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Evaluation Of Wound Healing Potential Of Fruit Peel Extract Of Trapa Natans In Excision Wound Model

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Abstract
The present study investigates the phytochemical composition and wound-healing potential of the methanolic extract of Trapa natans (water chestnut) peel. Fruits were collected, authenticated, and the peel was shade-dried, powdered, and extracted using petroleum ether and methanol. The extracts were subjected to preliminary phytochemical screening, revealing the presence of alkaloids, phenolics, flavonoids, tannins, saponins, triterpenoids, steroids, and carbohydrates, with the methanolic extract exhibiting a richer phytochemical profile. Quantitative analysis showed high total phenolic (84.18 mg GAE/g) and catechin content (82.41 mg CE/g). Acute toxicity studies indicated that the methanolic extract was safe up to 2000 mg/kg in Wistar rats, with no mortality or observable adverse effects. The wound-healing potential was evaluated using an excision wound model in rats. Topical application of 5% w/w methanolic extract ointment significantly enhanced wound contraction (58.86% on day 20) compared to control (8.99%) and vehicle control (13.18%), though slightly lower than the standard Povidone-Iodine ointment (81.06%). The observed wound-healing activity is attributed to the synergistic effects of phenolics, flavonoids, tannins, and saponins, which possess antioxidant, anti-inflammatory, and antimicrobial properties. These findings suggest that Trapa natans peel extract may serve as a natural therapeutic agent for promoting wound repair. Further studies are warranted to elucidate the underlying molecular mechanisms.

Keywords: Trapa natans; Methanolic extract, Phytochemical screening, Wound healing; Excision wound model, Phenolics, Catechin.

Introduction

Wound healing is a complex, multi-phasic biological process that restores the structural and functional integrity of injured tissues through coordinated hemostasis, inflammation, proliferation, and remodeling phases [1]. Imbalanced inflammation and excessive reactive oxygen species (ROS) are major contributors to impaired or chronic wounds; consequently, therapies that combine antimicrobial, anti-inflammatory, and antioxidant activities are of particular interest for accelerating repair and preventing complications [2].

Medicinal plants have long been a productive source of such multifunctional bioactives, with phytochemicals including polyphenols, flavonoids, tannins, terpenoids, and saponins reported to promote wound closure by modulating inflammation, scavenging free radicals, stimulating fibroblast proliferation and collagen synthesis, and exerting antimicrobial effects. This integrative action of phytoconstituents has motivated renewed preclinical research into traditional botanicals as affordable, locally available wound therapeutics [3].

Trapa natans L. (water chestnut; family Lythraceae), commonly known as water caltrop or singhara in South Asia, is an aquatic plant widely distributed across temperate and subtropical regions. Its edible kernels and pericarps are used both as food and in traditional medicine for ailments such as diarrhea, dysentery, and inflammatory conditions [4]. Phytochemical investigations of T. natans have identified a rich spectrum of phenolic compounds, flavonoids, tannins, lignans, and triterpenoids (including ursolic acid derivatives), which are associated with antioxidant and anti-inflammatory properties [5].

Several recent phytochemical and pharmacological studies have strengthened the biological rationale for evaluating T. natans in the context of tissue repair. Quantitative analyses indicate substantial total phenolic and flavonoid contents in different plant parts (fruit rind, kernel, and leaves), with robust free-radical scavenging and ferric-reducing antioxidant activities observed in vitro—attributes that are mechanistically relevant to reducing oxidative stress at wound sites. In addition, in vitro and in vivo work has documented antimicrobial and anti-inflammatory effects for extracts from the pericarp and leaves, further supporting their potential to curb wound infection and inflammation, two obstacles to optimal healing [6].

Direct evaluation of T. natans in wound models is emerging. An in vivo study using excision and incision wound models in rats reported that topical application of T. natans leaf extract significantly enhanced wound contraction, reduced epithelialization time, and improved tensile strength compared with control, suggesting a tangible pro-healing effect under experimental conditions [7].

Despite these encouraging results, gaps remain: standardized extraction methods, comprehensive phytochemical fingerprinting linked to bioactivity, dose–response relationships, and mechanistic studies (e.g., effects on cytokine profiles, collagen deposition, angiogenesis, and oxidative markers) are still limited or inconsistent across reports. Addressing these gaps with rigorous phytochemical characterization (qualitative and quantitative), in vitro bioassays (antioxidant, antimicrobial, anti-inflammatory), and well-controlled in vivo wound models will be essential to validate therapeutic potential and identify lead compounds or enriched fractions suitable for formulation [8].

Accordingly, the present study aims to (1) perform phytochemical screening and quantification of major phenolic/flavonoid constituents in selected Trapa natans extracts, and (2) assess in vivo wound-healing efficacy using a standardized excision model and biochemical endpoints. By linking phytochemical content to biological outcomes, this work seeks to provide a robust preclinical foundation for the development of T. natans-based topical therapeutics for wound management.

Material and Methods

Material

The fruits of Trapa natans were procured from local vendors in Bhopal and authenticated by a botanist at RB Science, Bhopal. The peel and pulp were separated, shade-dried, and powdered for extraction. Solvents including petroleum ether and methanol were used for sequential extraction. Chemicals and reagents for phytochemical analysis included Mayer's, Hager's, Wagner's, and Dragendorff's reagents for alkaloids; Folin–Ciocalteu reagent for total phenolics; vanillin and hydrochloric acid for tannins; and standard compounds such as gallic acid and catechin. Simple ointment base ingredients cetostearyl alcohol, wool fat, white soft paraffin, and hard paraffin were used for topical formulation. Povidone-iodine ointment (5% w/w) served as the standard drug in wound-healing studies. All chemicals were of analytical grade and procured from reputed suppliers.

Methods

Collection and Identification of Plant Material

Fresh fruits of Trapa natans were procured from local roadside vendors in Bhopal, Madhya Pradesh. The plant material was taxonomically identified and authenticated by a botanist at RB Science, Bhopal, and a voucher specimen was retained for reference.

Preparation of Plant Material for Extraction

The fruits were peeled manually, and both the pulp and peel were separated. These were shade-dried at room temperature to prevent degradation of thermolabile constituents, pulverized into coarse powder, and stored in airtight containers until further use [9].

Extraction of Fruit Peel

A weighed quantity of powdered peel (65 g) was first defatted with hexane at room temperature for 24 hours. The defatted marc was air-dried and then subjected to Soxhlet extraction using ethanol as solvent for

approximately 18 hours (hot continuous extraction). The resulting extract was filtered, concentrated by evaporation on a water bath, and further dried in a desiccator to remove residual moisture. The dried ethanolic extract was stored in an airtight desiccator until subjected to phytochemical screening and pharmacological evaluation [10].

Phytochemical Screening

The ethanolic extract was qualitatively analyzed for the presence of major secondary metabolites using standard phytochemical tests. The classes of compounds tested included triterpenes/steroids, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic acids. Characteristic color changes and/or precipitate formation were used as indicators of positive reactions [11].

Quantitative Estimation of Total Phenolics

The total phenolic content of the extract was determined using the Folin–Ciocalteu reagent method, with gallic acid as the reference standard. Briefly, 200 µL of the extract was mixed with 1.4 mL of distilled water followed by the addition of 100 µL Folin–Ciocalteu reagent. After incubation at room temperature for 15 minutes, 300 µL of 20% sodium carbonate solution was added, and the mixture was further incubated for 2 hours at room temperature. The absorbance was measured at 760 nm using a UV–Visible spectrophotometer. Standard gallic acid solutions (10–100 ppm) were prepared and treated similarly to generate the calibration curve. The results were expressed as milligrams of gallic acid equivalents (GAE) per 100 g of dry extract [12].

Estimation of Total Tannin Content

The total tannin content was estimated according to the method of Broadhurst, using catechin as the reference standard. A 400 µL aliquot of the extract was mixed with 3 mL of vanillin solution (4% in methanol) and 1.5 mL of concentrated hydrochloric acid. The mixture was incubated at room temperature for 15 minutes, and the absorbance was recorded at 500 nm. Standard catechin solutions (10–100 ppm) were processed under the same conditions to construct the calibration curve. The tannin content was expressed as grams of catechin equivalent (g catechin/100 g) of dry extract [13].

Fourier Transform Infrared (FT–IR) Spectral Analysis

The FT–IR spectrum of the extract was recorded using an FT–IR spectrophotometer. A small quantity of dried extract was triturated with dry potassium bromide (KBr), and the mixture was compressed into a translucent disc. The sample was scanned over the range of 4000–400 cm^{-1}, and the characteristic absorption bands were analyzed to identify functional groups present in the extract [14].

Pharmacological Evaluation

Animals

Healthy adult male Wistar rats weighing between 180–250 g were selected for the study. Animals were housed in polypropylene cages under standard laboratory conditions (12 h light/dark cycle, temperature maintained between 17–26 °C, and relative humidity 40–60%). They were fed a standard pellet diet with water provided ad libitum. Prior to experimentation, the rats were fasted for 12 hours with free access to water [15].

Acute Toxicity Study

The acute oral toxicity of the methanolic peel extract of Trapa natans was evaluated according to OECD guideline 423. Three rats were administered a single oral dose of 2000 mg/kg body weight. Animals were observed individually during the first 30 minutes, periodically over the next 24 hours, and thereafter once daily for 14 days. Observations included changes in skin, fur, eyes, and mucous membranes, as well as respiratory rate, cardiovascular (heart rate and blood pressure), autonomic (salivation, perspiration,

urination, defecation), and central nervous system (drowsiness, tremors, convulsions) parameters. Mortality, if any, was recorded throughout the study period.

Preparation of Test Samples and Standard Drug

The methanolic extract of Trapa natans peel was incorporated into a simple ointment base to obtain a 5% w/w formulation. For comparison, commercially available 5% Povidone-Iodine ointment was used as the standard reference drug.

Preparation of Simple Ointment Base

The simple ointment base was prepared by melting hard paraffin (5 g) and cetostearyl alcohol (5 g) in a porcelain dish over a water bath maintained at 70 °C. Wool fat (5 g) and white soft paraffin (85 g) were then added, and the mixture was stirred until homogenous. The molten base was allowed to cool with continuous stirring and subsequently stored in suitable containers for use.

Experimental Procedure for Wound Healing (Excision Model)

Experimental Design

Animals were randomly divided into four groups, with five rats in each group. The excision wound model was employed to assess wound-healing activity. Treatment allocation was as follows:

Table 1: Experimental Design

Group	Nomenclature	Treatment
I	Control	Untreated
II	Vehicle Control	Simple ointment base
III	Standard	Povidone-iodine ointment (5% w/w)
IV	Test	Trapa natans extract ointment (5% w/w)

Induction of Wound

On the day of wound induction, each rat was anesthetized by the open-mask method using short exposure to diethyl ether. The dorsal surface was shaved using an electric clipper, and the wound area was demarcated with methylene blue using a circular stainless-steel stencil. A full-thickness excision wound measuring 1.5 cm in diameter (≈2.25 cm^2) was created along the marked area using toothed forceps, a surgical blade, and pointed scissors. The wound was left open without suturing. All surgical procedures were performed under aseptic conditions. Beginning 24 hours after wound creation, the respective ointments were applied topically once daily to cover the wound area until complete healing. Wound size and wound contraction was monitored daily [15].

Measurement of Wound Contraction

The rate of wound healing was evaluated by assessing wound contraction at regular intervals. The wound area was traced on a transparent sheet and transferred to a graph paper to calculate the remaining wound size. Observations were recorded for all animals in each group until complete epithelialization was achieved, and the day of complete wound closure was noted.

The percentage of wound contraction was calculated using the formula:

$$\text{Percent wound contraction} = \frac{\text{Healed area}}{\text{Total area}} \text{ X } 100$$

Results and Discussion

The present study evaluated the phytochemical composition and wound-healing potential of the methanolic extract of Trapa natans peel. The extraction yield differed substantially between solvents, with methanol yielding 15.7% of the extract compared to 1.4% for petroleum ether (Table 2). This higher yield in methanol can be attributed to its polar nature, which efficiently extracts phenolic, flavonoid, tannin, and saponin

compounds, as supported by subsequent phytochemical screening. The petroleum ether extract primarily contained non-polar constituents such as triterpenoids and steroids, whereas the methanolic extract was rich in alkaloids, phenolics, flavonoids, tannins, and saponins (Table 3). This diverse chemical profile indicates that methanol is a suitable solvent for isolating bioactive constituents relevant to wound healing.

Quantitative analysis further confirmed the presence of high levels of phenolics (84.18 mg GAE/g) and catechins (82.41 mg CE/g) in the methanolic extract (Table 4). Both phenolics and catechins are well-known for their antioxidant properties, which play a crucial role in neutralizing reactive oxygen species at the wound site and accelerating tissue repair. The presence of tannins and flavonoids is also significant, as these compounds contribute to antimicrobial activity, collagen stabilization, and modulation of inflammation—key factors in promoting wound healing.

Acute toxicity studies revealed that the methanolic extract was safe at a dose of 2000 mg/kg, with no mortality or observable behavioral changes in rats (Table 5). This indicates a wide therapeutic window for topical application in wound healing experiments.

The wound-healing potential of the Trapa natans extract was evaluated using the excision wound model (Table 6). The test group treated with 5% methanolic extract ointment exhibited progressive wound contraction over 20 days, achieving 58.86% closure, compared to 81.06% in the standard Povidone-Iodine group. Although the extract did not match the standard drug, it significantly accelerated wound contraction compared to the control (8.99%) and vehicle control (13.18%) groups. The enhancement of wound closure in the extract-treated group can likely be attributed to its high content of phenolics, flavonoids, tannins, and saponins, which collectively contribute to antioxidant, anti-inflammatory, and antimicrobial actions.

The data suggest that the methanolic extract of Trapa natans peel is a promising natural agent for wound healing. Its efficacy is likely mediated by the synergistic effect of multiple phytochemicals that promote tissue regeneration, reduce oxidative stress, and protect against microbial infection. Further studies, including histopathological evaluation and mechanistic investigations, are warranted to fully elucidate the molecular pathways involved in its wound-healing activity.

Table 2: Extraction yield and appearance of extracts

Solvent	Yield (%)	Appearance	Texture
Petroleum ether	1.4	Yellow	Powder
Methanol	15.7	Brown	Sticky

Table 3: Phytochemical screening of Trapa natans extract

Phytochemical Class	Test / Reagent	Observation	Petroleum Ether Extract	Methanolic Extract
Alkaloids	Mayer's reagent	Cream-colored precipitate	–	+
	Hager's reagent	Yellow precipitate	–	+
	Wagner's reagent	Reddish-brown precipitate	–	+
	Dragendorff's reagent	Reddish-brown precipitate	–	+
Glycosides	Legal's test	Pink/red coloration	–	–
	Baljet test	Orange/yellow coloration	–	–
	Keller–Kiliani test	Reddish-brown color in acid layer	–	–
Phenolics	Ferric chloride test	Blue/green coloration	–	+
	Lead acetate test	Yellow coloration	–	+

Flavonoids	Shinoda test	Red coloration	–	+
	Alkaline reagent test	Yellow turning red upon acidification	–	+
Proteins	Biuret test	Violet coloration	–	–
	Ninhydrin test	Purple/blue coloration	–	–
Triterpenoids	Liebermann–Burchard test	Deep red coloration	+	+
	Salkowski test	Yellow coloration in lower layer	+	–
Carbohydrates	Molisch's test	Violet-colored ring	+	+
Steroids	Liebermann–Burchard test	Brown ring at junction; upper layer turns green	+	+
	Salkowski test	Red coloration in chloroform layer	+	–
Tannins	Lead acetate test	Yellow/red coloration	–	+
Saponins	Foam test	Persistent foam (1 cm)	–	+

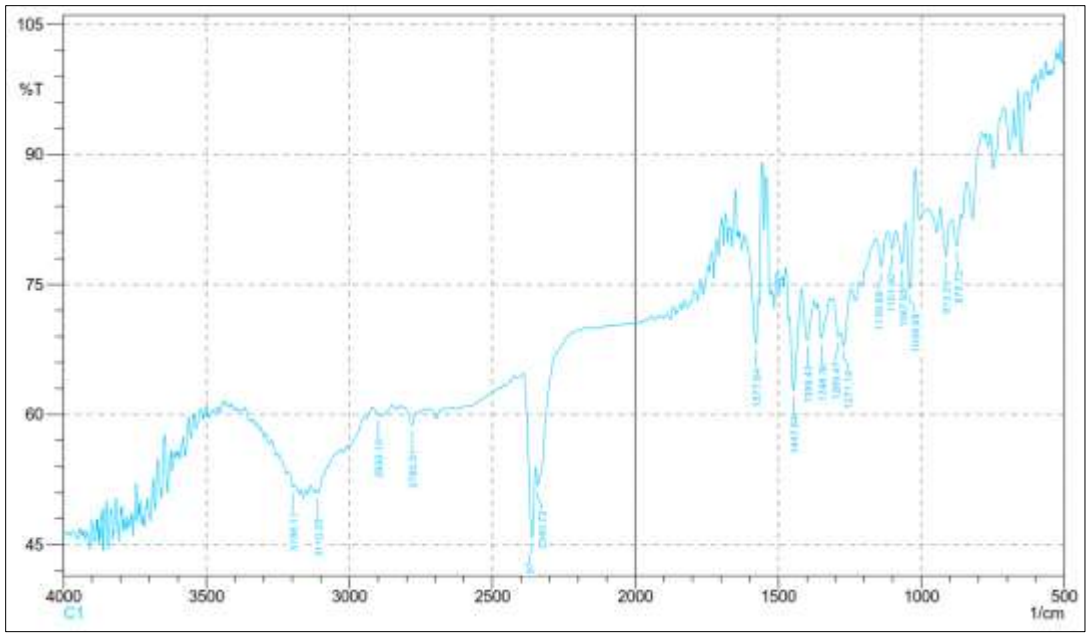

Figure 1: FTIR Spectra of the extract

Table 4: Quantitative Estimation of total phenolics and total tannin content

Parameter	Result (mg/g)	Standard Equivalent Used
Total Phenolic Content	84.18	Gallic Acid Equivalent (GAE)
Catechin Content	82.41	Catechin Equivalent (CE)

Table 5: Results of Acute Toxicity Study

Group	Extract	Number of Mice (n)	Dose (mg/kg)	Deaths (n)	Survivors (n)	Mortality (%)
1	Methanolic	3	2000	0	3	0

Table 6: Effect of Trapa natans Extract on Wound Contraction in Excision Model

Day	Control (Area mm²)	% Contraction	Vehicle Control (Area mm²)	% Contraction	Standard (Povidone-Iodine 5%) (Area mm²)	% Contraction	Test (Trapa natans 5%) (Area mm²)	% Contraction
0	100.16 ± 2.40	–	101.16 ± 1.47	–	101.16 ± 2.63	–	98.83 ± 1.83	–
5	99.33 ± 2.42	0.83	98.83 ± 1.32	2.30	88.16 ± 4.16	12.85	97.16 ± 1.32	1.69
10	97.00 ± 1.54	3.15	96.33 ± 1.50	4.77	65.16 ± 1.32	35.59	93.16 ± 1.47	5.74
14	94.00 ± 0.89	6.15	92.33 ± 1.03	8.73	50.50 ± 1.37	50.08	66.16 ± 2.99	33.06
20	91.16 ± 0.98	8.99	87.83 ± 1.94	13.18	19.16 ± 2.85	81.06	40.66 ± 3.07	58.86

Excision wound on test animals (Day of excision)

Excision wound on test animals (Day 20)

Figure 2: Images of wound healing activity

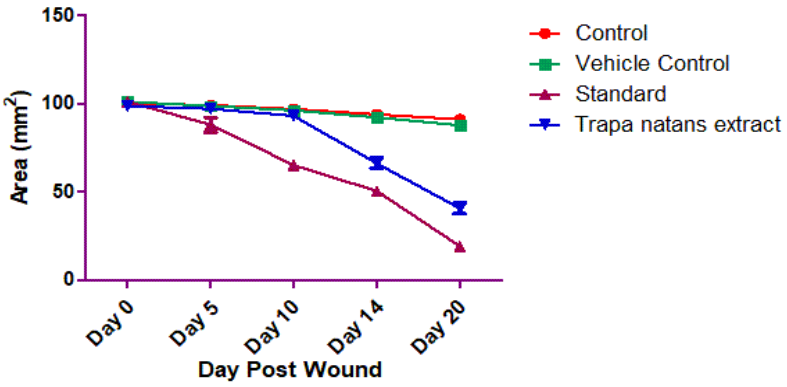

Figure 3: Wound healing efficacy of Trapa natans by in vivo excision model

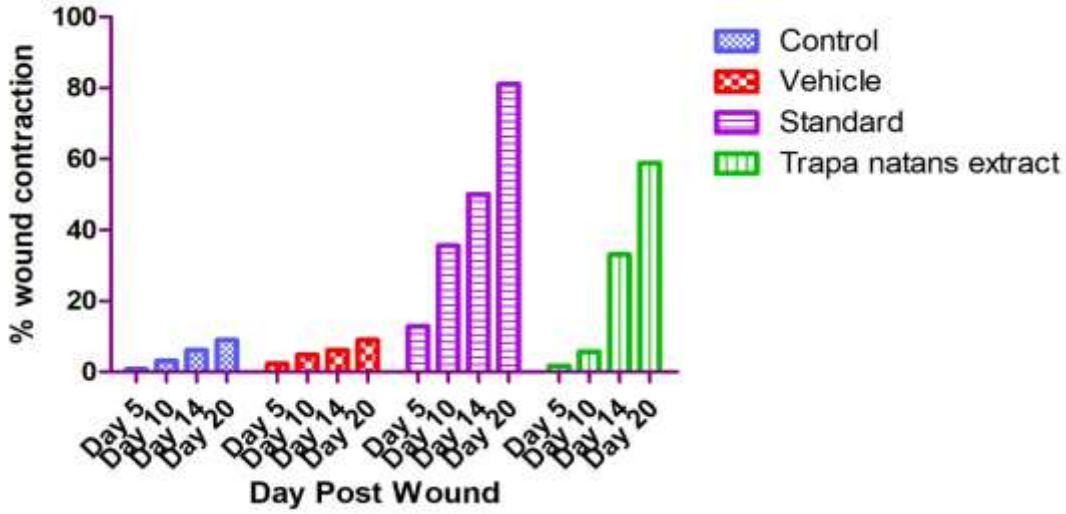

Figure 4: Wound healing efficacy of Trapa natans by in vivo excision model

Conclusion

The present study demonstrates that the methanolic extract of Trapa natans peel is rich in bioactive phytochemicals, including phenolics, flavonoids, tannins, alkaloids, and saponins, which are known to possess antioxidant, anti-inflammatory, and antimicrobial properties. Acute toxicity studies confirmed its safety at high doses, and topical application of the extract significantly enhanced wound contraction in the excision wound model, indicating a promising wound-healing potential. These findings suggest that Trapa natans peel extract could serve as a natural therapeutic agent for accelerating wound repair. Further studies, including histopathological and mechanistic investigations, are warranted to elucidate the molecular pathways underlying its wound-healing effects.

References

1. Shedoeva A, Leavesley D, Upton Z, Fan C. Wound healing and the use of medicinal plants. Evidence-Based Complementary and Alternative Medicine. 2019;2019:2684108.
2. Zhao R, Liang H, Clarke E, Jackson C, Xue M. Inflammation in chronic wounds. Int J Mol Sci. 2016;17(12):2085.
3. Pathak D, Mazumder A. A critical overview of challenging roles of medicinal plants in improvement of wound healing technology. DARU J Pharm Sci. 2024;32(1):379–419.
4. Palm N, Franzaring J, Schweiger AH. International Biological Flora: Trapa natans†: No. 5. J Ecol. 2024;112(10):2386–420.
5. Banu ZW, Dasgupta D, Hazarika I, Laloo D, Kalita JM. Trapa natans L.: A Journey from Traditional to Contemporary Therapies-A Review. Nat Prod J. 2023;13(8):122–34.
6. Ansari AP, Anwar N, Ahmed NZ, Makbul SA. Phytochemistry and Pharmacology of Aquatic Plants Used in Unani System of Medicine. In: Crude Drugs of Unani Medicine. Apple Academic Press; 2026. p. 231–79.
7. Pei J, Kanwal S, Sivaramakrishnan R, Katelakha K. Therapeutic potential of microalgae-derived natural compounds in diabetic wound healing: A comprehensive review. Heliyon. 2025;11(4).
8. Rutkowska M, Witek M, Olszewska MA. A Comprehensive Review of Molecular Mechanisms, Pharmacokinetics, Toxicology and Plant Sources of Juglanin: Current Landscape and Future Perspectives. Int J Mol Sci. 2024;25(19):10323.
9. Baraiya P, Chauhan P. Investigation of antioxidant potential of Petunia hybrida leaf extracts. Journal of Pharmacology and Biomedicine. 2021; 5(4): 415-421

10. Arora P, Arora V. Preliminary phytochemical screening of crude drugs In: A Textbook of Herbal Drug Technology, Pee Vee Books, Punjab 2019, pp 179-180
11. Tiwari P, Joshi A, Dubey BK. (2017). Total phenolic content, flavonoid concentration, antimicrobial and insecticidal screening of aqueous extracts of Annona squamosa (seeds), Azadirachta indica (leaves) and Lavandula angustifolia (flower). J Pharmacol Biomed, 1(1), 30-43.
12. Sharma T, Gamit R, Acharya R, Shukla VJ. (2023). Quantitative estimation of total tannin, alkaloid, phenolic, and flavonoid content of the root, leaf, and whole plant of Byttneria herbacea Roxb. Ayu, 42, 143-147.
13. https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl423.pdf; assessed on 17/05/2021
14. Gaur R, Azizi M, Gan J., Hansal P, Harper K, Mannan R, Panchal A, Patel K, Patel MK, Patel N, Rana J, Rogowska A. (2009). Simple ointment: Formulated preparations. British Pharmacopoeia. 3.
15. Morton, J.J.P, Malone, M.H. Evaluation of vulnerary activity by an open wound procedure in rats. Arch. Int. Pharmacodyn 1972, 196: 117-126.