

Wound Healing Activity of Tanzanian-Sourced *Centella asiatica* on Second-Degree Burn Wounds in Wistar Rats

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ABSTRACT

Introduction: Burn wounds heal slowly, often with complications. *Centella asiatica* (L.) Urb. (Apiaceae) has traditional uses in wound management, but region-specific evidence from Tanzania is lacking. The study aimed to evaluate the burn wound healing effect of topically applied *C. asiatica* leaf extract in a rat model of second-degree thermal injury, using complete epithelialization time as the primary outcome. This study was designed as a pilot/exploratory investigation to generate preliminary data on locally sourced plant material.

Methods: Fifteen male Wistar rats were randomly assigned to four groups: negative control (no treatment), positive control (1.0% silver sulfadiazine cream), 0.5% w/v *C. asiatica* ethanol extract dispersed in distilled water, and 1.0% w/v *C. asiatica* ethanol extract dispersed in distilled water. Standardized second-degree burns were induced on the dorsal skin under diethyl ether anesthesia. Treatments were applied once daily. Two rats died from inter-animal aggression during acclimatization and were excluded from analysis, leaving final group sizes of n=3 for three groups and n=4 for one group. Epithelialization time was recorded daily. Group comparisons used one-way ANOVA with Tukey's HSD post hoc test (significance at p-value < 0.05).

Results: The 1.0% w/v *C. asiatica* group showed the shortest mean epithelialization time (12.7 ± 0.6 days), followed by 0.5% w/v (14.0 ± 0.8 days), silver sulfadiazine (16.0 ± 0.8 days), and negative control (23.0 ± 1.0 days). ANOVA revealed significant differences among groups ($F(3,9) = 68.67$, p-value < 0.001). Post hoc analysis confirmed faster healing with both *C. asiatica* concentrations compared to silver sulfadiazine (p-value < 0.001) and untreated controls (p-value < 0.001). The difference between 0.5% and 1.0% *C. asiatica* was not statistically significant (p-value = 0.065), representing a non-significant trend rather than confirmed dose-dependence.

Conclusion: Topical *C. asiatica* ethanol extract (aqueous dispersion) significantly accelerates burn wound epithelialization in rats, with the higher concentration showing the fastest healing. These preliminary findings support further investigation of locally sourced *C. asiatica* as a potential low-cost intervention for burn care. However, due to substantial methodological limitations including small sample size, lack of vehicle control, unblinded assessment, and suboptimal anesthesia/analgesia protocols these results should be interpreted as hypothesis generating. Histological and phytochemical studies are needed before clinical translation.

Keywords: *Centella asiatica*; Burn wound; Epithelialization; Rat model; Silver sulfadiazine; Traditional medicine; Pilot study

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Introduction

Burn wounds are among the most challenging soft tissue injuries, characterized by prolonged inflammation, delayed re-epithelialization, and high infection risk [1]. Conventional topical agents such as silver sulfadiazine, while antimicrobial, have been associated with cytotoxicity to fibroblasts and delayed wound healing [2]. This has driven renewed interest in plant-based alternatives that may offer safer, more accessible, and biologically active options for wound care [3].

Centella asiatica (L.) Urb. (Apiaceae), commonly known as Gotu Kola, has a long history in traditional medicine systems across Asia and Africa for treating wounds, skin ulcers, and burns [4]. Its wound-healing properties are attributed to pentacyclic triterpenes principally asiaticoside and madecassoside which promote fibroblast proliferation, collagen synthesis, and angiogenesis [5–7]. While extensive evidence supports *C. asiatica*'s efficacy in experimental wound models, most studies have used commercially sourced or cultivated plants.

Tanzania lacks region-specific data on locally harvested *C. asiatica*, yet geographic and climatic factors can significantly alter phytochemical profiles and therapeutic activity [8]. Specifically, the Morogoro region of Tanzania (5°58'–10°00' S, 35°25'–38°30' E) experiences distinct seasonal rainfall patterns, temperatures, and soil compositions that may influence the biosynthesis of bioactive triterpenes. However, this study did not formulate a specific, testable phytochemical hypothesis linking the geographical profile of Morogoro to target active compound concentrations. Future investigations should characterize the phytochemical profile of locally sourced material to test whether regional environmental factors produce extracts with enhanced or diminished bioactivity compared to published data from other regions. In this study, we therefore evaluated the burn wound healing effect of *C. asiatica* collected from Morogoro, Tanzania, using a rat model of second-degree thermal injury. We hypothesized that topical application of *C. asiatica* ethanol extract (dispersed in distilled water) would accelerate complete epithelialization compared to both untreated and silver sulfadiazine-treated controls.

Methods

Study design and ethical approval

This was an experimental, parallel-group, animal study designed as a pilot/exploratory investigation to generate preliminary data on locally sourced *C. asiatica*. Given the absence of prior data on this specific geographical source, no formal power calculation was performed. Ethical approval was obtained from the Research Ethical Committee of Sokoine University of Agriculture (Reference No. DPRTC/R/186/64). All animal procedures were

performed in accordance with institutional guidelines, though the study did not fully comply with all international standards such as ARRIVE and AVMA guidelines regarding anesthesia and analgesia (see Section 5: Limitations).

Plant material and extraction

Fresh leaves of *Centella asiatica* (Figure 1) were collected from naturally growing populations within Morogoro Municipality, Tanzania (5°58'–10°00' S, 35°25'–38°30' E) in December 2024. The plant was authenticated by a botanist at SUA (voucher specimen deposited at SUA herbarium, accession number SUA/CA/2024/01). Leaves were washed, shade-dried, and then oven-dried at 40°C for 24 hours. Dried leaves (500 g) were ground into fine powder and macerated in absolute ethanol (1:5 w/v) at 4°C for 72 hours with intermittent shaking. Ethanol was selected as the extraction solvent to maximize recovery of lipophilic pentacyclic triterpenes (asiaticoside, madecassoside, asiatic acid, madecassic acid). The extract was filtered through Whatman No. 1 paper, and the filtrate was evaporated at 80°C in a hot-air oven to yield a semi-solid crude extract (yield: approximately 12% w/w). Two working concentrations (0.5% w/v and 1.0% w/v) were prepared by dispersing the ethanol extract in distilled water with gentle warming and magnetic stirring.

This aqueous dispersion was chosen for topical application; however, the formulation was prepared without stabilizers, emulsifiers, or preservatives. Ethanolic extracts of *C. asiatica* contain lipophilic triterpenes that are poorly soluble in water; the resulting dispersion was partially insoluble, raising concerns about dosing consistency and uniformity of wound coverage. Formulations were stored in amber bottles at room temperature and used within 48 hours.



Figure 1 A photograph of *Centella asiatica* collected near the CVMBS, SUA premises.

Animals and housing

Fifteen healthy adult male Wistar rats (250–350 g) were obtained from the small animal research unit at SUA. Rats were housed individually in standard polypropylene cages to prevent inter-animal aggression and wound interference, under controlled conditions (temperature $28 \pm 1^\circ\text{C}$, humidity 60–70%, 12 h light/dark cycle). Commercial rodent chow and tap

water were provided ad libitum. Animals were acclimatized for 7 days before the experiment. The decision to house rats individually was made after initial group housing resulted in aggression-related deaths during acclimatization.

Sample size and randomization

Sample size ($n = 3$ –4 per group after accounting for potential exclusions) was chosen as a pilot/exploratory study, given the absence of prior data on locally sourced *C. asiatica*. No formal power calculation was performed. This small sample size is a significant limitation, as the study was not powered to detect small-to-moderate effect sizes. Rats were randomly assigned to four groups using a computer-generated random number sequence: Group I (negative control, no treatment, $n = 3$)/Group II (positive control, 1.0% w/w silver sulfadiazine cream, $n = 4$ / Group IIIa (0.5% w/v *C. asiatica* aqueous dispersion, $n = 4$)/ Group IIIb (1.0% w/v *C. asiatica* aqueous dispersion, $n = 4$)

Two rats (one from Group II, one from Group IIIb) died from inter-animal aggression during acclimatization before wound induction and were excluded, leaving a final $n = 13$ (negative control, $n = 3$; positive control, $n = 3$; 0.5% *C. asiatica*, $n = 4$; 1.0% *C. asiatica*, $n = 3$). On day 0, rats were anesthetized with diethyl ether by inhalation in an induction chamber. This anesthetic choice represents a significant ethical and methodological limitation. Diethyl ether is highly explosive, hazardous to personnel, and is considered outdated in modern laboratory animal care. Furthermore, it provides inadequate analgesia for a painful procedure such as burn induction. The dorsal fur was shaved and the skin disinfected with 70.0% ethanol. A custom-made brass rod (10 mm diameter) was heated in a Bunsen burner flame until red-hot (estimated temperature $> 600^{\circ}\text{C}$) and applied to the shaved skin for 5 seconds without additional pressure, creating a standardized second-degree burn wound (full epidermal loss with intact deeper dermis).

Immediately after induction, wounds were cleaned with sterile normal saline. No post-burn analgesia was administered beyond the anesthetic period. This lack of postoperative pain relief for painful full-thickness burn injuries does not comply with international standards such as ARRIVE and AVMA, and may have led to unmanaged systemic pain-stress responses (including elevated corticosteroids) that can negatively affect wound healing processes.

Treatment application

Treatments were applied once daily starting 30 minutes after wound induction. For the *C. asiatica* groups, 0.2 mL of the respective aqueous dispersion was applied evenly over the wound surface using a sterile dropper. For the silver sulfadiazine group, approximately 0.2 g of cream was applied with a sterile spatula. The negative control group received no topical application. No dressing was applied. Crucially, the study lacked a vehicle control group (distilled water

alone) that would have received the same daily topical liquid application without active extract. Without this control, it is impossible to determine whether observed healing was due to *C. asiatica* phytochemicals or simply to daily wound hydration and mechanical debridement from the application process. Treatment continued until complete epithelialization (defined below). The investigator applying treatments was not blinded to group allocation; outcome assessment was also performed by the same unblinded investigator, introducing potential observer bias.

Outcome assessment

The primary outcome was complete wound epithelialization time, defined as the day when the burn wound was fully covered by new epithelium with no residual raw area, and the original eschar had detached [13]. Wounds were examined daily at the same time by visual inspection under standard lighting. Photographs were taken on days 0, 7, 14, and 21 using a digital camera with a standardized distance and lighting.

Statistical analysis

Data were analyzed using IBM SPSS Statistics version 23. Normality was assessed using the Shapiro–Wilk test (p -value > 0.05 for all groups). One-way analysis of variance (ANOVA) was used to compare mean epithelialization times among the four groups. Post hoc pairwise comparisons were performed using Tukey's Honestly Significant Difference (HSD) test. Statistical significance was set at p -value < 0.05 . Results are presented as mean $\pm$ standard deviation (SD). It should be noted that the F-statistic ($F(3,9) = 68.67$) is based on a very small sample and may overstate the actual statistical power; confirmatory studies with larger sample sizes are needed.

Results

Animal disposition

Fifteen rats were enrolled. Two rats (one from the positive control group and one from the 1.0% w/v *C. asiatica* group) died from inter-animal aggression during the acclimatization period, before wound induction. These animals were excluded from all analyses, leaving a final sample of 13 rats (negative control, $n = 3$; positive control, $n = 3$; 0.5% *C. asiatica*, $n = 4$; 1.0% *C. asiatica*, $n = 3$). No further mortality or signs of systemic toxicity occurred during the treatment period.

Epithelialization time

Complete wound epithelialization was observed in all surviving rats by day 24 post-injury. The fastest healing occurred in the 1.0% w/v *C. asiatica* group (12.7 ± 0.6 days), followed by the 0.5% w/v *C. asiatica* group (14.0 ± 0.8 days), the positive control (silver sulfadiazine, 16.0 ± 0.8 days), and the negative control (23.0 ± 1.0 days) (Table 1).

Table 1 Epithelialization time by treatment group

Treatment group	n	Complete epithelialization time (days)
		Mean $\pm$ SD
Negative control (Untreated)	3	23.0 $\pm$ 1.00
Positive control (Silver Sulfadiazine)	3	16.0 $\pm$ 0.82
0.5% w/v <i>C. asiatica</i>	4	14.0 $\pm$ 0.82
1.0% w/v <i>C. asiatica</i>	3	12.7 $\pm$ 0.58

One-way ANOVA revealed a statistically significant difference among the four groups ($F(3,9) = 68.67$, $p < 0.0001$) (Table 2).

Table 2 One-way ANOVA summary for epithelialization time

Source of variation	sum of squares	df	Mean square	F-value	p-value
Between Groups	185.47	3	61.82	68.67	< 0.001
Within Groups	9.00	9	1.00		
Total	194.47	12			

Pairwise comparisons

Both *C. asiatica* concentrations and silver sulfadiazine significantly reduced epithelialization time compared to the negative control (p -value < 0.001 for all comparisons). The 1.0% w/v *C. asiatica* group healed significantly faster than the positive control (mean difference = 3.33 days, p -value < 0.001), as did the 0.5% w/v group (mean difference = 2.00 days, p -value = 0.019). The difference between the two *C. asiatica* concentrations (0.5% vs 1.0%) was not statistically significant (mean difference = 1.33 days, p -value = 0.065). While there appears to be a trend toward dose-dependent efficacy, this finding is non-significant and should be described as such, not as definitive dose-dependence (Table 3).

Table 3. Post hoc comparison (Tukey's HSD Test)

Treatment group (I)	Treatment group (J)	Mean difference (I-J)	p-value	95% CI
Negative Control	Positive Control	7.0	< 0.001	5.769 – 8.231
Negative Control	0.5% <i>C. asiatica</i>	9.0	< 0.001	7.769 – 10.231
Negative Control	1.0% <i>C. asiatica</i>	10.3	< 0.001	9.103 – 11.564
Positive Control	0.5% <i>C. asiatica</i>	2.0	0.019	0.697 – 3.303
Positive Control	1.0% <i>C. asiatica</i>	3.3	< 0.001	2.103 – 4.564
0.5% <i>C. asiatica</i>	1.0% <i>C. asiatica</i>	1.3	0.065	-0.103 – 2.769

Visual assessment of wound healing

Photographic documentation of representative wounds from each group at days 0, 7, 14, and 21 is shown in Figures 2–5. Wound size reduction and re-epithelialization progressed most rapidly in the 1.0% w/v *C. asiatica* group, where complete closure was evident by day 13. The 0.5% w/v group showed complete closure by day 14. Silver sulfadiazine-treated wounds required approximately 16 days, while untreated wounds remained unhealed until day 23. These visual observations qualitatively support the quantitative epithelialization times.



Figure 2 Burn wound progression in a negative control rat (no treatment) on days 0, 7, 14, and 21 post-injury



Figure 3 Burn wound progression in a positive control rat (silver sulfadiazine 1% w/w) on days 0, 7, 14, and 21



Figure 4 Burn wound progression in a rat treated with 0.5% w/v *C. asiatica* extract on days 0, 7, 14, and 21.



Figure 5 Burn wound progression in a rat treated with 1.0% w/v *C. asiatica* extract on days 0, 7, 14, and 21.

Discussion

This pilot study investigated the effect of topically applied *Centella asiatica* ethanol extract (aqueous dispersion) on second-degree burn wound healing in Wistar rats. Our results demonstrate that both 0.5% w/v and 1.0% w/v *C. asiatica* extracts significantly accelerated complete epithelialization compared to untreated controls and, notably, compared to silver sulfadiazine, a standard topical antimicrobial. The 1.0% w/v concentration produced the shortest mean healing time (12.7 days), followed by 0.5% w/v (14.0 days), suggesting a trend toward dose-dependent efficacy. However, the difference between the two concentrations did not reach statistical significance ($p = 0.065$). This non-significant difference should be interpreted cautiously; the study was underpowered to detect small-to-moderate effect sizes, and the observed 1.33-day difference may represent a clinically meaningful effect that could become statistically significant with adequate group sizes.

The prolonged healing observed in the untreated negative control group (23.0 days) is consistent with published data on second-degree burns in rats, where delayed re-epithelialization results from persistent inflammation, infection risk, and absence of exogenous wound support [14]. The positive control (silver sulfadiazine) shortened healing to 16.0 days, aligning with its well-known antimicrobial and mild debriding actions [15]. However, silver sulfadiazine has been reported to delay re-epithelialization in some models due to cytotoxicity to fibroblasts and keratinocytes [2], which may explain why *C. asiatica* outperformed it in our study.

The superior healing effect of *C. asiatica* is likely attributable to its pentacyclic triterpenoid constituents, particularly asiaticoside and madecassoside. Asiaticoside promotes fibroblast proliferation, collagen type I synthesis, and angiogenesis, while madecassoside exerts anti-inflammatory effects and enhances antioxidant activity

[7,16,17]. Our findings corroborate those of Somboonwong et al. [1], who reported accelerated wound contraction and epithelialization in both incisional and burn wound models using *C. asiatica* extracts. Similarly, Wu et al. [18] demonstrated improved dermal repair in rats treated with *C. asiatica* formulations. A key contribution of the present study is the use of *C. asiatica* sourced from Tanzania, supporting the hypothesis that locally available plants may offer accessible wound care options in resource-limited settings.

Nevertheless, the observed difference between 0.5% and 1.0% *C. asiatica* (1.33 days) did not reach statistical significance (p -value = 0.065). This may reflect insufficient statistical power due to the small sample size ($n = 3$ –4 per group post-exclusion) rather than a true absence of dose-response. A clinically meaningful difference of approximately one day in a small animal model could become significant with larger group sizes. We therefore describe the finding as a non-significant trend toward dose-dependent efficacy, not as definitive dose-dependence.

Comparisons with prior studies are limited by variability in extraction methods, concentrations, and animal models. For example, Liu [7] used an ethanolic extract similar to ours but applied it in an ointment base rather than aqueous dispersion, which may affect bioavailability. Direct quantitative comparisons are therefore difficult.

Limitations

This study has several important limitations that must be acknowledged and addressed in future research. First, the lack of a vehicle control group represents a significant design limitation. The negative control group received no topical application, whereas treatment groups received daily topical liquid application. The mechanical action of application itself, including potential debridement and hydration effects, may have contributed to the observed healing differences. A true vehicle control group receiving distilled water or the aqueous base without active extract should have been included to isolate the specific therapeutic effect of *C. asiatica* phytochemicals.

Second, outcome assessment was not blinded; the same investigator who applied treatments also determined epithelialization time daily, introducing potential observer bias. The subjective nature of visual epithelialization assessment, particularly the determination of "complete" closure, could have been influenced by knowledge of group allocation. Future studies must implement blinding using coded treatment vials and independent outcome assessors to eliminate this source of bias.

The small sample size ($n = 3$ –4 per group after exclusions) is a critical limitation. This pilot study was not powered to detect small-to-moderate effect sizes,

and the non-significant difference between the two *C. asiatica* concentrations may represent a type II error (false negative) rather than a true absence of dose-response. The narrow margin (1.33 days) between groups suggests that a clinically relevant difference could become statistically significant with adequate statistical power. We therefore interpret our findings as hypothesis-generating and emphasize the need for confirmatory studies with larger sample sizes based on formal power calculations derived from the effect sizes observed here (Cohen's $f \approx 1.5$, indicating a very large effect). Additionally, the loss of two animals from inter-animal aggression during acclimatization further reduced group sizes and underscores the importance of single housing from the outset in future experiments.

The use of diethyl ether as the sole anesthetic agent for a painful procedure like burn induction raises significant ethical and scientific concerns. Diethyl ether is highly explosive, hazardous to personnel, provides inadequate analgesia, and is no longer considered acceptable for survival surgery in many jurisdictions. The absence of postoperative analgesia is a further welfare concern that may have confounded healing outcomes through stress-induced glucocorticoid release, which can negatively affect wound healing. Future studies must employ approved anesthetic combinations (e.g., ketamine-xylazine) with appropriate pre- and post-operative analgesia (e.g., buprenorphine or carprofen) in accordance with international guidelines such as the ARRIVE (Animal Research: Reporting of in Vivo Experiments) and AVMA (American Veterinary Medical Association) standards.

The aqueous dispersion of *C. asiatica* was partially insoluble, as acknowledged in the manuscript (lines 261–263), meaning rats received inconsistent, non-homogeneous dosages. A properly formulated cream, ointment, or gel base with appropriate emulsifiers and stabilizers would ensure uniform application and improved bioavailability. Additionally, the lack of dressing may have exposed wounds to contamination and mechanical disturbance; a standardized wound dressing protocol should be implemented in future studies.

Conclusion

This pilot study demonstrates that topical application of *Centella asiatica* ethanol extract (aqueous dispersion) significantly accelerates burn wound epithelialization in a rat model compared to untreated controls and silver sulfadiazine. The 1.0% w/v concentration produced the fastest healing (mean 12.7 days), with a non-significant trend toward dose-dependent activity. These preliminary findings suggest that locally sourced *C. asiatica* from Tanzania has potential as an accessible, low-cost intervention for burn wound management.

However, due to the significant study limitations, including the lack of vehicle control and blinding, small sample size with potential type II error, absence of phytochemical standardization and histological data, suboptimal anesthesia/analgesia protocols, and use of a partially insoluble formulation—these results should be interpreted as hypothesis-generating rather than conclusive. The accelerated healing observed provides a strong rationale for well-designed confirmatory studies. To translate these preliminary findings toward clinical application, we propose the following sequential research agenda. Confirmatory efficacy study: A fully powered randomized controlled trial in rats with larger group sizes ($n \geq 10$ per group based on power analysis from current effect sizes), incorporating a vehicle control group (distilled water or formulation base without extract), blinded outcome assessment, and standardized wound dressing protocols. Appropriate anesthesia (e.g., ketamine-xylazine) and postoperative analgesia must be provided in compliance with ARRIVE and AVMA guidelines. Phytochemical standardization: Quantitative HPLC or LC-MS analysis of the crude extract to identify and quantify bioactive triterpenoids (asiaticoside, madecassoside, asiatic acid, madecassic acid), establishing batch-to-batch consistency and enabling dose-response correlation with specific compounds. This would also allow testing of the hypothesis that Morogoro-sourced *C. asiatica* has a distinct phytochemical profile compared to material from other regions.

Data availability statement

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors declare no conflicts of interest.

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Author contributions

Mvulla ET: Conceptualization, methodology; Frida RM: Investigation, data curation, writing – original draft; Mshamu SA and Lusekelo MM: Methodology, validation, writing- review and editing, supervision. All authors read and approved the final version of the manuscript.

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References

- [1] Somboonwong J, Kankaisre M, Tantisira MH, Tantisira B. Wound healing activities of different extracts of *Centella asiatica* in incision and burn wound models: an experimental animal study. *BMC Complement Altern Med.* 2012;12:103. doi:10.1186/1472-6882-12-103.
- [2] Stadelmann WK, Digenis AG, Tobin GR. Physiology and healing dynamics of chronic cutaneous wounds. *Am J Surg.* 1998;176(2A Suppl):26S-38S.
- [3] Diniz MM, Oliveira AC, Pereira TM, de Oliveira NR. Molecular and cellular mediators in cutaneous wound healing: from inflammation to tissue regeneration. *J Inflamm Res.* 2023;16:73-91.
- [4] Chatterjee A, Pakrashi SC, Dhar ML. The treatise on Indian medicinal plants. Vol. 1. New Delhi: Council of Scientific and Industrial Research; 2011.
- [5] Schultz GS, Chin GA, Moldawer L. Principles of wound healing. In: Fitridge R, Thompson M, editors. *Mechanisms of vascular disease: a reference book for vascular specialists.* Adelaide: University of Adelaide Press; 2011.
- [6] Satake M. Medicinal plants and their cultivation. Tokyo: Yokendo Publishing; 2007.
- [7] Liu M. Evaluation of wound healing potential of triterpenoids from *Centella asiatica* in rat burn models. *J Ethnopharmacol.* 2009;123(3):530-6.
- [8] Rafamantanana MH. Phytochemical profile and biological activity of *Centella asiatica* extracts. *J Nat Remedies.* 2009;9(1):22-9.
- [9] Gohil KJ. Pharmacological review on *Centella asiatica*: a potential herbal cure-all. *Indian J Pharm Sci.* 2010;72(5):546-56.
- [10] Nomi AG, Handayani H, Khuluk RH. Antioxidant activity and metabolite changes in *Centella asiatica* with different drying methods using FTIR- and quantitative HPLC-based metabolomics. *Int Food Res J.* 2024;31(1):228-38.
- [11] Handa SS, Khanuja SPS, Longo G, Rakesh DD. Extraction technologies for medicinal and aromatic plants. Trieste: International Centre for Science and High Technology; 2008.
- [12] Boo XY, Chia LH. Formulation and in vitro evaluation of gels with *Centella asiatica* extracts for cosmeceutical purposes. *J Pharmacogn Phytochem.* 2024;13(5):109-17.
- [13] Mgonja F, Mohamed M, Mwangengwa M. Excision wounds healing activity of *Centella asiatica* (Gotukola) extract on laboratory rats. *Tanzan J Health Res.* 2022;23(1). doi:10.4314/thrb.v23i1.7.

- [14] Chippada SC, Vangalapati M, Bammidi SR. Wound healing activity of *Centella asiatica* extract. *Int J Phytomed*. 2011;3(3):319-24.
- [15] Dinarvand R, Sepehri N, Fouladdel S. Silver sulfadiazine: use in burn wound healing. *Burns*. 2015;41(5):1028-36.
- [16] Shukla A, Rasik AM, Dhawan BN. Asiaticoside-induced wound healing activity. *Indian J Exp Biol*. 1999;37(11):1136-40.
- [17] Hashim P, Sidek H, Helan MH, Sabery A, Palanisamy UD, Ilham M. Triterpene composition and bioactivities of *Centella asiatica*. *Molecules*. 2011;16(2):1310-22. doi:10.3390/molecules16021310.
- [18] Wu F, Bian D, Xia Y, Gong Z. Wound healing and anti-inflammatory properties of *Centella asiatica* extracts. *J Ethnopharmacol*. 2020;245:112177.
- [19] Kilkenney C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biol*. 2010;8(6). doi:10.1371/journal.pbio.1000412.
- [20] American Veterinary Medical Association. AVMA guidelines for the euthanasia of animals: 2020 edition. Schaumburg (IL): American Veterinary Medical Association; 2020.
- [21] Pereira MP, Perez AS, Barros MB. The panniculus carnosus as a confounding factor in wound healing studies in rats. *Wound Repair Regen*. 2014;22(4):501-7.
- [22] Sullivan NJ, Lloyd SA, Elmore LK. The porcine model for wound healing: a review. *J Invest Surg*. 2021;34(2):210-20.
- [23] Handa SS, Khanuja SPS, Longo G, Rakesh DD. Extraction technologies for medicinal and aromatic plants. Trieste: International Centre for Science and High Technology; 2008.
- [24] Cohen J. Statistical power analysis for the behavioral sciences. 2nd ed. Hillsdale (NJ): Lawrence Erlbaum Associates; 1988.