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Formulation and Evaluation of Nanostructured Lipid Carrier (NLC) Based Gel of Leucas Aspera Extract for Wound Healing Activity: A Research

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ABSTRACT:

Wound healing is a complex biological process that involves inflammation, tissue regeneration, and remodelling. Delayed wound healing due to microbial infection and oxidative stress remains a significant clinical challenge. *Leucas aspera*, a medicinal herb widely used in traditional medicine, possesses antimicrobial, anti-inflammatory, antioxidant, and wound healing properties owing to its rich phytoconstituents. However, the therapeutic efficacy of its extract may be limited by poor skin penetration and low bioavailability. Nanostructured Lipid Carriers (NLCs) are advanced lipid-based nanocarriers that enhance drug stability, improve skin permeation, and provide controlled release of bioactive compounds. The present study aims to formulate and evaluate an NLC-based gel containing *Leucas aspera* extract for topical wound healing application. The NLC dispersion will be prepared using glyceryl monostearate, oleic acid, and Tween 80 by the hot homogenization followed by ultrasonication method and subsequently incorporated into a Carbopol 984 gel base. The prepared formulation will be evaluated for particle size, polydispersity index, zeta potential, entrapment efficiency, pH, viscosity, spread ability, drug content, in vitro drug release, stability, and wound healing activity. The developed NLC gel is expected to provide sustained release, enhanced skin penetration, improved therapeutic efficacy, and accelerated wound healing compared to conventional herbal gel formulations. This study may offer a promising herbal nanocarrier-based topical formulation for effective wound management.

Keywords: *Leucas aspera*, Nanostructured Lipid Carrier (NLC), Wound Healing, Topical Drug Delivery, Herbal Gel, Skin Permeation, Controlled Drug Release.

1.INTRODUCTION:

Wound healing is a complex biological process involving four phases: haemostasis, inflammation, proliferation, and remodelling. Excessive inflammation delays healing; therefore, anti-inflammatory agents are important for effective wound repair.

Conventional therapies such as corticosteroids and NSAIDs are effective but may cause systemic side effects with long-term use, creating a need for safer plant-based alternatives.

Leucas aspera (Thumbai), a medicinal plant of the Lamiaceae family, possesses anti-inflammatory, antimicrobial, antioxidant, and wound-healing properties, making it a promising natural therapeutic agent.

Baicalin, a flavonoid from *Scutellaria radix*, exhibits antioxidant, antibacterial, anti-inflammatory, and antidiabetic activities. However, its poor water solubility and low bioavailability limit its therapeutic effectiveness.

Nanostructured Lipid Carriers (NLCs) improve baicalin stability, drug loading, controlled release, skin penetration, and wound-site retention. Incorporating NLCs into a topical gel enhances local drug delivery and accelerates wound healing while minimizing systemic side effects.

1.1 Plant Profile:

1.1.1 *Leucas Aspera*

S.No	Category	Classification
1	Kingdom	plantae
2	Family	Lamiaceae
3	Genus	Leucas
4	Species	Leucas Aspera
5	Common Name	Thumbai/Leucas
6	Tamil Name	தும்பை

Table 1: Taxonomic Classification of *Leucas aspera*

Leucas aspera (Thumbai) is an annual medicinal herb belonging to the family Lamiaceae. It is widely distributed throughout India and other tropical regions. The plant has white flowers and is traditionally used in Ayurveda and Siddha medicine for the treatment of wounds, skin diseases, fever, inflammation, insect bites, and infections. It contains several bioactive constituents such as flavonoids, alkaloids, terpenoids, tannins, saponins, phenolic compounds, and essential oils, which exhibit antioxidant, antimicrobial, anti-inflammatory, and wound-healing activities. These pharmacological properties make *Leucas aspera* a promising medicinal plant for topical wound-healing formulations.



Figure: 1 *Leucas aspera*

1.2 Acute Wound:

An acute wound is a wound that heals through the normal stages of wound healing within an expected period, usually 4–6 weeks. It is caused by surgery, trauma, burns, cuts, or abrasions and progresses through the four healing phases: hemostasis, inflammation, proliferation, and remodeling. Acute wounds generally heal completely without complications when proper treatment is provided.

Examples: Surgical incisions, Cuts (lacerations), Abrasions, Burns, Puncture wounds.

1.3 Nanostructured Lipid Carrier (NLC) Gel:

Nanostructured Lipid Carrier (NLC) gel is an advanced topical drug delivery system in which drug-loaded nanostructured lipid carriers are incorporated into a gel base. NLCs are composed of a mixture of solid lipids and liquid lipids, which improve drug loading, stability, and controlled drug release. Incorporating NLCs into a gel enhances skin adhesion, hydration, and localized drug delivery, making it highly suitable for wound healing and other topical applications.

1.3.1 Ideal Properties of NLC Gel:

- Should have high drug loading capacity.
- Should provide controlled and sustained drug release.
- Should possess good physical and chemical stability.
- Should have small, uniform particle size with low polydispersity.
- Should enhance drug penetration and bioavailability.
- Should maintain a skin-friendly pH (around 5.5–7.0).

1.3.2 Advantages of NLC Gel:

- Improves drug stability and protects the drug from degradation.
- Provides sustained and controlled drug release.
- Enhances skin penetration and bioavailability.
- Maintains a moist wound environment and promotes faster healing.
- Reduces systemic side effects by delivering the drug directly to the wound site.
- Suitable for both hydrophilic and lipophilic drugs.

1.3.3 Disadvantages of NLC Gel:

- Physical instability during long-term storage (particle aggregation or gel separation).
- Requires specialized equipment and optimized formulation methods for preparation.
- Higher production cost compared with conventional gels.
- Risk of microbial contamination if preservatives are inadequate.
- Some lipids or surfactants may cause skin irritation or allergic reactions in sensitive individuals.

2.MATERIALS AND METHODS:

2.1 Plant Collection and Authentication

The fresh leaves of leucas aspera plant were collected from local vendors in Kanchipuram district, Tamilnadu, India in the month of July 2026. The plant material was identified and authenticated by central council for research in siddha, Chennai, ministry of AYUSH, government of India, on 03.07.2026

2.2 Preparation of Plant Extract

The collected leucas aspera plant were washed with water to remove dust and debris. Then they were dried at room temperature.

The dried leucas aspera plant were made into a powder using a grinder. Passed through a sieve.

The leucas aspera powder was packed into a container. Extracted with ethanol in a Soxhlet apparatus.

The leucas aspera extract was then concentrated and dried to get a -solid extract, which was stored in a container at a cool temperature until it was used again.

2.3 Preliminary Phytochemical Screening

The ethanolic extract of Leucas aspra plant was subjected to qualitative chemical tests using standard procedures to identify the presences of Alkaloids, Glycosides , Terpenoids , Flavonoids , Steroids, Tannins, Saponin.

2.4 Excipients profile

S.No	Ingredient	use
1	Leucas aspera	Wound healing and anti- inflammatory
2	Glyceryl monostearate	Solid lipid
3	Oleic acid	Liquid lipid
4	Tween 80	Surfactant
5	Carbopol 984	Gelling agent
6	Triethanolamine	pH adjuster
7	Methyl paraben sodium	Preservative
8	Propylene glycol	Humectant

Table: 2 Excipients uses and their function

2.5 Equipment used

S.No	Equipment	Manufacture
1	Soxhlet extraction apparatus	Rohin enterprise
2	High shear homogeniser	Glan
3	Digital pH meter	Lab junction
4	Magnetic stirrer	Remi equipment
5	Digital weighing balance	Glun
7	Heating mantle	Prima series
8	Probe sonicator	Godrej

Table:3 Equipment used

2.6 Methods of preparation

Method: High shear homogeniser

Step I: Preparation of Nanostructured Lipid Carrier (NLC)

1. Preparation of lipid phase:

Glyceryl monostearate (1.17 g), oleic acid (0.50g), Leucas aspera extract (0.3 g) were accurately weighed and transferred into a suitable vessel. The lipid phase was heated to 70°C until the lipid components were completely melted and uniformly mixed.

2. Preparation of aqueous phase:

Tween 80 (0.83g) was dissolved in distilled water, and the aqueous phase was heated separately to 70°C.

3. Formation of NLC dispersion:

The hot aqueous phase was slowly added to the molten lipid phase with continuous mixing.

The resulting mixture was subjected to high-shear homogenization using a high-shear.

homogenizer at 10,000 rpm for 5 minutes to obtain a uniform NLC dispersion.

Step II: Preparation of Gel Base

Carbopol 940 was dispersed in distilled water and allowed to hydrate and swell for 30 minutes.

Propylene glycol, methyl paraben (MPs) and propyl paraben (PPs) were added to the hydrated Carbopol dispersion and mixed uniformly.

Triethanolamine was added gradually with continuous stirring to neutralize the Carbopol and adjust the pH to 6.5–7.0. A clear and uniform gel base was obtained.

Step III: Incorporation of NLC into Gel Base

The prepared NLC dispersion was slowly incorporated into the prepared Carbopol gel base with gentle and continuous stirring. The mixture was stirred at 500 rpm for 10 minutes to obtain a homogeneous NLC gel.

Care was taken to minimize the formation of air bubbles during mixing. The final formulation was stored in a suitable container for further evaluation.



1. Fresh leucas aspera plants are collected.



2. Leaves dried in shade And powdered coarsely.



3. Powdered plant material extracted Using ethanol by Soxhlet apparatus.



4. plant extracted heated by Heating Mantle to remove ethanol.



5. Plant extract (API)



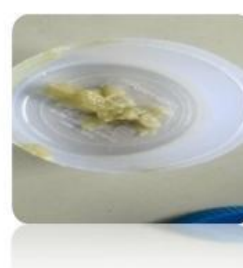
6. Preparation of NLC Carrier.



7. preparation of gel base.



8. Hot aqueous phase is added To lipid phase and homogeneized At high speed.



9. Formation of leucas aspera (NLC) gel.

Figure 2 : Prepration of NLC gel

3. EVALUATION

3.1 Physical examination

The developed formulation (NLC) was examined visually for its physical appearance, color, and consistency.

3.2 pH of NLC

The pH of the finally developed formulation was checked by calibrated pH meter and pH strip. The 1 gm. of NLC was diluted with 10 ml purified DW, therefore pH was measured at ambient temperature.

3.3 Spread ability

One of the criteria for an NLC gel to meet the ideal quantities is that it should possess good spread ability. It is the term expressed to denote the extent of area to which gel readily spreads on application to skin or affected part. The therapeutic efficacy of a formulation also depends upon its spread ability.

Spread ability of NLC gel and marketed gel was measured in terms of diameter of NLC gel circle produced when NLC is placed between two glass plates of definite weight.

A weighed quantity (350 mg) of NLC gel or gels was taken on one glass plate, and another glass plate was dropped from a distance of 5 cm. The diameter of the circle of spread NLC gel was measured. It is calculated by using the formula:

$$S=M.L/T$$

Where,

S= spread ability

M= weight tied to upper slide. L= length of glass slide.

T= time taken to separate the slides complete



Figure 3: Spread ability

3.4 Extrudability

Extrudability will be assessed by filling the gel into a standard collapsible tube and measuring the weight/percentage of gel extruded on application of a standard load, expressed qualitatively as excellent, good or poor.

3.5 Drug Content

An accurately weighed quantity of gel will be dissolved/extracted in a suitable solvent, filtered, and the concentration of the marker constituent determined by UV-Visible spectrophotometry/HPLC against a validated calibration curve, to confirm uniformity of drug content.

3.6 Particle Size

These are critical parameters affecting stability and biological interactions. Dynamic Light Scattering (DLS) is used to determine the mean particle size (typically 100-500 nm)

For example, studies have reported NLCs with particle sizes around 137 nm to 199 nm and PDI values below 0.3.

3.7 Zeta Potential

This measures the surface charge of the nanoparticles and indicates their colloidal stability. A higher absolute zeta potential (e.g., above ± 30 mV) typically suggests greater electrostatic repulsion between particles, thus preventing aggregation.

Values such as -34.3 mV have been reported for stable NLCs.

3.8 In vitro Release Studies

Franz diffusion cells are typically used to assess the release profile of the *Leucas aspera* extract from the NLC gel. This helps to understand the drug's sustained release characteristics.

3.9 In vitro Anti-inflammatory Activity

This involves assays to confirm the anti-inflammatory potential of the *Amaranthus spinosus* extract within the NLC gel.

Inhibition of albumin denaturation.

4. RESULT AND DISCUSSION

4.1 Phytochemical test

S.No	Phytoconstituent	Test performed	Observed change	Result
1	Alkaloids	Mayer's test	Creamy white precipitate	+
2	Glycosides	Legal's test	Pink to red colour	+
3	Terpenoids	Salkowski test	Red colour	+
4	Flavonoid	Shinoda test	Orange to red	+
5	Steroids	Libermann-Burchard's test	Greenish colour	+
6	Tannins	FeCl ₃ test	Brownish green colour	+
7	Saponin	Frothing test	Frothing test	+

Table 4: Preliminary phytochemical screening of leucas aspera plant extract

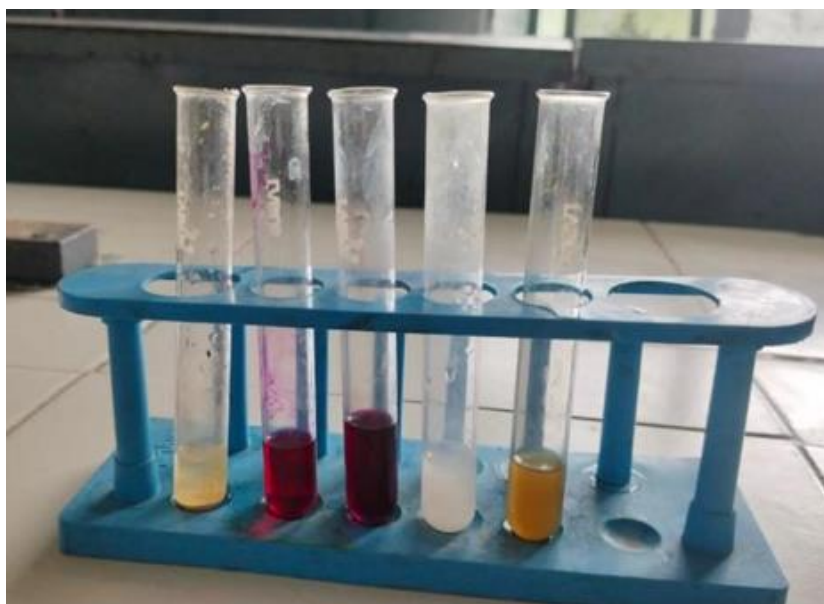


Figure 4: Preliminary phytochemical screening of leucas aspera plant extract

4.2 Physicochemical evaluation of NLC gel

Parameter	Result	Observation
Appearance	Smooth, Greenish gel	Uniform
pH	6.0	Suitable for skin
Viscosity(cP)	32,850cP	Good consistency
Spreading ability (g.cm)/sec)	32.71g.cm/sec	Easily spreadable
Drug content (%)	90%	Uniform drug distribution

Table 5: Physicochemical evaluation of NLC gel

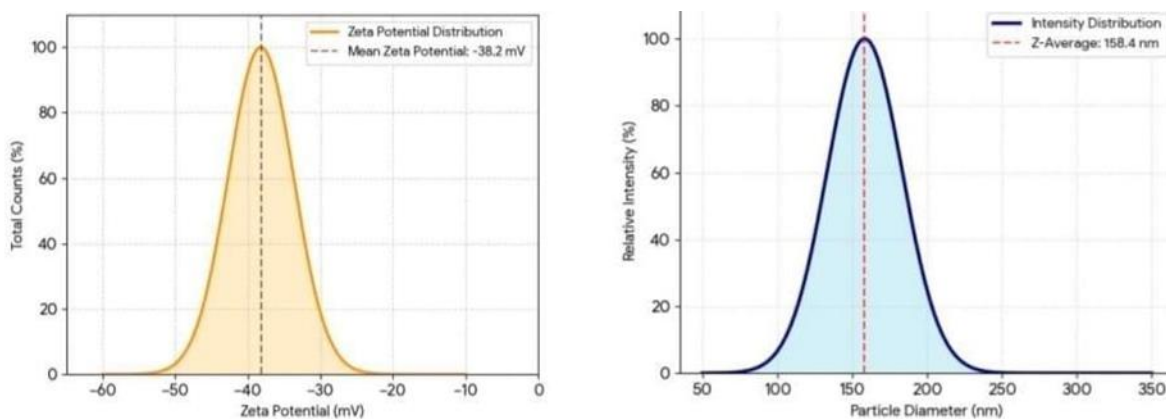


Figure 5: Practical size distribution and zeta potential

4.3 In vitro drug release study

FEANZ DIFFUTION TEST

Time (hrs)	Cumulative % Drug Release
0	0
1	15.2%
2	28.5 %
4	42.1 %
6	55.4 %
8	66.8 %
10	75.2 %
12	82.4 %

Table 6: Drug release profile

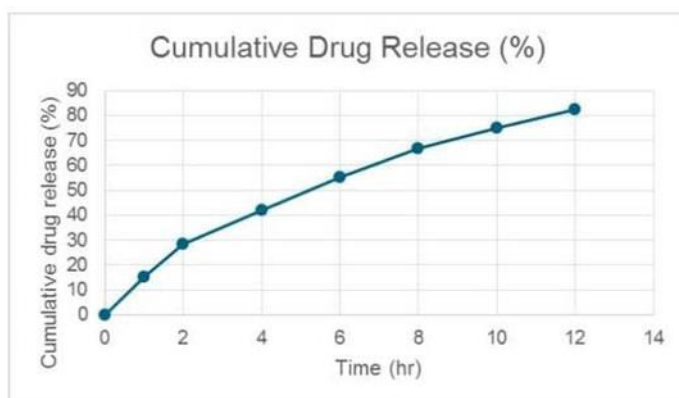


Figure 6: Drug release profile

4.4 *in vitro* anti-inflammatory activity (protein denaturation method)

Concentration ($\mu\text{g/mL}$)	% Inhibition	Standard (Diclofenac)
50	32.0%	-
100	45.0%	-
200	52 %	-
400	58 %	-
800	62.0%	60.9%

Table7: In vitro anti inflammatory activity

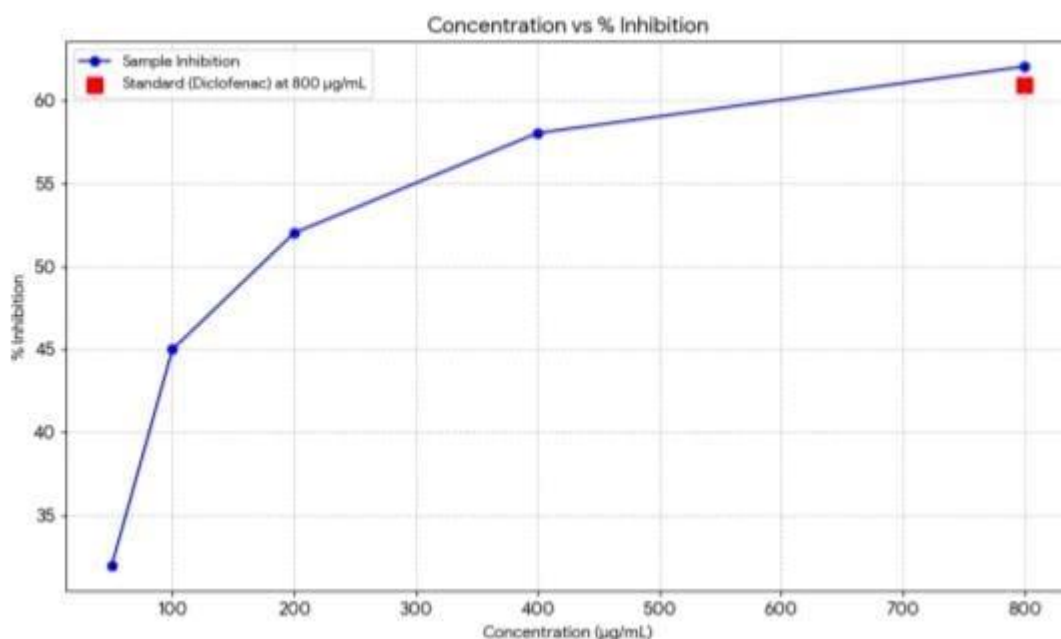


Figure 7: protein denaturation test % inhibition

5. CONCLUSION

➤ The present research work was focused on the formulation and evaluation of a Nanostructured Lipid Carrier (NLC) based gel containing *Leucas aspera* extract for potential topical wound healing application. Wound healing is a complex physiological process involving haemostasis, inflammation, proliferation and tissue remodelling, and excessive inflammation, microbial infection and oxidative stress may delay the normal healing process. Therefore, the development of a topical formulation possessing anti-inflammatory, antioxidant, antimicrobial and wound-healing potential is of considerable importance. *Leucas aspera*, a traditionally used medicinal plant, was selected for the present investigation due to its reported pharmacological properties and the presence of several bioactive phytoconstituents.

➤ The ethanolic extract of *Leucas aspera* was prepared by Soxhlet extraction and subjected to preliminary phytochemical screening. The screening confirmed the presence of important phytoconstituents including alkaloids, glycosides, terpenoids, flavonoids, steroids, tannins and saponins. The presence of these phytoconstituents supports the potential therapeutic usefulness of the plant extract in topical wound management, particularly because several of these constituents are associated with antioxidant, antimicrobial and anti-inflammatory activities.

➤ The NLC formulation was developed using glyceryl monostearate as the solid lipid, oleic acid as the liquid lipid and Tween 80 as the surfactant. The NLC dispersion was prepared by the high-shear homogenization method and subsequently incorporated into a Carbopol-based gel system. The incorporation of NLCs into the gel provides a suitable topical dosage form with the potential to improve local retention, enhance skin delivery and provide controlled release of the incorporated herbal constituents. The developed formulation was subsequently evaluated for its important physicochemical and performance characteristics.

➤ The prepared NLC gel exhibited a smooth, uniform and greenish appearance, indicating satisfactory physical characteristics. The formulation showed a pH of 6.0, which was considered suitable for topical application and compatible with the skin. The viscosity of the formulation was found to be 32,850 cP, indicating good consistency for application as a topical gel. The spreadability value was reported as 32.71 g·cm/sec, demonstrating that the formulation

could be spread readily over the skin. The drug content was found to be 90%, indicating satisfactory and relatively uniform distribution of the extract within the formulation. These physicochemical properties suggest that the developed NLC gel possesses suitable characteristics for topical administration.

➤ Particle size and zeta potential are important parameters for assessing the quality and stability of NLC systems. The developed formulation was evaluated for these parameters, as particle size influences the interaction of the formulation with the skin and zeta potential provides an indication of colloidal stability. The in-vitro drug release study was performed using a Franz diffusion cell to assess the release behaviour of the extract from the NLC gel. The release study provides useful information regarding the ability of the formulation to maintain and control the release of the incorporated herbal constituents.

➤ Furthermore, the in-vitro anti-inflammatory activity of the developed formulation was investigated by the protein denaturation method. This evaluation was included to determine the anti-inflammatory potential of the *Leucas aspera* extract incorporated in the NLC gel. The combination of the phytochemical constituents of *Leucas aspera* with the NLC-based delivery system provides a promising approach for localized topical application.

➤ Overall, the present study demonstrates the feasibility of developing a *Leucas aspera* extract-loaded NLC gel as a novel herbal topical formulation for wound management. The formulation showed satisfactory physical appearance, skin-compatible pH, suitable viscosity, good spreadability and acceptable drug content. The NLC-based approach may offer advantages over conventional herbal gels by improving the delivery and retention of bioactive constituents at the wound site while providing a controlled release profile. Thus, the developed formulation represents a promising candidate for topical wound-healing applications. However, further detailed studies including formulation optimization, long-term stability studies, comprehensive in-vitro release and permeation studies, and suitable in-vivo wound-healing investigations are required to establish its therapeutic effectiveness and safety. The present work therefore provides a useful foundation for further development of *Leucas aspera* extract-loaded NLC gel as a potential herbal nanocarrier-based topical formulation for effective wound management.

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Disclosure of conflict of interest:

There is no conflict of interest.

Author contribution:

M. Swathika, S. Thamizharasi, V. Theerthagiri, B. Uma mageshwari had principal responsibility for the conception, design of the study and drafting of manuscript. Dr.G.Marriyappan, was responsible for the revision of the manuscript for important intellectual content, Dr. J. Karthi, Dr. S. Swarnalatha contributed towards reviewing of the literature. All authors agreed on the final version of manuscript.

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