

Phytochemical Study and Evaluation of the Anti-Sickling and Anti-Inflammatory Activities of Total Extracts From *Tetracera poggei* Leaves

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ABSTRACT

Introduction

Tetracera poggei is traditionally used in Congolese medicine to manage inflammatory disorders, but scientific evidence regarding its pharmacological properties remains scarce.

Purpose

This study evaluated the microscopic features, phytochemical composition, and in vitro anti-sickling and anti-inflammatory activities of aqueous and organic leaf extracts of *T. poggei*.

Methods

Microscopic analysis of powdered leaves was conducted using Steinmetz reagent to identify diagnostic histological structures. Qualitative phytochemical screening and thin-layer chromatography (TLC) were used to characterize secondary metabolites. Total polyphenols, flavonoids, and anthocyanins were quantified spectrophotometrically. Anti-sickling activity was assessed using the Emmel test on blood samples collected from 10 homozygous sickle cell (SS) patients. Anti-inflammatory activity was evaluated via the thermal denaturation inhibition of ovalbumin. Data were analyzed in triplicate and expressed as mean $\pm$ standard deviation (SD). Group comparisons were performed using Student's t-test and one-way analysis of variance (ANOVA), with statistical significance set at $p < .05$.

Results

Microscopic examination revealed sclerenchyma fibers, calcium oxalate crystals in collenchyma, smooth trichomes, amyloiferous parenchyma, and starch-rich mesophyll cells. Phytochemical screening confirmed the presence of polyphenols, tannins, anthocyanins, alkaloids, saponins, flavonoids, quinones, and terpenes. Quantitative analysis yielded 28.13 ± 1.18 mg GAE/g of polyphenols, 0.070 ± 0.001 mg QE/g of flavonoids, and 0.47 ± 0.14 mg QE/g of anthocyanins. In the Emmel test, extracts restored the normal biconcave morphology of erythrocytes under hypoxia. At 25 mg/mL, the organic extract inhibited protein denaturation by $91.60\% \pm 0.02\%$, compared to $80.20\% \pm 0.02\%$ for the aqueous extract and $99.7\% \pm 0.1\%$ for diclofenac sodium. The difference between the aqueous and organic extracts was statistically significant ($p < .05$).

Conclusion

Tetracera poggei leaf extracts exhibit notable phytochemical diversity, in vitro anti-sickling activity, and concentration-dependent anti-inflammatory potential. These findings support its traditional use and highlight the need for further in vivo and toxicity evaluations.

INTRODUCTION

Throughout history, humans have relied on nature to meet essential needs, including medical care. The therapeutic use of plants is ancient and has evolved alongside humanity. Although much of the 20th century focused on synthetic molecules, the screening of natural sources has led to the discovery of numerous pharmacological agents that play an important role in disease treatment (Mpiana et al., 2008). The World Health Organization recognizes traditional and complementary medicine as an essential component of global healthcare (Ngbolua et al., 2011).

Recent studies have reinforced this perspective, highlighting the anti-sickling potential of African medicinal plants such as *Pergularia daemia*, *Canna indica*, and *Petiveria alliacea* (Iyekowa et al., 2023), and systematically validating plant-derived compounds for managing sickle cell disease (Amponsah et al., 2024). Similarly, polyphenols and flavonoids from medicinal plants continue to be recognized for their anti-inflammatory and antioxidant properties (Riaz et al., 2023; Wen, Zhang, & Li, 2019). In the Congolese context, several species have demonstrated antioxidant and hypoglycemic activities, supporting their ethnopharmacological importance (Masengo, Djolu, & Ngbolua, 2023).

Tetracera poggei, belonging to the Dilleniaceae family, is widely used in Congolese traditional medicine to treat various conditions (Muanyishay et al., 2018). Despite this ethnopharmacological relevance, scientific evidence regarding its pharmacological properties remains limited. While related species have been reported to manage conditions such as malaria, dysentery, gonorrhea, fever, and diuresis, no prior studies have evaluated both the anti-sickling and anti-inflammatory activities of *T. poggei*. This gap justifies the present investigation.

Inflammation is a natural defense mechanism against infection or injury, characterized by redness, swelling, heat, and pain. It involves enzymes such as lipoxygenases and cyclooxygenases (COX-1 and COX-2), which synthesize pro-inflammatory mediators from arachidonic acid (Delarue, 2001). Conventional treatments with glucocorticoids and nonsteroidal anti-inflammatory drugs (NSAIDs) are effective but associated with adverse effects,

including gastrointestinal lesions and renal toxicity (Gaziano & Gibson, 2006; Surdej et al., 2005; Yougbaré-Ziérou et al., 2016). These limitations highlight the need for safer, plant-derived alternatives (Wen, Zhang, & Li, 2019).

Given its traditional use and phytochemical richness, *T. poggei* represents a promising candidate for pharmacological evaluation. The aim of this study was to conduct a histomorphological and phytochemical analysis and to assess the in vitro anti-sickling and anti-inflammatory activities of aqueous and organic leaf extracts of *T. poggei*.

METHODS

Plant Material Collection and Preparation

Leaves of *Tetracera poggei* were selected based on ethnobotanical reports from the population of Lukala in the Kongo Central province, Democratic Republic of the Congo (DRC). Fresh leaves were harvested in November 2023 in the commune of Mont-Ngafula (Kimwenza), Kinshasa, DRC (4°27'25.2"S, 15°17'37.7"E). The species was authenticated and deposited under voucher specimen number R. Devred 2745 at the Herbarium of the National Institute for Agronomic Studies and Research (INERA), University of Kinshasa (UNIKIN). The samples were shade-dried at ambient temperature (27 ± 2°C) in the Natural Substances and Macromolecules Laboratory for 2 weeks and ground into a fine powder.

Collection of Sickle Cell Blood Samples

Blood samples were collected from 10 homozygous sickle cell (SS) patients (n = 10) diagnosed at the Centre de Médecine Mixte et d'Anémies SS (CMMASS) in Kinshasa, DRC. Inclusion criteria were confirmed SS genotype status and no history of blood transfusion within the preceding 3 months. Sickle cell status was verified using a baseline Emmel test prior to experimental assays. Ethically, full confidentiality and anonymity were maintained throughout the sampling process.

Microscopic Analysis of Powdered Material

Microscopic characterization of *T. poggei* leaf powder was carried out according to the protocol described by Mutwale et al. (2018) with slight modifications. Powdered samples were mounted on microscope slides using Steinmetz reagent. Diagnostic histological features—

including fibers, collenchyma, calcium oxalate crystals, trichomes, parenchyma, and starch grains—were identified under an Optika optical microscope equipped with a digital imaging system. All observations were performed in triplicate.

Preparation of Extracts and Preliminary Phytochemical Screening

Powdered leaves were extracted using solvents of varying polarity (water and organic solvents) at a ratio of 1:10 (w/v) under continuous agitation. The resulting mixtures were filtered, and the liquid extracts were stored at 4°C prior to analysis.

Qualitative phytochemical screening was performed using standard colorimetric and precipitation methods (Mpiana et al., 2008). Tannins were tested using ferric chloride solution (FeCl₃). Alkaloids were detected using Mayer's reagent. Saponins were quantified using the foam index method. The presence of polyphenols, flavonoids, anthocyanins, quinones, and terpenes was evaluated using established pharmacognostic protocols. All tests were executed in triplicate.

Thin-Layer Chromatography (TLC) Profiling

Thin-layer chromatography (TLC) was conducted on silica gel 60 F₂₅₄ plates to profile secondary metabolites in methanolic and ethyl acetate extracts. Mobile phases included:

- a) **PM1:** Ethyl acetate/formic acid/glacial acetic acid/water
- b) **PM2:** Ethyl acetate/formic acid/methanol/water
- c) **PM3:** Dichloromethane / formic acid / acetone
- d) **Toluene/ether (1:1 v/v) and Toluene/ethyl acetate (9:1 v/v)**

Metabolites were visualized under ultraviolet light (254 nm and 366 nm) and sprayed with specific reagents: Neu's reagent for flavonoids and phenolic acids; 10% sulfuric acid for iridoids; phosphovanillin for anthocyanins; ethanolic KOH for coumarins and anthraquinones; and vanillin-sulfuric acid for terpenes. D-catechin, thymol, menthol, and oleanolic acid served as reference standards.

Quantitative Phytochemical Analysis

Quantification of major bioactive groups was performed using spectrophotometric methods in triplicate:

1. **Total Polyphenol Content (TPC):** Evaluated via the Folin-Ciocalteu method. Absorbance was measured at 765 nm, and results were expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g).
2. **Total Flavonoid Content (TFC):** Determined using the aluminum chloride (AlCl₃) colorimetric assay. Absorbance was recorded at 415 nm, and results were expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g).
3. **Total Anthocyanin Content (TAC):** Measured spectrophotometrically following standard analytical routines and expressed as mg QE/g dry extract.

Evaluation of In Vitro Anti-Sickling Activity (Emmel Test)

Anti-sickling activity was determined using the Emmel test as described by Mpiana et al. (2008) and Tshilanda et al. (2015). Erythrocyte sickling was induced under hypoxic conditions using a 2% sodium metabisulfite solution. SS blood was mixed with extract dilutions prepared in normal saline (0.9% NaCl) at 50 µg/mL. Normal saline served as the negative control. Slides were sealed and monitored under 400x magnification at t = 0 min and t = 60 min. Reversion of falciform erythrocytes to normal biconcave morphology was quantified across five random fields per preparation (n = 10 patients, performed in triplicate).

In Vitro Anti-Inflammatory Activity (Protein Denaturation Method)

Anti-inflammatory potential was quantified via the inhibition of thermal denaturation of ovalbumin (Kumari et al., 2014). The reaction mixture contained fresh egg albumin, phosphate-buffered saline (PBS, pH 6.8), and plant extracts at varying concentrations (12, 16, and 25 mg/mL). Diclofenac sodium was used as the reference drug, and distilled water served as the control. Mixtures were incubated at 37°C for 15 minutes and then heated at 70°C for 5 minutes. Absorbance was recorded spectrophotometrically after cooling. Percentage inhibition was calculated as:

$$\text{Inhibition (\%)} = (1 - A_{\text{sample}} / A_{\text{control}}) \times 100$$

Statistical Analysis

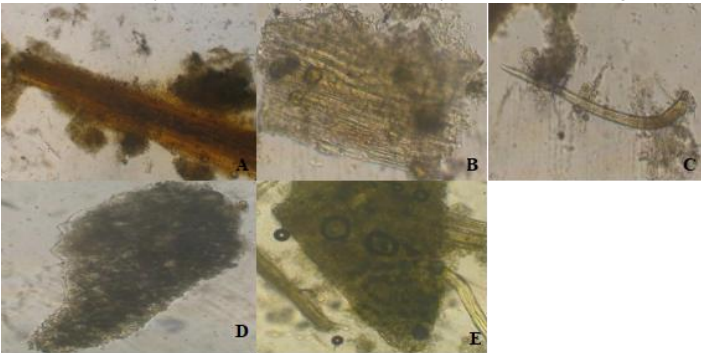
Data were compiled in Microsoft Excel 2021 and analyzed using R and SPSS. Values are expressed as mean ± standard deviation (SD). Differences between two groups were evaluated using Student’s t-test for independent samples, whereas comparisons across multiple concentrations were performed using one-way ANOVA. Statistical significance was established at $p < .05$.

RESULTS

Microscopic Characterization

Histological examination of *T. poggei* leaf powder confirmed diagnostic botanical features (Figure 1). Key diagnostic structures included lignified fibers, collenchyma containing solitary and clustered calcium oxalate crystals, smooth unicellular trichomes, amyliiferous parenchyma, and mesophyll tissues densely filled with starch granules.

Figure 1:
Micrographic Characteristics of *Tetracera poggei* Leaf Powder
(A) Sclerenchyma fibers; (B) Collenchyma with calcium oxalate crystals; (C) Smooth trichomes; (D) Amyliiferous parenchyma; (E) Mesophyll cells rich in starch grains.



Phytochemical Composition

Qualitative screening demonstrated the presence of polyphenols, gallic and catechic tannins, anthocyanins, leucoanthocyanins, quinones, alkaloids, saponins, flavonoids, and triterpenes. Quantitative estimations confirmed that total polyphenols constituted the majority of secondary metabolites in *T. poggei* leaves (Table 1).

Table 1:
Secondary Metabolite Content in *Tetracera poggei* Leaves

Parameter	Concentration (mg/g dry extract)
Total Polyphenol Content (\text{mg GAE/g})	28.13 ± 1.18
Total Flavonoid Content (\text{mg QE/g})	0.070 ± 0.001
Total Anthocyanin Content (\text{mg QE/g})	0.47 ± 0.14

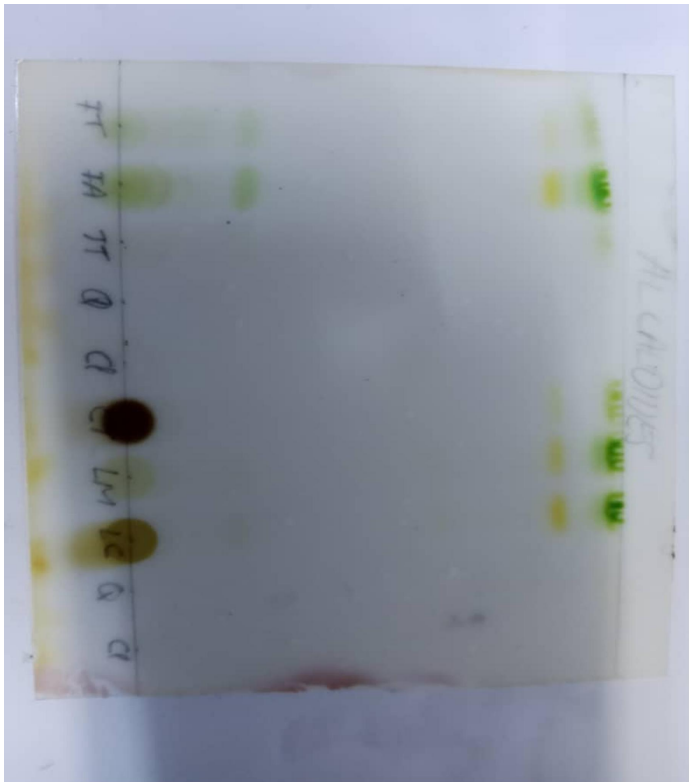
Note. Data are presented as mean ± SD (n = 3). GAE = gallic acid equivalents; QE = quercetin equivalents.

Thin-Layer Chromatographic Fingerprinting

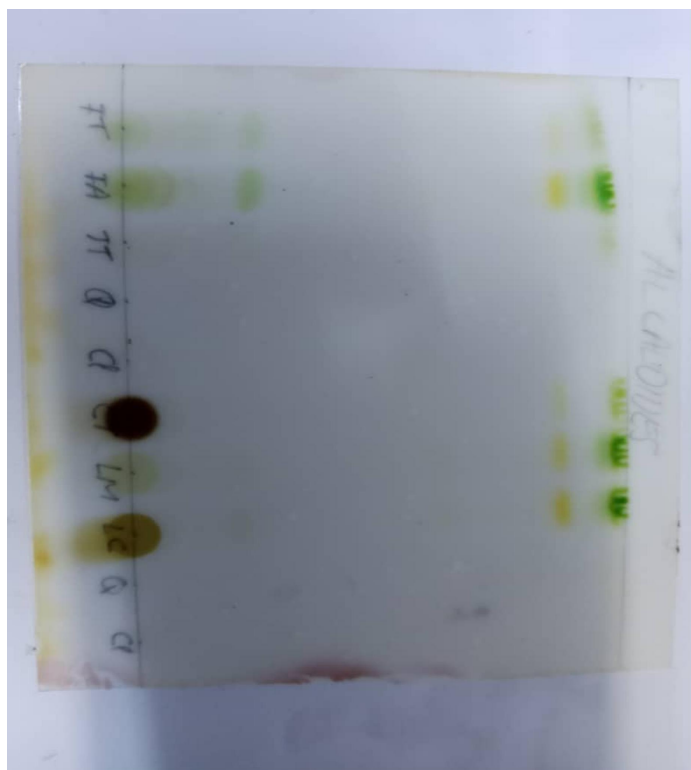
Chromatographic separation confirmed distinct chemical bands across extracts (Figures 2–8):

1. **Alkaloids:** Clear bands resolved in methanolic extracts (Figure 2).
2. **Anthocyanins and Coumarins:** Identified under UV light following derivatization with phosphovanillin and ethanolic KOH, respectively (Figures 3 and 4).
3. **Flavonoids and Phenolic Acids:** Exhibited characteristic blue and yellow-orange fluorescence under Neu's reagent (Figure 5).
4. **Iridoids, Terpenes, and Anthraquinones:** Visible spot patterns matching reference standards were confirmed (Figures 6, 7, and 8).

Figure 2:
Thin-layer chromatogram of alkaloids detected in methanolic extracts of *T. poggei* leaves



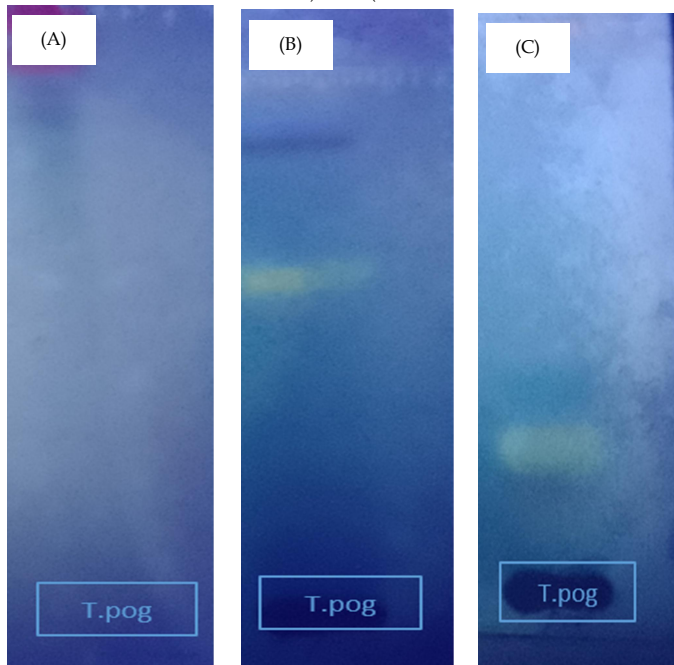
TLC chromatogram of anthocyanin derivatives from *T. poggei* leaf extracts, revealed under UV light after derivatization.



COUMARINES

FT TA TT CT LM LC

TLC chromatograms of flavonoid extracts (A, B) and phenolic acids (C). Mobile phases: PM1 (ethyl acetate-formic acid-glacial acetic acid-water), PM2 (ethyl acetate-formic acid-methanol-water), PM3 (dichloromethane-formic acid-acetone).



TLC chromatogram of methanolic extracts of *T. poggei* leaves showing iridoid derivatives (mobile phase: ethyl acetate-methanol-water).



Figure 7:
TLC chromatogram of ethyl acetate extracts of *T. poggei* leaves with terpene control (mobile phase: toluene-ethyl acetate 9:1; detection: vanillin-sulfuric acid reagent).

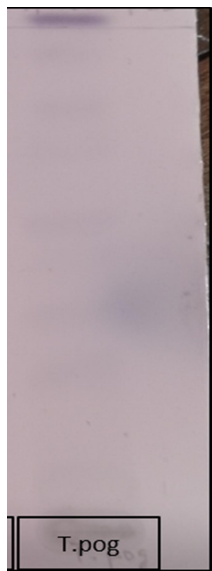
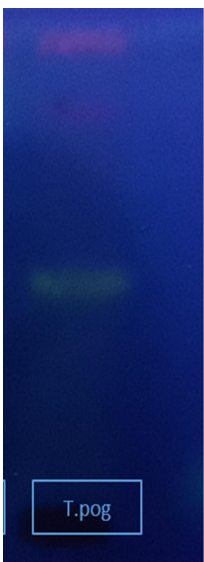


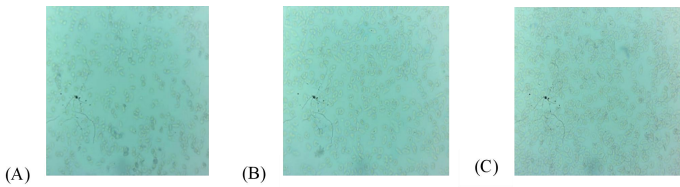
Figure 8:
TLC chromatogram of methanolic extracts of *T. poggei* leaves showing anthraquinones (mobile phase: ethyl acetate-methanol-water; detection: alcoholic KOH).



Anti-Sickling Activity

In the Emmel test, untreated SS blood under hypoxic conditions exhibited widespread falciform transformation (**Figure 9A**). Incubation with 50 µg/mL of *T. poggei* leaf extracts restored the normal biconcave, discoid morphology of sickled red blood cells (**Figures 9B and 9C**). The organic extract showed more pronounced morphological normalization than the aqueous extract.

Figure 9:
Micrographs of Sick Erythrocytes Under Hypoxic Conditions (Emmel Test)
(A) Untreated SS blood (negative control, 0.9% NaCl) showing characteristic sickle cells;
(B) SS blood treated with 50 µg/mL aqueous extract showing partial shape normalization;
(C) SS blood treated with 50 µg/mL organic extract showing marked restoration of biconcave red blood cells



Anti-Inflammatory Activity

Tetracera poggei leaf extracts inhibited heat-induced ovalbumin denaturation in a dose-dependent manner (**Table 2**). The organic extract showed higher inhibition values than the aqueous extract at all tested concentrations, with statistically significant differences observed at each concentration ($p < .05$, $p < .01$, and $p < .05$, respectively). At 25 mg/mL, the organic extract achieved $91.60\% \pm 0.02\%$ inhibition, approaching the efficacy of the standard drug diclofenac sodium ($99.7\% \pm 0.1\%$).

Table 2:
Inhibition of Heat-Induced Ovalbumin Denaturation by *Tetracera poggei* Extracts

Test Concentration (mg/mL)	Aqueous Extract (% Inhibition)	Organic Extract (% Inhibition)	Diclofenac Sodium (% Inhibition)	p-value (Organic vs. Aqueous)
12	64.40 ± 0.10	72.20 ± 0.02	88.20 ± 0.16	$< .05$
16	45.00 ± 0.20	73.00 ± 0.32	91.60 ± 0.02	$< .01$
25	80.20 ± 0.02	91.60 ± 0.02	99.70 ± 0.10	$< .05$

Note. Data represent mean $\pm$ SD (n = 3). Group differences between organic and aqueous extracts were evaluated using Student's t-test.

DISCUSSION

Histomorphological Analysis

Microscopic profiling of plant drugs is an essential step for quality control, standard setting, and botanical verification (**Mutwale et al., 2018**). The presence of calcium oxalate crystals, trichomes, fibers, and amyliiferous tissue in *T. poggei* leaf powder provides key diagnostic markers for authentication. Similar histomorphological features have been observed in other Congolese medicinal plants, such as *Tetracera rosiflora* (**Muanyishay et al., 2018**), *Picralima nitida* (**Akoubet Ouayogodé et al., 2021**), *Ficus exasperata* (**Masengo, Ngbolua, et al., 2023**), and *Uvariadendron molundense* (**Djolu et al., 2023**).

Phytochemical Profile

Phytochemical screening showed that *T. poggei* leaves contain diverse secondary metabolites, including polyphenols, tannins, flavonoids, alkaloids, saponins, and terpenes. These findings align with reports on *T. rosiflora* (Muanyishay et al., 2018) and related taxa such as *Aframomum alboviolaceum* (Djeussi et al., 2013). Quantitative analysis revealed a high polyphenol content (28.13 ± 1.18 mg GAE/g), whereas flavonoids were present in lower concentrations (0.070 ± 0.001 mg QE/g). Variations in secondary metabolite yields relative to other Congolese flora, such as *Ficus exasperata* (Masengo, Ngbolua, et al., 2023), may stem from environmental variables, soil composition, leaf maturity, harvesting periods, and processing parameters (Ngbolua et al., 2011; Ngbolua et al., 2012).

Anti-Sickling Activity

The Emmel test showed that *T. poggei* extracts reversed the sickling of SS erythrocytes induced by sodium metabisulfite. Polyphenolic constituents, particularly anthocyanins and flavonoids, have been previously identified as major drivers of anti-sickling activity in plant extracts (Mpiana et al., 2008; Tshilanda et al., 2015; Tshilanda et al., 2016). These compounds can interact with hemoglobin S (HbS) molecules, increase oxygen affinity, inhibit intracellular polymerization, and stabilize erythrocyte membranes against oxidative cross-linking (Gbolo et al., 2022; Mpiana et al., 2010; Ngbolua et al., 2015).

Anti-Inflammatory Potential

Denaturation of tissue proteins is a well-established cause of inflammatory and autoimmune disorders. NSAIDs like diclofenac sodium prevent protein denaturation, thereby mitigating inflammatory cascades (Kumari et al., 2014). In this study, *T. poggei* leaf extracts significantly inhibited heat-induced ovalbumin denaturation in a concentration-dependent manner, with the organic extract reaching 91.60% inhibition at 25 mg/mL. This activity is likely driven by polyphenols and flavonoids, which are known to interact with protein structures, scavenger reactive oxygen species, and suppress cyclooxygenase pathways (Middleton et al., 2000; Riaz et al., 2023; Wen, Zhang, & Li, 2019).

Limitations

Several limitations of this study should be noted. First, blood samples were obtained from a small donor cohort ($n = 10$). Second, the anti-sickling and anti-inflammatory activities were evaluated exclusively in vitro; in vivo pharmacodynamics and pharmacokinetic profiles remain to be established. Third, toxicological assessments and exact IC_{50} determinations were not performed. Future studies should focus on bio-guided isolation of active compounds, in vivo efficacy studies, and comprehensive toxicity profiling.

CONCLUSION

This study establishes the histomorphological baseline and phytochemical profile of *Tetracera poggei* leaves. Extracts of *T. poggei* exhibited significant in vitro anti-sickling activity by restoring the normal biconcave shape of hypoxic SS erythrocytes, alongside concentration-dependent anti-inflammatory potential through the inhibition of protein denaturation. These empirical findings provide scientific support for the traditional use of *T. poggei* in Congolese folk medicine. Further studies involving bio-guided isolation, in vivo modeling, and toxicological profiling are recommended to characterize the active pharmacological constituents.

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Use of Artificial Intelligence (AI) Declaration: The authors declare that no generative artificial intelligence was used in the conception and design of the study, data collection, data analysis, interpretation of results, or the generation or modification of the primary research data. Artificial intelligence-assisted language tools were used solely to improve the English translation, grammar, spelling, and overall linguistic clarity of the manuscript. All scientific content, interpretations, conclusions, and the final version of the manuscript were critically reviewed, verified, and approved by the authors, who take full responsibility for the content of this article.

Authors' Contributions:

- i. **Conceptualization:** C. M. Lukanda
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- vii. **Writing** – review & editing: C. M. Lukanda, A. K. Ngoyi, W. B. Balela, P. L. Lokadi, F. L. Lumba, S. L. Ndiku, N. N. Kabamba, P. M. Tshimankinda

viii. **Supervision:** P. M. Tshimankinda

Data Availability Statement: The datasets generated and analyzed during the current study are not publicly available because they contain information that could compromise participant confidentiality. However, anonymized data supporting the findings of this study are available from the corresponding author upon reasonable request, subject to approval by the relevant institutional ethical authorities and applicable institutional policies.

Ethical Approval: This study was conducted under the research protocol entitled 'Activités biologiques, phytochimie et culture in vitro de deux espèces du genre *Tetracera*,' which received ethical approval from the Comité de Bioéthique de l'Institut Supérieur des Techniques Médicales de Kinshasa (ISTM-KINSHASA), Democratic Republic of the Congo, under Ethical Opinion No. 005/CBE/STM/KIN/RDC/PMBBL/2023, dated 23 February 2023, pursuant to Decision No. 055/ESU/ISTM/DG/2022 dated 08 October 2022. The ethical approval authorized the conduct of the research from 23 February 2023 to 23 February 2024.

Anonymity & Informed Consent: Written informed consent was obtained from the participants or their legally authorized representatives for the collection and use of blood samples for research purposes."

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Conflicts of Interest: The authors declare that they have no conflicts of interest.

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