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# Comparative Pharmacological Analysis of Certain Secondary Metabolite-Containing Fractions from Georgian Flora Species of Primula

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## ABSTRACT

Background: The genus *Primula L.*, comprising approximately 577 species worldwide, includes 22 species recorded in Georgia. Notably, *Primula saguramica Gavr.* is a Georgian endemic, while *Primula woronowii Losinsk*. Phytochemical investigations of Primula species have revealed a variety of bioactive compounds, including flavonoids, saponins, and phenolic compounds, which are known to possess antioxidant, anti-inflammatory, antimicrobial, expectorant, and antirheumatic properties.

Objectives: This study aimed to conduct a preliminary pharmacological evaluation of secondary metabolite-containing fractions of different polarities obtained from three Primula species growing in Georgia: *P. macrocalyx Bunge, P. woronowii Losinsk*., and *P. saguramica Gavr*.

Methods: Based on recent TLC screening, in vitro analyses, and literature data, the analgesic and anti-inflammatory potential of crude extracts and their corresponding fractions were assessed in vivo using the "hot plate" and carrageenan-induced paw edema models, respectively. All animal studies were conducted by the 3Rs principles and were approved by the TSMU Ethics Committee on Animal Research (Approval # AP-56-22).

Results: The in vivo experiments demonstrated analgesic and anti-inflammatory efficacy of the fractions, with activity ranging from 22% to 120% and 26% to 84%, respectively, compared to the control group. Variability in pharmacological activity is presumed to be influenced by the flavonoid-to-terpenoid ratio within specific fractions. These findings support the rationale for a detailed investigation of the most active fractions to isolate and identify the individual compounds responsible for the observed effects.

Conclusions: The study confirms the analgesic and anti-inflammatory potential of secondary metabolite-rich fractions from Primula species. Activity differences appear to be influenced by the flavonoid-to-terpenoid ratio, suggesting the need for further phytochemical analysis to isolate and characterize the active constituents.

Keywords: Analgesic; anti-inflammatory; *P. Macrocalyx; P. Saguramica; P. Woronowii;* secondary metabolites.

## BACKGROUND

In Georgia, the family Primulaceae is represented by seven genera and 46 species.[1] Among the 22 Primula species recorded in the country, two—*Primula abchasica* and *Primula saguramica*—are endemic to Georgia, while ten species are endemic to the Caucasus region.[2-4] This study focused on *Primula macrocalyx Bunge*, *P. woronowii Losinsk., and P. saguramica Gavr. Previous* investigations of various Primula species have identified a range of secondary metabolites, particularly flavonoids, which demonstrate biological activities such as antiallergic, antiviral, anti-inflammatory, and vasodilatory effects.[5-7] Although numerous synthetic anti-inflammatory and analgesic agents are available, their use is often limited by side effects and complications. This has driven the search for alternative therapeutics derived from natural sources, mainly plant-based secondary metabolites. Accordingly, our study aimed to compare the analgesic and anti-inflammatory efficacy of fractions obtained from the selected Primula species.

## METHODS

Specimens of all three target plant species were collected during their active flowering phase in the floristic region of Kartli in 2020–2021. Voucher specimens (*P. woronowii* – TBPH-21166, *P. macrocalyx* – TBPH-21329, *P. saguramica* – TBPH-21749) are deposited in the herbarium of the Iovel Kutateladze Institute of Pharmacochemistry at TSMU.[8] The plant materials were air-dried, ground into powder, and extracted three times with 80% ethanol. The resulting extracts were concentrated and subjected to freeze-drying. Further chromatographic separation was performed using a Diaion HP-20 column, eluted with a water-methanol gradient ranging from 0% to 100% methanol.[9] This process yielded four fractions from each species: P.m1, P.m2, P.m3, P.m4 from P. macrocalyx; P.w1, P.w2, P.w3, P.w4 from P. woronowii; and P.s1, P.s2, P.s3, P.s4 from P. saguramica. Before in vivo testing, all fractions were dissolved in distilled water.

### Experimental animals

Outbred albino CD1 mice weighing 28±2 g (n=40) were used in the study. The animals were obtained from the animal facility of the I. Kutateladze Institute of Pharmacochemistry, Tbilisi State Medical University (TSMU), and acclimatized for one week in the Department of Preclinical Pharmacological Research under standard laboratory conditions (temperature:


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20±2°C; humidity: 55–65%; 12/12-hour light/dark cycle). Animals were provided granulated food (4 g/animal/day) and water ad libitum. All procedures complied with the EU Directive 2010/63 and the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health[10,11] and were approved by the TSMU Ethics Committee on Animal Research (Approval No. AP-56-2022).

### Assessment of analgesic activity: hot plate assay

Fractions were dissolved in distilled water and administered intraperitoneally to mice (n=6 per group) at a dose of 50 mg/kg. Control animals (n=6) received 0.4 mL of normal saline via the same route. Analgesic activity was evaluated using the hot plate assay. Mice were individually placed in a transparent cylindrical chamber with a metal surface maintained at 50±2°C. The latency to the first nociceptive response (hind paw licking or jumping) was recorded at baseline (before injection), 30 minutes, and 60 minutes post-administration. Animals with baseline latencies exceeding 15 seconds were excluded. The analgesic effect was calculated using the formula:

$$E\ (\%) = ((Tn - T_o) / T_o) \times 100,$$

where $T_o$ is the baseline latency, and Tn is the latency at 30 or 60 minutes post-injection.[12]

### Assessment of anti-inflammatory activity: carrageenan-induced paw edema assay

The same group distribution and dosing regimen as described above were applied. Acute inflammation was induced by subplantar injection of 50 µL of 1% carrageenan solution in normal saline into the right hind paw of each mouse. One hour before carrageenan injection, animals received intraperitoneal injections of either 0.5 mL of normal saline (control) or 0.5 mL of the test compound solution (50 mg/kg). Paw thickness was measured with a digital micrometer immediately before carrageenan injection (baseline) and 2 hours afterward. Anti-inflammatory efficacy was calculated as the percentage inhibition of inflammation using the formula:

$$E\ (\%) = [1 - (\Delta T_{exp} / \Delta T_{on})] \times 100,$$

where $\Delta Tc_{on}$ and $\Delta T_{exp}$ represent the mean increase in paw thickness (in conventional units) in control and experimental groups, respectively.[13]

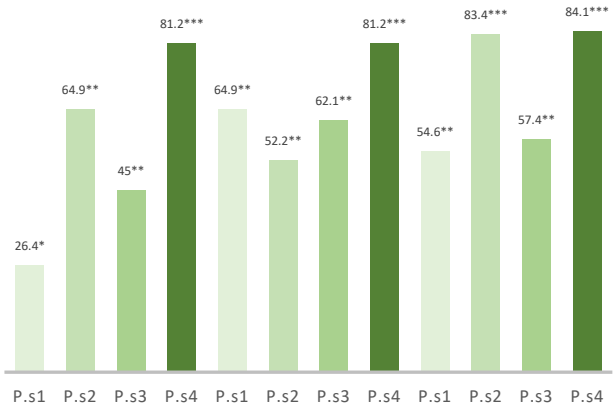
## RESULTS

In the "Hot-plate" analgesic model, the control group exhibited a baseline pain response latency of 11.5 seconds, which slightly decreased to 10.9 seconds at 30 minutes following administration of the control substance - indicating no significant change (Tab.1). In contrast, animals treated with various fractions of *P. saguramica* showed a notable increase in latency times. Among these, the group receiving the 100% methanol extract (P.s4) demonstrated the highest analgesic activity, with an increase in latency time of up to 119% relative to baseline ($p<0.001$), indicating a statistically significant analgesic effect (Fig.1).

TABLE 1. Analgesic activity - "Hot-plate" assay

| Group | Reaction time (sec) | |
|---|---|---|
| | Baseline | 30 min |
| Control | 11.5 | 10.9 |
| P.s1 | 11.2 | 17.1 |
| P.s2 | 11.3 | 14.0 |
| P.s3 | 11.4 | 24.6 |
| P.s4 | 11.8 | 25.2 |
| P.w1 | 10.9 | 24.3 |
| P.w2 | 11.0 | 17.4 |
| P.w3 | 11.9 | 20.5 |
| P.w4 | 11.6 | 16.8 |
| P.m1 | 11.4 | 16.2 |
| P.m2 | 12.0 | 14.4 |
| P.m3 | 11.9 | 14.9 |
| P.m4 | 10.0 | 17.0 |

FIGURE 1. Analgesic effects of fractions from *P. woronovii, P. macrocalyx*, and *P. saguramica.* * p<0.05; ** p<0.01; *** p<0.001 vs control.




In the carrageenan-induced paw edema model used to evaluate anti-inflammatory activity, all treatment groups exhibited an apparent inhibitory effect on inflammation. One hour post-carrageenan injection, the groups treated with 100% methanolic fractions (P.s4, P.w4, P.m4) showed the most pronounced anti-inflammatory response, with an edema reduction of up to 84% compared to the control group (Tab.2 and Fig.2).

Overall, all tested fractions of *P. saguramica* displayed varying degrees of analgesic and anti-inflammatory activity. Notably, the 100% methanol extract (P.s4) consistently exhibited the most potent effects in both in vivo models, highlighting its promising pharmacological profile.

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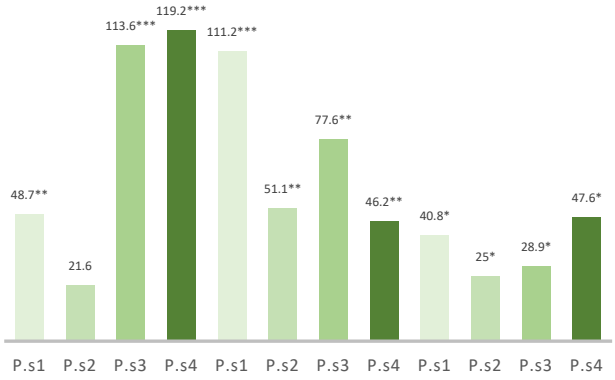
TABLE 2. Anti-inflammatory activity - Carrageenan-induced paw edema assay

| Group | Thickness of the paw (µm) | |
|---|---|---|
| | Baseline | 120 min |
| Control | 192.00 | 327.25 |
| P.s1 | 183.60 | 283.40 |
| P.s2 | 193.00 | 240.40 |
| P.s3 | 186.20 | 261.80 |
| P.s4 | 186.60 | 212.40 |
| P.w1 | 198.40 | 245.40 |
| P.w2 | 193.20 | 257.60 |
| P.w3 | 195.60 | 238.20 |
| P.w4 | 199.40 | 225.20 |
| P.m1 | 197.4 | 259.8 |
| P.m2 | 192.80 | 215.60 |
| P.m3 | 199.2 | 256.4 |
| P.m4 | 204.6 | 226.4 |

FIGURE 2. Anti-inflammatory effects of fractions from *P. woronovii, P. macrocalyx, and P. saguramica.* *p<0.05; **p<0.01; ***p<0.001 vs control




## DISCUSSION

The findings of this investigation demonstrate the pharmacological potential of *P. saguramica* fractions, notably the 100% methanolic one, which exhibited significant analgesic and anti-inflammatory properties. The increased hot-plate latency indicates a central analgesic mechanism, potentially mediated through interactions with pain-modulating pathways, consistent with previous findings from plant-based studies.[14] The anti-inflammatory efficacy of the MeOH fractions in the carrageenan-induced paw edema model further supports their potential therapeutic applications. The marked reduction in edema formation, particularly during the first hour, suggests that early-phase inflammatory mediators such as histamine, serotonin, and prostaglandins may be inhibited. This type of response is typical for plant extracts rich in bioactive phytochemicals such as flavonoids, saponins, and terpenoids, which have been recognized for their anti-inflammatory effects in other Primula species.[15] The superior performance of the 100% MeOH fraction in both models suggests it may contain a higher concentration or more potent profile of active compounds. The ratio of flavonoids to terpenoids could play a key role in modulating the observed biological activities, as both classes have demonstrated synergistic pharmacological effects. These patterns reinforce ethnopharmacological knowledge and underline the therapeutic potential of *P. saguramica*, aligning with previously reported findings on other Primula species. However, the variation in activity among different fractions emphasizes the importance of isolating and identifying individual active constituents.

## CONCLUSIONS

This study provides experimental evidence supporting the analgesic and anti-inflammatory properties of *Primula saguramica* fractions, particularly the 100% methanol fraction. These findings align with traditional medicinal uses and previously reported pharmacological activities of related Primula species. The results highlight the potential of this Georgian endemic plant as a valuable source of bioactive compounds suitable for pharmaceutical development. Given the promising pharmacological profile observed, further phytochemical investigations are recommended to isolate and identify the specific active constituents responsible for the effects. Such studies could pave the way for the development of novel, plant-based therapeutic agents for managing pain and inflammation. Additionally, future research should investigate the underlying mechanisms of action, potential toxicity, and efficacy in chronic models of inflammation and pain to validate the therapeutic potential of *P. saguramica* extracts fully.

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