari R. Phytochemical screening, total flavonoid and total phenolic content and antioxidant activity of different parts of *Caesalpinia bonduc* (L.) Roxb. Pharmacogn J. 2018;10(1):123–7. doi:10.5530/pj.2018.1.22
 37. Mncube SC, Gololo SS, Mogale MA. Seasonal variations of phytochemical content and antioxidant activity of *Senna italica* leaves. Asian J Chem. 2020;32(9):2371–4. doi:10.14233/ajchem.2020.22739
 38. Njeru SN, Muema JM. In vitro cytotoxicity of *Aspilia pluriseta* Schweinf. extract fractions. BMC Res Notes. 2021;14:57. doi:10.1186/s13104-021-05472-4
 39. More GK, Makola RT. In-vitro analysis of free radical scavenging activities and suppression of LPS-induced ROS production in macrophage cells by *Solanum sisymbriifolium* extracts. Sci Rep. 2020;10(1). doi:10.1038/s41598-020-63491-w
 40. Kao TK, Ou YC, Raung SL, Lai CY, Liao SL, Chen CJ. Inhibition of nitric oxide production by quercetin in endotoxin/cytokine-stimulated microglia. Life Sci. 2010;86(9–10):315–21. doi:10.1016/j.lfs.2009.12.014
 41. Iwalewa EO, McGaw LJ, Naidoo V, Eloff JN. Inflammation: The foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. Afr J Biotechnol. 2007;6(25):2868–85. doi:10.5897/AJB2007.000-2457
 42. Pattoo M, Belewa V, Somai B. Phytochemical constituents of *Tulbaghia violacea* Harv extract and its antifungal potential against *Cryptococcus neoformans* and *Cryptococcus gattii*. Nat Prod J. 2019;9:330–40. doi:10.2174/2210315509666181214154345
 43. Checkouri E, Ramin-Mangata S, Diotel N, Viranaicken W, Marodon C, Reignier F, et al. Protective Effects of Medicinal Plant Decoctions on Macrophages in the Context of Atherosclerosis. Nutrients. 2021;13(1):280. doi:10.3390/nu13010280
 44. Khumalo GP, Loa-Kum-Cheung W, Van Wyk BE, Feng Y, Cock IE. Leaf extracts of eight selected southern African medicinal plants modulate pro-inflammatory cytokine secretion in LPS-stimulated RAW 264.7 macrophages. Inflammopharmacology. 2024;32(2):1607–20. doi:10.1007/s10787-023-01420-9
 45. Marques RV, Sestito SE, Bourgaud F, Miguel S, Cailotto F, Reboul P, et al. Anti-Inflammatory Activity of *Bryophytes* Extracts in LPS-Stimulated RAW264.7 Murine Macrophages. Molecules. 2022;27(6):1940. doi:10.3390/molecules27061940
 46. Madike LN, Takaidza S, Ssemakalu CC, Pillay M. The effect of extracts of *Tulbaghia violacea* on the proliferation of a murine macrophage cell line. S Afr J Bot. 2020;130:185–97. doi:10.1016/j.sajb.2019.12.025
 47. Lamichhane G, Pandeya PR, Lamichhane R, Yun HD, Shrivastava AK, Cheon JY, et al. Evaluation of anti-inflammatory potential of extract, fractions and major compounds of *Ponciri Fructus* in LPS-induced RAW 264.7 cells. Curr Res Biotechnol. 2023;6:100138. doi:10.1016/j.crbiot.2023.100138
 48. Zagórska-Dziok M, Ziemlewska A, Wójciak M, Sowa I, Wąsik-Szczepanek E, Nizioł-Lukaszewska Z. Comparison of Cytotoxicity and Antioxidant, Antibacterial, and Anti-Inflammatory Activity of Aqueous and Ethanolic Extracts from *Malus domestica*, *Prunus armeniaca*, and *Prunus cerasus* Leaves. Molecules. 2025;30(10). doi:10.3390/molecules30102085
 49. Basheer AS, Abas F, Othman I, Naidu R. Role of Inflammatory Mediators, Macrophages, and Neutrophils in Glioma Maintenance and Progression: Mechanistic Understanding and Potential Therapeutic Applications. Cancers (Basel). 2021;13(16):4226. doi:10.3390/cancers13164226
 50. Hämäläinen M, Nieminen R, Vuorela P, Heinonen M, Moilanen E. Anti-Inflammatory Effects of Flavonoids: Genistein, Kaempferol, Quercetin, and Daidzein Inhibit STAT-1 and NF-κB Activations, Whereas Flavone, Isorhamnetin, Naringenin, and Pelargonidin Inhibit only NF-κB Activation along with Their Inhibitory Effect on iNOS Expression and NO Production in Activated Macrophages. Mediat Inflamm. 2007;2007:1–10. doi:10.1155/2007/45673
 51. Aree T, Chaichit S, Junlatat J, Kiattisin K, Intharuksa A. Bridging Phytochemistry and Cosmetic Science: Molecular Insights into the Cosmeceutical Promise of *Crotalaria juncea* L. Int J Mol Sci. 2025;26(16):7716. doi:10.3390/ijms26167716
 52. Jochum K, Miccoli A, Sommersdorf C, Poetz O, Braeuning A, Tralau T, Marx-Stoelting P. Comparative case study on NAMS: towards enhancing specific target organ toxicity analysis. Arch Toxicol. 2024;98(11):3641–58. doi:10.1007/s00204-024-03839-7
 53. Soonthornsit N, Pitaksutheepong C, Hemstapat W, Utaisincharoen P, Pitaksutheepong T. In Vitro Anti-Inflammatory Activity of *Morus alba* L. Stem Extract in LPS-Stimulated RAW 264.7 Cells. Evid Based Complement Altern Med. 2017;2017: 3928956. doi:10.1155/2017/3928956
 54. Karpuzoglu E, Ahmed SA. Estrogen regulation of nitric oxide and inducible nitric oxide synthase (iNOS) in immune cells: Implications for immunity, autoimmune diseases, and apoptosis. Nitric Oxide. 2006;15(3):177–86. doi:10.1016/j.niox.2006.03.009
 55. Yang S, Zhang J, Chen D, Ding J, Zhang Y, Song L. Quercetin Supplement to Aspirin Attenuates Lipopolysaccharide-Induced Pre-eclampsia-Like Impairments in Rats Through the NLRP3 Inflammasome. Drugs R D. 2022;22(4):271–9. doi:10.1007/s40268-022-00402-6
 56. Alharbi HOA, Alshebremi M, Babiker AY, Rahmani AH. The Role of Quercetin, a Flavonoid in the Management of Pathogenesis Through Regulation of Oxidative Stress, Inflammation, and Biological Activities. Biomolecules. 2025;15(1):151. doi:10.3390/biom15010151