

Pharmacological Evaluation of Alkaloid-Rich Extracts from *Gloriosa superba* for Analgesic and Anti-Inflammatory Activity

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Abstract: This study explores the pharmacological potential of *Gloriosa superba* Linn., emphasizing its analgesic and anti-inflammatory effects. Ethanolic extracts prepared from the plant leaves, seeds, tubers, stems, and flowers were subjected to phytochemical screening, revealing alkaloids, flavonoids, tannins, steroids, saponins, and glycosides. Alkaloids, identified as the major constituents, were isolated from the tubers and quantified by UV-visible spectrophotometry, with a maximum absorbance at 260 nm using colchicine as the reference standard. FTIR analysis confirmed characteristic functional groups of alkaloids, and HPTLC profiling showed multiple alkaloid bands under UV light at 254 and 366 nm. *In vivo* assays demonstrated significant pharmacological activities. The tail flick latency test in Wistar rats showed a dose-dependent increase in reaction time (60 min), with 100 mg/kg and 200 mg/kg extracts producing 31.9% and 49.3% analgesia, respectively. In the formalin-induced peritonitis model, anti-inflammatory effects were observed with 41.30% and 56.12% inhibition of peritoneal exudate formation at the same doses, comparable to Ibuprofen (52.73%). These findings suggest that *Gloriosa superba* tuber extracts, rich in colchicine and other phytochemicals, have potential as analgesic and anti-inflammatory agents.

Keywords: analgesic activity; anti-inflammatory activity; alkaloids; HPTLC profiling; tail-flick test.

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1. Introduction

Medicinal plants have long served as valuable sources of therapeutic agents, providing the foundation for modern drug discovery. Among these, *Gloriosa superba* L., a member of the family Colchicaceae, stands out for its remarkable pharmacological potential [1]. Commonly known as the flame lily or glory lily, this perennial climbing herb is native to tropical and subtropical regions. It has been extensively used in traditional systems of medicine such as Ayurveda, Siddha, and Unani. The plant is particularly prized for its tubers and seeds, which are known to contain potent alkaloids with significant biological activities [2].

In recent years, the search for plant-derived compounds with strong analgesic and anti-inflammatory effects has gained momentum, especially as synthetic drugs often produce

undesirable side effects [3]. *Gloriosa superba* has emerged as a promising candidate in this context due to the presence of bioactive alkaloids, notably colchicine and gloriosine. These alkaloids are known to modulate inflammatory mediators and pain pathways, providing scientific justification for the plant's traditional use in managing conditions such as arthritis, rheumatism, and joint pain. However, while colchicine is well recognized for its therapeutic relevance, the contribution of other associated alkaloids and their synergistic effects within the plant matrix remains inadequately explored [4-6]. Most prior studies have focused on isolating individual compounds or assessing general bioactivities, without establishing a direct relationship between alkaloid concentration and biological response. A comprehensive understanding of this relationship is essential for optimizing extraction processes, identifying the most active plant fractions, and guiding potential therapeutic applications [7].

Pain and inflammation are complex physiological responses involving mediators such as prostaglandins, histamines, bradykinins, and cytokines [8,9]. Plant-derived alkaloids exhibit analgesic effects by suppressing peripheral pain mediators and modulating central nervous pathways. At the same time, their anti-inflammatory activity mainly results from inhibiting cyclooxygenase and lipoxygenase enzymes, thereby reducing prostaglandin and leukotriene synthesis [10]. In *Gloriosa superba*, the alkaloid colchicine binds to tubulin, preventing microtubule formation and leukocyte migration to inflamed tissues, effectively minimizing inflammation and pain [11-13]. Additional alkaloids and phytoconstituents enhance these effects by stabilizing cell membranes, inhibiting nitric oxide release, and scavenging reactive oxygen species. Collectively, these mechanisms highlight the therapeutic potential of *Gloriosa superba* as a natural source of analgesic and anti-inflammatory agents [14,15]. The study aimed to investigate the phytochemical composition and pharmacological potential of *Gloriosa superba* through *in vitro* and *in vivo* analyses. Bioactive compounds were extracted from tubers and characterized using phytochemical screening, UV-Vis spectrophotometry, FTIR spectroscopy, and HPTLC analysis to quantify total alkaloids, identify functional groups, and detect key chromatographic markers. Furthermore, ethanolic extracts were evaluated for analgesic and anti-inflammatory activities in albino Wistar rats using the tail-flick assay and the formalin-induced peritonitis model to establish a correlation between alkaloid content and pharmacological efficacy.

2. Materials and Methods

2.1. Sample collection.

Gloriosa superba was collected from an agricultural farm near Oddanchatram, Dindugal District, Tamil Nadu (Lat: 10.4874432"N; Long: 77.7616978 "E). Label plastic bags with date, location, and part of the plant to be used for sampling. The plant specimen was taxonomically identified and authenticated by Dr. P. Vijayakanth, Head, Department of Botany, Arignar Anna College, Krishnagiri - 635 115, India, affiliated to Periyar University. A voucher specimen (No. RGRIT/AAC/2024/031) was deposited in the institutional herbarium for future reference. Healthy and representative parts of the plant should be selected. The collection of leaves, seeds, rhizome, stem, and flowers of the plant should be carefully cut or dug using sterilized tools. The samples should be stored in labelled bags without overcrowding right away after collection.

2.2. *Compounds extraction.*

Fresh plant leaves, seeds, rhizomes (tubers), stems, and flowers were collected and properly cleaned with tap water, then with distilled water, to remove dust and germs from their surfaces before being shade-dried for 10 days. The dry components were ground into a fine powder for subsequent screening.

2.3. *Phytochemical screening.*

Phytochemical analysis was carried out to identify the secondary metabolites and organic molecules present in the solvent extracts of the leaves, seeds, rhizomes, stems, and flowers of *G. superba* using standard procedures. 10 mL of ethanol was used for the extraction process for each. The crude extracts were kept in desiccators once the solvent was removed with the support of a rotary evaporator. Alkaloids, flavonoids, steroids, saponins, tannins, and glycosides were isolated from crude extracts through screening. The presence of phytochemicals is indicated by a (+) and their absence by a (-) in the qualitative data.

Phytochemical analysis of the ethanolic extracts of *Gloriosa superba* was performed using established qualitative methods to detect key secondary metabolites. The froth test indicated the presence of saponins by the formation of stable foam upon vigorous shaking with distilled water. Tannins were identified by a color change to blue-black or green upon the addition of ferric chloride solution. The presence of flavonoids was confirmed by a yellow coloration after treatment with dilute sodium hydroxide, which decolorized upon subsequent acidification. Alkaloids were detected by the appearance of an orange-red precipitate following the addition of Dragendorff's reagent. In the Salkowski reaction, the formation of a red layer at the interface after adding concentrated sulfuric acid confirmed the presence of steroids. Cardiac glycosides were indicated by a brown ring at the interface in the Keller-Kiliani test, following sequential treatment with acetic acid, ferric chloride, and sulfuric acid.

2.4. *Alkaloid extraction and estimation.*

Cleaning, chopping, and grinding the shade-dried tubers into a fine powder was the first step. 5 g of the powdered plant material was extracted with 50 mL of ethanol containing a few drops of hydrochloric acid (HCl), with a solvent-to-sample ratio of 10:1 (v/w) to enhance alkaloid dissolution. The mixture was placed on a mechanical shaker at ambient temperature ($25 \pm 2^\circ\text{C}$) for 8 hours, or alternatively left overnight (12 hours) to ensure thorough extraction. The combination proceeds to pass through Whatman No. 1 filter paper, and the collected liquid is then concentrated. To prepare the sample for spectrophotometric analysis, the alkaloids in the acidic extract are converted to their base form by adjusting the pH to alkaline using sodium hydroxide. This step helps in partitioning the alkaloids, which are then extracted with an organic solvent like chloroform. After being separated, the chloroform layer was dried by evaporation. The remaining residue, which contains the alkaloids, was then dissolved in a suitable ethanol solvent, and the absorbance of the extract was recorded in the wavelength range of 200–350 nm using a UV-visible spectrophotometer (PC-Based Double Beam UV-VIS Spectrophotometer 2202, Systronics, India) equipped with 1 cm quartz cuvettes. Ethanol was used as the solvent blank to calibrate the baseline before measurement. The total alkaloid content in the sample was quantified using a standard calibration curve constructed from known concentrations of colchicine (10–100 $\mu\text{g/mL}$), with each measurement performed in triplicate to ensure accuracy.

2.4.1. Spectral analysis of alkaloids by FTIR.

The presence of both organic and inorganic compounds in the sample was determined using FT-IR spectroscopy. The specific functional groups were identified based on infrared absorption frequencies within the range of 400–4000 cm^{-1} . A Spectrum 100 PerkinElmer FT-IR spectrometer was used to record FT-IR spectra. Each spectrum represented an average of multiple scans, with a resolution ranging from 4 cm^{-1} to 6000 cm^{-1} . The FT-IR analysis indicated that carboxylic and hydroxyl groups were responsible for the structural characteristics observed.

2.4.2. Alkaloids in HPTLC.

HPTLC was carried out on 7.0 × 10.0 cm precoated silica gel 60 F₂₅₄ aluminum plates (Merck, Germany) using a CAMAG Linomat V sample applicator equipped with a 100 μL Hamilton syringe. The sample solution (5 μL per band) was applied as 6 mm-wide bands, positioned 10 mm from the bottom edge and 15 mm apart to prevent overlapping during development. Chromatographic development was performed in a CAMAG Automatic Development Chamber (ADC 2) previously saturated with the mobile phase (methanol: chloroform, 9:1 v/v) for 20 minutes at room temperature ($25 \pm 2^\circ\text{C}$) and relative humidity of 40–50%. The migration distance was maintained at 80 mm. After development, the plates were air-dried and visualized using a CAMAG TLC Visualizer 2. Detection was performed at 254 nm (short-wave UV) and 366 nm (long-wave UV). At 254 nm, UV-active compounds appeared as dark bands on a fluorescent green background, while at 366 nm, fluorescent components were observed as bright bands on a dark background. The R_f values were determined and expressed as the mean of three replicates, with R_f reproducibility maintained within ± 0.02 , confirming method consistency.

2.5. *In vivo* activities.

2.5.1. Procurement of test animals.

Male albino Wistar rats, each weighing between 120 and 150 grams, were procured from Biogen Laboratory Animal Facility, Bengaluru, India. The animals were housed at the Department of Pharmacy's animal facility, Sri Lakshmi Narayana Institute of Medical Sciences, Osudu Agaram Village, Villianur Commune, Kudapakkam Post, Villianur, Puducherry 605502, India. Animals were maintained under standard laboratory conditions (temperature $25 \pm 2^\circ\text{C}$, relative humidity $55 \pm 10\%$, and 12 h light/dark cycle) with free access to standard pellet diet and water ad libitum. Standard drugs such as Butorphanol and Ibuprofen were obtained from Sigma-Aldrich, India. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) under reference number 932/Po/Pc/S/06/CPCSEA, dated 12/08/2024.

2.5.2. Experimental design.

Acute oral toxicity of the ethanolic extract of *Gloriosa superba* was evaluated according to OECD Guideline 425, as described [13]. Healthy adult albino Wistar rats (120–150 g) were fasted overnight prior to dosing, and their body weights were recorded to determine accurate dosage. An initial oral dose of 100 mg/kg body weight was administered to a single rat. Upon survival, four additional rats were treated sequentially and observed for signs of

toxicity or mortality for 7 days. No deaths or toxic effects were observed, suggesting that the LD₅₀ value was greater than 100 mg/kg. Based on this, doses of 100 mg/kg and 200 mg/kg were selected for subsequent pharmacological evaluations. Animals were randomly assigned to different experimental groups using a simple randomization method (computer-generated random numbers). The investigator performing the data analysis was blinded to the treatment allocations to minimize bias. Each group consisted of six rats (n = 6), a commonly accepted sample size for preliminary pharmacological evaluations to achieve sufficient statistical power while adhering to the 3R principles (Reduction, Replacement, and Refinement) in animal experimentation. All experimental data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism version 9.0. Differences between groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. A value of P < 0.05 was considered statistically significant, while P < 0.01 and P < 0.001 were considered highly significant and very highly significant, respectively.

2.5.3. Analgesic activity – Tail-Flick method.

Analgesic activity was assessed using the tail-flick method [14]. Healthy albino Wistar rats of either sex (120–150 g) were randomly divided into four groups (n = 6). Group I (Control): Received 0.5% CMC (vehicle, p.o.(oral)). Group II (Standard): Received Ibuprofen (15 mg/kg body weight, p.o.(oral)). Group III: Received *G. superba* ethanolic extract (100 mg/kg body weight, p.o.). Group IV: Received *G. superba* ethanolic extract (200 mg/kg body weight, p.o.).

Tail flick latency was measured using an analgesiometer that applied radiant heat approximately 2–3 cm from the tail tip. The latency time (reaction time to tail withdrawal) was recorded at 0, 30, 60, 90, and 120 minutes post-treatment. A cut-off time of 10 seconds was established to prevent tissue injury. Increased latency compared to the control group indicated analgesic activity. Percentage analgesia was calculated at each time point using the following formula:

$$\% \text{Analgesia} = \left[\frac{\text{Treated latency} - \text{Control latency}}{\text{Cut-off time} - \text{Control latency}} \right] \times 100 \quad (1)$$

2.5.4. Anti-inflammatory activity – Formalin-induced Peritonitis model.

Anti-inflammatory activity was evaluated using a formalin-induced peritonitis model [15]. Albino Wistar rats were randomly divided into four groups of six animals each. Group I served as the control, while Group II received Ibuprofen (15 mg/kg body weight) as the reference drug. Groups III and IV were treated with ethanolic extracts of *G. superba* at doses of 100 mg/kg and 200 mg/kg, respectively. All treatments were administered one hour before the induction of inflammation. Peritonitis was induced by intraperitoneal injection of 1 mL of 1% formalin solution under sterile conditions. Four hours after injection, animals were sacrificed, and their abdominal cavities were carefully opened to collect the peritoneal exudate. The volume of fluid recovered from each group was measured. Anti-inflammatory activity was determined by comparing exudate volume in treated groups with that in the control group, and the percentage inhibition of inflammation was calculated accordingly.

$$\% \text{ Inhibition} = (V_c - V_t) \times 100 / V_c \quad (2)$$

Where V_c represents the average exudate volume in the control group, and V_t denotes the volume observed in treated groups.

3. Results and Discussion

Medicinal plants traditionally used in healthcare often serve as valuable leads for the discovery of new therapeutic agents. In this context, crude solvent and aqueous extracts, along with the derived fractions of *Gloriosa superba* Linn. (Figure 1), were evaluated for their biological properties, particularly their analgesic and anti-inflammatory potential.

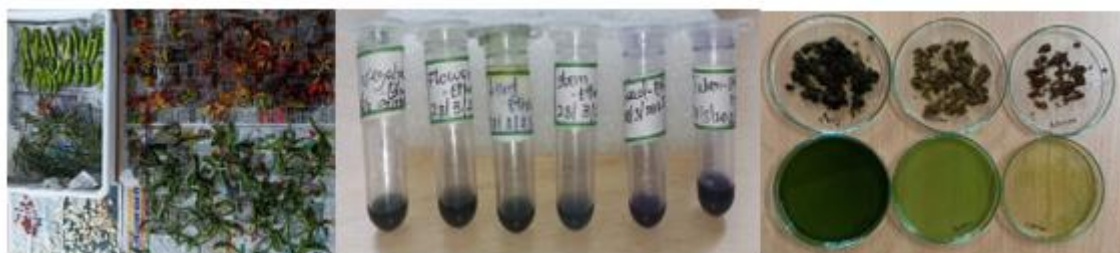


Figure 1. Healthy and representative parts of *Gloriosa superba* and ethanolic extracts.

3.1. Phytochemical screening of *Gloriosa superba*.

The phytochemical screening was carried out to identify the secondary metabolites present in the aqueous extracts of leaves, seeds, tubers, stem, and flowers of *Gloriosa superba*. Standard procedures were followed for the identification of alkaloids, steroids, tannins, saponins, flavonoids, and glycosides, and the results are presented herewith in Table 1. The findings from this study align with previous ethnobotanical and pharmacological research, reinforcing the medicinal significance of this plant and backing up its traditional applications. All the solvent extracts, along with their various fractions, showed notable analgesic effects. This can be linked to the presence of secondary metabolites, such as alkaloids, flavonoids, saponins, and tannins, which have been documented in *Gloriosa superba*.

Table 1. Qualitative Phytochemical Analysis of *Gloriosa superba* ethanolic extracts.

Phytochemicals	Leaves	Seeds	Tubers	Stem	Flowers	Observation
Alkaloids	+++	+++	+++	++	++	The orange-red precipitate
Steroids	++	++	++	++	++	Green blue formation
Tannins	+	+	+	+	+	Blue-green coloration
Saponins	+++	+++	+++	++	+++	Formation of foam on the top of the test tubes
Flavonoids	++	++	++	++	++	The yellow solution turned colorless
Glycosides	-	++	++	-	-	The reddish brown ring at interphase

“+++” = High presence, “++” = Moderate presence, “+” = Low presence, “-” = Absent

The process of extracting ethanol-HCl, adjusting the pH, and partitioning with chloroform successfully produced a concentrated alkaloid fraction from the tubers of *Gloriosa superba*. When we analyzed it by UV-Visible spectrophotometry, we observed significant absorbance between 220 and 280 nm, with a notable peak at 260 nm, suggesting the presence of alkaloids. To ensure accuracy, we created a standard calibration curve using colchicine, a well-known alkaloid found in *Gloriosa superba*, at concentrations ranging from 10 to 100 µg/mL. The absorbance values for these standards were linear, with a correlation coefficient (R^2) of 0.997, confirming reliable quantification (Table 2). The strong UV absorbance at 260 nm (Figure 2) and the notable alkaloid content reported in this study are consistent with earlier research highlighting the rich alkaloid profile of *Gloriosa superba*, especially colchicine [16].

Table 2. Total alkaloid content of tuber extract from *Gloriosa superba*.

Parameter	Observation
Weight of tuber powder used	5.0 g
Volume of ethanol-HCl extract	50 mL
Maximum absorbance wavelength (λ_{max})	260 nm
Calibration curve R^2 value	0.997
Alkaloid content in the extract	23.4 mg/g dry weight
Type of standard used	Colchicine

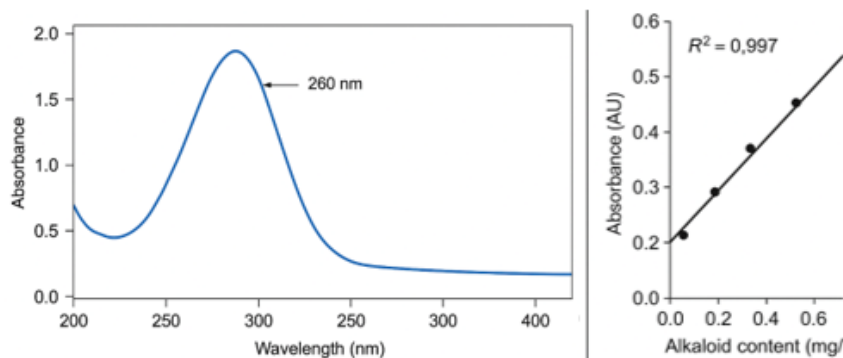


Figure 2. UV-Visible absorption peak at 260 nm, corresponding to the λ_{max} of colchicine standard.

3.2. FTIR analysis.

The FTIR analysis of *Gloriosa superba* ethanolic extract revealed several distinct absorption bands corresponding to important functional groups commonly found in phytochemicals, particularly alkaloids and secondary metabolites. The spectrum was captured over $4000\text{--}400\text{ cm}^{-1}$, and we observed significant peaks indicative of various bioactive compounds. A broad and strong peak around 3300 cm^{-1} points to O–H stretching vibrations, suggesting the presence of hydroxyl groups, which are usually found in phenols and alcohols. The sharp peaks near 2920 cm^{-1} are linked to C–H stretching vibrations of aliphatic --CH_2 groups, commonly seen in fatty acids and alkaloid structures. A notable peak at 1627 cm^{-1} indicates C=O stretching vibrations, suggesting the presence of carbonyl functional groups such as ketones or amides (Figure 3). Furthermore, a peak in the $1350\text{--}1250\text{ cm}^{-1}$ range is associated with C–N stretching, confirming the presence of amines or alkaloid-like structures, which supports the extraction of nitrogen-containing compounds such as colchicine from the tubers [17]. The presence of these groups underscores the pharmacological significance of the tuber extract, particularly regarding its analgesic and anti-inflammatory properties.

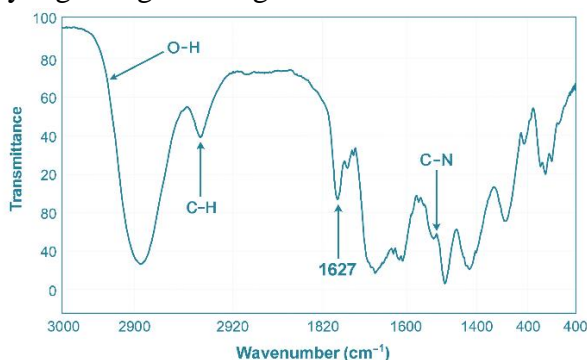


Figure 3. Characteristic absorption bands corresponding to various functional groups present in the extract.

3.3. Alkaloids in HPTLC.

HPTLC profiling of the alkaloid extract from *Gloriosa superba* tubers revealed a variety of distinct bands under UV light at 254 nm and 366 nm, suggesting the presence of several

alkaloid components. At 254 nm, we identified six prominent bands with Rf values ranging from 0.01 to 0.98. The most significant area was noted for Band 3, which had 5767 units at an Rf of 0.60, indicating it's a major alkaloid component. Following closely was Band 4, with an area of 6478 units at Rf 0.12, suggesting it has a strong intensity and concentration in the lower part of the plate. Band 2 also stood out with the highest volume of 464.4, hinting at a dense and possibly complex alkaloid spot at a higher Rf of 0.98. At 366 nm, we observed fluorescence or changes in the absorption spectra of the alkaloids, with six corresponding bands (Figure 4). Band 2 again showed high intensity, with an area of 3388 units at Rf 0.79, while Band 1 shifted to a higher Rf of 0.88, likely due to differences in polarity or structural features upon exposure to UV light. Bands 4 and 6 also had substantial areas of 2464 and 3388 units, respectively, further confirming the presence of multiple types of alkaloids [18].

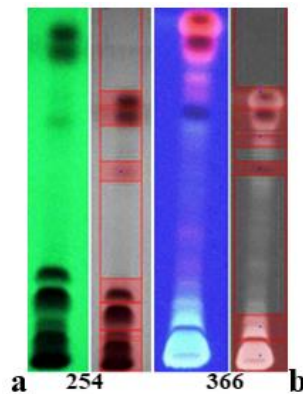


Figure 4. HPTLC chromatograms of *Gloriosa superba* ethanolic extract compared with colchicine standard (a) Visualization under 254 nm showing quenching bands; (b) 366 nm fluorescence revealing alkaloid-rich fractions.

3.4. Tail Flick latency.

The pain-relieving effects of the ethanolic extract from *Gloriosa superba* were assessed using the tail flick method on albino Wistar rats.

Table 3. Tail Flick latency (in seconds) and percentage analgesia.

Time (min)	Control (Vehicle)	Standard (Butorphanol, 46 mg/kg)	<i>G. superba</i> (100 mg/kg)	% Analgesia	<i>G. superba</i> (200 mg/kg)	% Analgesia
0	2.1 ± 0.2	2.3 ± 0.3	2.1 ± 0.2	–	2.2 ± 0.1	–
30	2.4 ± 0.3	7.8 ± 0.4**	4.6 ± 0.3*	29.2%	5.9 ± 0.4**	44.1%
60	2.5 ± 0.2	8.5 ± 0.3**	5.0 ± 0.2*	31.9%	6.7 ± 0.3**	49.3%
90	2.3 ± 0.3	8.0 ± 0.2**	4.8 ± 0.4*	29.9%	6.2 ± 0.3**	46.4%
120	2.1 ± 0.1	7.5 ± 0.3**	4.2 ± 0.3*	25.0%	5.5 ± 0.2**	41.1%

*Values expressed as mean ± SEM (n = 6). *P < 0.05, **P < 0.01 compared to control group.

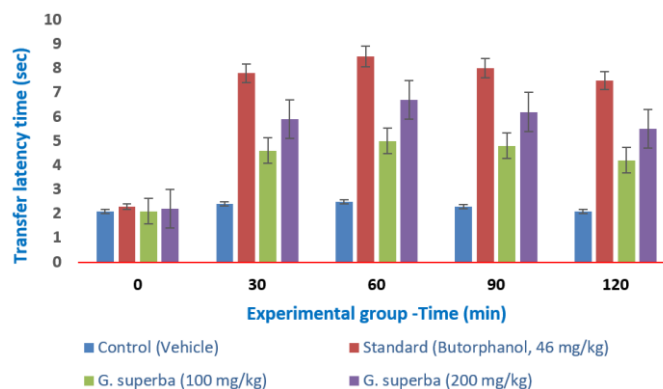


Figure 5. Dose-dependent analgesic effect of *Gloriosa superba* in the tail flick test compared with the standard Butorphanol-treated group.

We measured reaction times (latency to tail flick) at 0, 30, 60, 90, and 120 minutes after administration for all groups. Both doses of the *Gloriosa superba* extract (100 mg/kg and 200 mg/kg) led to a gradual increase in tail-flick latency over time, with the 200 mg/kg group demonstrating significantly longer latency than both the control group and the 100 mg/kg group (Figure 5). Meanwhile, the group that received the standard drug, Butorphanol (46 mg/kg), exhibited the most pronounced analgesic effect throughout the observation period (Table 3).

The current study has shown that the ethanolic extract from *Gloriosa superba* tubers has notable analgesic properties that depend on the dosage, as assessed through the tail-flick method. The longer tail-flick latency, especially at the 100 mg/kg dose, suggests that this extract may act centrally to relieve pain, possibly by modulating spinal reflexes or interacting with opioid receptors. Moreover, the analgesic effects observed in this study are supported by phytochemical evidence that *Gloriosa superba* contains various active compounds, including alkaloids, flavonoids, and phenolic acids, all of which are recognized for their ability to modulate pain [19,20]. The enhanced effectiveness at 200 mg/kg likely reflects a higher concentration of these compounds, resulting in stronger pharmacological effects.

3.5. Formalin-induced peritonitis in rats.

The anti-inflammatory effects of the ethanolic extract from *Gloriosa superba* were evaluated using the formalin-induced peritonitis model in albino Wistar rats. When the extract was administered at 100 mg/kg and 200 mg/kg, there was a notable decrease in peritoneal exudate volume compared to the control group, suggesting that it effectively reduces inflammation. Additionally, the standard drug Ibuprofen at 15 mg/kg also demonstrated a significant inhibitory effect. The 200 mg/kg dose showed a 56.12 % reduction in inflammation, which is quite close to the standard anti-inflammatory drug Ibuprofen (15 mg/kg), which achieved a 52.73% reduction (Figure 6). Even the lower dose of 100 mg/kg demonstrated significant activity, with a 48.57% reduction (Table 4).

Table 4. Effect of *Gloriosa superba* extract on formalin-induced peritonitis in rats.

Group	Treatment	Dose (mg/kg)	Mean exudate volume (mL)	% Inhibition
I	Control (Vehicle)	–	3.85 ± 0.12	–
II	Ibuprofen	15	1.82 ± 0.09**	52.73%
III	<i>G. superba</i> Extract	100	2.34 ± 0.11*	41.30%
IV	<i>G. superba</i> Extract	200	1.98 ± 0.10**	56.12%

*Values are expressed as mean ± SEM (n = 6). *P < 0.05, **P < 0.01 vs. control group.

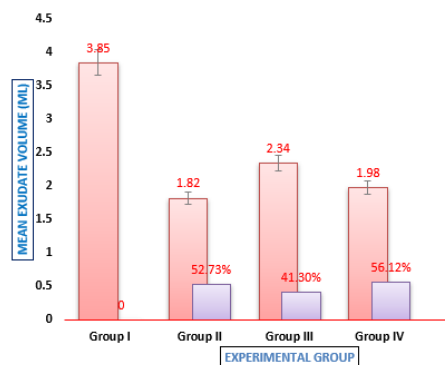


Figure 6. Dose-dependent reduction in exudate volume, with 41.30% inhibition at 100 mg/kg and 56.12% inhibition at 200 mg/kg, closely comparable to Ibuprofen (52.73%).

These results indicate that the *G. superba* extract exhibits a dose-dependent anti-inflammatory effect, suggesting it contains pharmacologically active compounds that modulate

inflammatory pathways. The reduction in exudate volume suggests that the *G. superba* extract may have disrupted these early inflammatory mechanisms [21-23].

Mechanistically, this reduction in peritoneal exudate suggests that *G. superba* extract suppresses early-phase inflammatory mediators such as histamine and serotonin, responsible for increased vascular permeability, as well as late-phase mediators like prostaglandins and bradykinin, which sustain inflammatory exudation [10]. The extracts' high alkaloid and flavonoid content likely contributes to inhibition of cyclooxygenase (COX) activity, thereby reducing prostaglandin synthesis, while also stabilizing lysosomal membranes and decreasing leukocyte infiltration mechanisms similar to those of non-steroidal anti-inflammatory drugs like Ibuprofen.

3.6. Discussion.

The phytochemical investigation of *Gloriosa superba* revealed a rich presence of secondary metabolites, predominantly alkaloids, flavonoids, saponins, tannins, and steroids. Among these, alkaloids, particularly colchicine and its derivatives, are considered the principal bioactive compounds responsible for the plant's pharmacological activity. The UV–Vis spectrum showing a sharp λ_{max} at 260 nm and the FTIR signals at 1627 cm^{-1} (C=O stretching) and 1350 cm^{-1} (C–N stretching) confirm the presence of colchicine-like structures, consistent with previous chemical profiling studies [24]. Colchicine is a tricyclic alkaloid that binds to tubulin, inhibiting its polymerization into microtubules. This mechanism disrupts cell division and inflammatory cell migration, explaining the plant's long-recognized anti-inflammatory and analgesic potential [25,26]. The strong linearity of the calibration curve ($R^2 = 0.997$) in UV quantification further validates the extract's high alkaloid concentration (23.4 mg/g dry weight), suggesting a pharmacologically active dose range for in vivo studies.

The FTIR and HPTLC profiles further substantiate the mechanistic basis for these activities. The presence of multiple C–N, C=O, and O–H peaks, as well as several fluorescent bands under 254 nm and 366 nm, reflects a mixture of nitrogenous and phenolic alkaloids structurally related to colchicine [27]. These compounds may interact with prostaglandin pathways by inhibiting cyclooxygenase (COX) enzymes, thereby reducing prostaglandin synthesis [28]. Additionally, colchicine and its analogs are known to suppress neutrophil chemotaxis and adhesion by altering cytoskeletal dynamics through microtubule depolymerization. The observed inhibition of peritoneal exudate formation in the formalin-induced peritonitis model is consistent with this mechanism, suggesting that the extract acts on early vascular and cellular events of inflammation, limiting leukocyte infiltration and exudate accumulation [29]. These actions collectively support both central and peripheral pathways for analgesia and the control of inflammation.

The tail-flick latency data corroborate the central role of these mechanisms. The extract significantly increased the pain threshold in a dose-dependent manner, with 200 mg/kg showing near-equivalent efficacy to Butorphanol. Since colchicine and related alkaloids are known to influence neural microtubule dynamics and modulate neurotransmitter release, it is plausible that they contribute to pain suppression by affecting synaptic transmission in spinal reflex arcs [30]. Furthermore, flavonoids and saponins in the extract may synergistically potentiate these effects by inhibiting nitric oxide synthase and reducing oxidative stress, mechanisms known to enhance opioid mediated analgesia [31]. Thus, the analgesic and anti-inflammatory responses observed in this study reflect the multi-target pharmacology of *G. superba* [32], primarily mediated through tubulin interference, prostaglandin inhibition, and

neuronal modulation, mirroring the established pharmacodynamics of colchicine and its analogs.

4. Conclusions

The present study concluded that the ethanolic tuber extract of *Gloriosa superba* Linn. exhibits potent analgesic and anti-inflammatory activities, validating its traditional medicinal use. Phytochemical screening and spectroscopic analyses (UV–Vis, FTIR, and HPTLC) confirmed the presence of colchicine and related rich alkaloids, along with flavonoids, tannins, and saponins, which are likely responsible for the observed pharmacological effects. *In vivo* studies showed that the 200 mg/kg extract increased tail flick latency significantly and produced 56.12% inhibition of inflammation, an effect comparable to Ibuprofen (52.73%). These findings suggest that *G. superba* exerts its effects through both central analgesic mechanisms and prostaglandin-mediated anti-inflammatory pathways. Future research should focus on isolating and characterizing individual alkaloids, elucidating their molecular and receptor-level mechanisms, and conducting toxicological and pharmacokinetic evaluations to establish safe and effective therapeutic formulations.

Author Contributions

Conceptualization, S.K. and S.T.; methodology, S.T.; software, S.T.; validation, S.T., M.R., and P.A.; formal analysis, M.R.; investigation, S.K.; resources, S.K.; data curation, P.A.; writing—original draft preparation, M.R.; writing—review and editing, M.R.; visualization, M.R.; supervision, S.K.; project administration, S.T.; funding acquisition, S.T. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

Department of Pharmacy, Sri Lakshmi Narayana Institute of Medical Sciences, Osudu Agaram Village, Villianur Commune, Kudapakkam Post, Villianur, Puducherry 605502, India. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) under reference number 932/Po/Pc/S/06/CPCSEA, dated 12/08/2024.

Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

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