Formulation and Evaluation of Pickering-Type Lipid Emulsions Prepared with a Solid Lipid Nanoparticle Suspension Loaded with a *Combretum glutinosum* Perr. ex DC. (Combretaceae) Extract: *In Vivo* Wound Healing Activity

Formulation et évaluation d'émulsions lipidiques de type Pickering préparées à partir d'une suspension de nanoparticules lipidiques solides chargée en extrait de *Combretum glutinosum* Perr. ex DC. (Combretaceae) : activité cicatrisante *in vivo*

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Abstract

Background: *Combretum glutinosum* is traditionally used in West Africa for wound healing, but the physicochemical instability of its bioactive compounds may limit therapeutic applications. **Objective:** To develop solid lipid nanoparticles (SLNs) and SLN-based Pickering-type emulsions containing a hydrophilic extract of *C. glutinosum* and evaluate their wound-healing activity. **Methods:** SLNs were prepared by hot spontaneous emulsification using Suppocire® C and Solutol® HS15. Pickering-type emulsions were formulated with SLN suspensions and an oil phase. Three formulations containing 0%, 0.2%, and 0.8% (w/w) plant extract were evaluated. Wound healing was monitored for 28 days in Wistar rats with second-degree burns using Kamoshida scoring. **Results:** SLNs exhibited a mean hydrodynamic diameter of approximately 40 nm and spherical morphology. Emulsions showed no macroscopic phase separation over 29 days, although pH increased during storage. Mean wound closure times were 22, 25, and 26 days for formulations containing 0.8%, 0.2%, and 0% extract, respectively. These differences were descriptive and were not statistically tested. **Conclusion:** SLN-based Pickering-type emulsions offer a promising approach for topical delivery of C. glutinosum extracts. However, encapsulation efficiency remains undetermined, and the observed wound-healing differences require confirmation through adequately powered studies.

Keywords: solid lipid nanoparticles; Pickering emulsions; *Combretum glutinosum*; wound healing; topical drug delivery; West Africa.

Résumé

Contexte : *Combretum glutinosum* est traditionnellement utilisé en Afrique de l'Ouest pour ses propriétés cicatrisantes, mais l'instabilité physicochimique de ses composés bioactifs peut limiter ses applications thérapeutiques. **Objectif :** Développer des nanoparticules lipidiques solides (NLS) et des émulsions de type Pickering contenant un extrait hydrophile de *C. glutinosum*, puis évaluer leur activité cicatrisante. **Méthodes :** Les NLS ont été préparées par émulsification spontanée à chaud avec la Suppocire® C et le Solutol® HS15. Les émulsions ont été formulées à partir de suspensions de NLS et d'une phase huileuse. Trois formulations contenant 0 %, 0,2 % et 0,8 % (m/m) d'extrait ont été évaluées. La cicatrisation a été suivie pendant 28 jours chez des rats Wistar présentant des brûlures du second degré, à l'aide du score de Kamoshida. **Résultats :** Les NLS présentaient un diamètre hydrodynamique moyen d'environ 40 nm et une morphologie sphérique. Aucune séparation macroscopique de phases n'a été observée pendant 29 jours, malgré une augmentation du pH au cours du stockage. Les délais moyens de fermeture des plaies étaient respectivement de 22, 25 et 26 jours pour les formulations à 0,8 %, 0,2 % et 0 % d'extrait. Ces différences descriptives n'ont pas fait l'objet d'une analyse statistique. **Conclusion :** Les émulsions de type Pickering à base de NLS constituent une approche prometteuse pour l'administration topique d'extraits de C. glutinosum. Toutefois, l'efficacité d'encapsulation reste à déterminer et les différences de cicatrisation observées nécessitent une confirmation sur des effectifs suffisants.

Mots-clés : nanoparticules lipidiques solides ; émulsions de Pickering ; Combretum glutinosum ; cicatrisation ; administration topique ; Afrique de l'Ouest.

1. Introduction

Medicinal plants are an important source of natural active compounds, particularly in developing countries where they are the population's first-line therapeutic option. In West Africa, *Combretum glutinosum* Perr. ex DC. (Combretaceae), commonly known as "Kinkéliba" in Senegal, is a plant species with a wide geographical distribution whose leaves, bark and roots have long been the subject of ancestral therapeutic use for treating skin wounds, infections, gastrointestinal disorders and inflammatory conditions [1-4].

In-depth phytochemical investigations have highlighted the richness of *C. glutinosum* in bioactive secondary metabolites, notably flavonoids, tannins, saponins, alkaloids and triterpenes, to which wound healing, antimicrobial, antioxidant and anti-inflammatory activities are attributed [1,5-9]. These pharmacological properties, validated by preclinical studies, give this plant extract a real therapeutic potential for the treatment of skin lesions.

However, the effective clinical exploitation of these extracts faces major obstacles: physicochemical instability of the active compounds (sensitivity to oxidation, hydrolysis and light), low bioavailability and lack of release control. These limitations can be overcome by encapsulation in innovative colloidal systems [10,11]. Among these systems, solid lipid nanoparticles (SLNs) represent a platform of choice. First described in the early 1990s, they consist of a lipid matrix that is solid at room temperature, stabilised by surfactants, and have a size ranging between 40 and 1000 nm [12]. Their biocompatibility, low toxicity and ability to encapsulate hydrophilic or lipophilic active substances make them ideal carriers for topical and transdermal applications [13-15].

Pickering emulsions constitute an innovative alternative to conventional surfactant-stabilised emulsions. Stabilised by solid particles adsorbed at the oil–water interface, they form a rigid mechanical barrier conferring increased resistance to coalescence and improved skin tolerance [16-18]. Their use to encapsulate sensitive plant extracts opens particularly interesting therapeutic prospects.

In this context, the present study aims to develop and characterize SLNs and Pickering-type emulsions prepared with a hydrophilic extract of *C. glutinosum*, and then to evaluate *in vivo* the wound healing activity of the resulting formulations in an experimental second-degree burn model in the Wistar rat.

2. Materials and Methods

2.1. Plant raw material

C. glutinosum Perr. ex DC. (Combretaceae) leaves were collected in November 2024 in the Thiès region, Senegal (14°47′ N, 16°56′ W), in a Sudano-Sahelian savannah area. The site was selected because of the natural occurrence and abundance of *C. glutinosum* in this zone and because of the traditional use of the species by local populations. Botanical identification was carried out on morphological characters at the Department of Botany and Pharmacognosy, Faculty of Medicine, Pharmacy and Odontology, Cheikh Anta Diop University of Dakar, and a voucher specimen was deposited in the reference herbarium under the number CG-UCAD-2024-11. The accepted scientific name of the species was checked against Plants of the World Online (Royal Botanic Gardens, Kew). After harvesting, the leaves were cleaned and dried in the shade at room temperature (25 ± 2 °C) for 10 days, away from direct light and in a properly ventilated room. The dried plant material was ground in a laboratory knife mill and sieved through a 500 µm sieve to obtain a reasonably homogeneous powder. The powder was extracted by maceration in distilled water at a drug/solvent ratio of 1:10 (w/v) for 24 h at room temperature (25 ± 2 °C) under intermittent stirring. The macerate was filtered successively through gauze and through Whatman No. 1 filter paper (11 µm). The aqueous filtrate was concentrated under reduced pressure on a Büchi Rotavapor® R-210 (BÜCHI Labortechnik AG, Switzerland) at a bath temperature of 40 °C and a pressure of about 100 mbar. The concentrate was frozen at −20 °C for 24 h and then freeze-dried for 48 h in a Christ Alpha 1-4 LDplus freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Germany) at a condenser temperature of about −55 °C and a pressure below 0.1 mbar. The extraction yield, calculated relative to the initial mass of dry plant powder, was 14.8 % (w/w). The freeze-dried aqueous extract of *C. glutinosum* (ECG) was packaged in hermetically closed amber glass vials and stored in a desiccator at 4 ± 2 °C, protected from light and moisture, for a maximum of three months before use in the formulation experiments. No systematic phytochemical screening and no quantitative assay

of specific bioactive markers were performed on the ECG batch used in this study; the absence of phytochemical standardisation of the extract is accordingly treated as a limitation and is taken into account in the interpretation of the results.

2.2. Formulation of the solid lipid nanoparticles (SLNs)

2.2.1. Reagents and materials

Suppocire® C (Gattefossé, France), a fatty material composed of esters of mono-, di- and triglycerides of saturated fatty acids (C10–C18), was used as the lipid matrix. Suppocire® C (Gattefossé, Saint-Priest, France) is a hard fat of the Adeps solidus type (Ph. Eur.), composed predominantly of mono-, di- and triglycerides of saturated fatty acids; the material used in this study was from batch No. 23C041, with a melting range of 33–36 °C according to the manufacturer's certificate of analysis. Solutol® HS15 (BASF, Ludwigshafen, Germany), also known as Kolliphor® HS15, was used as the non-ionic surfactant (batch No. 82367451). It consists mainly of macrogol 15 hydroxystearate (CAS No. 70142-34-6), monographed in the European Pharmacopoeia as Macrogol 15 Hydroxystearate and in USP–NF as Polyoxyl 15 Hydroxystearate, with an HLB value of approximately 14–16, occurring as a white to yellowish paste at room temperature and becoming liquid at approximately 30 °C. Distilled water constituted the aqueous phase. The devices used include a BIOBLOCK vortex mixer, a DK SONIC ultrasonic bath (42 kHz), magnetic stirrers and a Zetasizer Nano (Malvern Instruments, United Kingdom).

2.2.2. Preparation method

SLNs were prepared by hot spontaneous emulsification according to the following protocol. Suppocire® C and Solutol® HS15 were melted together at 80 °C, then homogenised at 1000 rpm at 90 °C. The aqueous phase (distilled water), preheated to 90 °C, was then gradually added to the molten lipid phase under continuous stirring at 1000 rpm, inducing the spontaneous formation of nanometric droplets. The dispersion was maintained under stirring for 5 min at 90 °C to ensure complete homogenization, then allowed to cool progressively to room temperature (25 ± 2 °C) over approximately 1 h. Cooling below the melting range of Suppocire® C promoted lipid crystallisation and the formation of the SLNs [19,20]. Under continuous stirring, the molten oil phase induced the spontaneous formation of nanometric droplets. Controlled cooling to room temperature led to lipid crystallisation and to the formation of the SLNs [19,20]. The composition of the SLN suspension is given in Table 1.

Table 1. Composition of the SLNs.

Component	Quantity
Suppocire® C	105 mg
Solutol® HS15	195 mg
Distilled water	690 µL

2.3. Formulation of the Pickering emulsions

The SLN suspension was used as the aqueous phase. The oil phase consisted of medium-chain triglycerides (MCT, Labrafac® WL 1349, Gattefossé, France). Six formulations (T1 to T6) were prepared at 25 °C by varying the oil/SLN ratio, with the addition of 0.5 mL of lanolin as a co-stabiliser (Table 2). Formulations T1–T6 constituted a preliminary screening series designed to identify the oil/SLN ratio providing the most satisfactory macroscopic stability; these screening formulations were not applied to the animals. The compositions were initially recorded volumetrically. For comparison on a mass basis, masses were calculated from the recorded volumes using densities of 0.94 g/mL for Labrafac® WL 1349, 0.94 g/mL for lanolin and an estimated 1.00 g/mL for the predominantly aqueous SLN suspension. The 4.0 mL of SLN suspension used in each formulation thus corresponded to approximately 4.00 g and the 0.5 mL of lanolin to approximately 0.47 g, while the volumes of 0.5, 1.5, 2.5, 3.5, 4.5 and 5.5 mL of Labrafac® WL 1349 corresponded to approximately 0.47, 1.41, 2.35, 3.29, 4.23 and 5.17 g, respectively, giving total batch masses of 4.94, 5.88, 6.82, 7.76, 8.70 and 9.64 g for T1 to T6. The oil content therefore ranged from 9.5 % (w/w) in T1 to 53.6 %

(w/w) in T6. The use of a solid-particle suspension as the stabilising phase follows the principles described for particle-stabilised emulsions [16-18]; the interfacial localisation of the SLNs was not experimentally demonstrated in the present study, and these systems are consequently referred to as Pickering-type emulsions. Emulsification was carried out by vortexing (2 min) followed by ultrasonic treatment (42 kHz, 3 min), performed in three consecutive cycles, each consisting of 2 min of vortex mixing followed by 3 min of

ultrasonication at 42 kHz, corresponding to a total processing time of 15 min, including 9 min of effective ultrasonication. All formulations were prepared under identical operating conditions (same speed, same duration, same temperature).

Table 2. Composition of the Pickering emulsions (T1–T6).

Component	T1	T2	T3	T4	T5	T6
SLNs (mL)	4	4	4	4	4	4
MCT oil (mL)	0.5	1.5	2.5	3.5	4.5	5.5
Lanolin (mL)	0.5	0.5	0.5	0.5	0.5	0.5

2.4. Preparation of the extract-loaded topical formulations

Based on the preliminary screening results, formulation T4 was selected as the most suitable compromise between oil/SLN ratio and macroscopic stability, and was used as the basis for developing the final topical vehicle. The screening formulations T1–T6 were used exclusively for formulation optimisation and were not administered to the animals; only the final formulations F0, F1 and F2 (Table 3) were evaluated in vivo. For the final formulations, the medium-chain triglycerides used during screening were replaced by sweet almond oil in order to obtain a vehicle better suited to repeated application to injured skin, sweet almond oil being an emollient and skin-conditioning agent of established pharmaceutical and cosmetic use. The MCT phase was therefore treated as a model oil phase for optimisation of the oil/SLN ratio. The ratio selected on MCT was not re-optimised after substitution with sweet almond oil, and the oil/SLN ratio of the final vehicle (24 mL of oil for 20.5 mL of SLN suspension) differs from that of T4 (3.5 mL for 4.0 mL); both points are acknowledged as limitations of the formulation development. Three final batches were prepared: an extract-free vehicle (F0) and two extract-loaded formulations, F1 and F2 (Table 3). Quantities of the liquid components were originally recorded volumetrically; using densities of 1.00 g/mL for distilled water and for the predominantly aqueous SLN suspension, 0.94 g/mL for lanolin and 0.91 g/mL for sweet almond oil, the corresponding masses were approximately 3.50 g of distilled water, 3.29 g of lanolin, 21.84 g of sweet almond oil and 20.50 g of SLN suspension, to which 3.00 g of PEG was added, giving a calculated vehicle mass of approximately 52.13 g before addition of ECG. A discrepancy was identified between the nominal extract concentrations initially assigned to F1 and F2 and the concentrations calculated from the recorded masses of the formulation components. Based on the reconstructed vehicle mass of approximately 52.13 g, the 71 mg and 313 mg of ECG actually weighed would correspond to approximately 0.136% and 0.600% (w/w), respectively, rather than the nominal concentrations of 0.2% and 0.8% (w/w). Because the original formulation records do not presently allow this discrepancy to be resolved unequivocally, the extract loads are reported on the basis of the masses actually weighed. The nominal designations of 0.2% and 0.8% are retained only where they correspond to the original experimental coding and should not be interpreted as analytically verified final concentrations.The extract was first dissolved in distilled water under moderate stirring. The molten lanolin was then incorporated hot into the aqueous phase, followed by sweet almond oil. The SLNs and polyethylene glycol (PEG) were added last, and the preparation was homogenised with an IKA® LABORTECHNIK RW16 basic overhead stirrer until macroscopically homogeneous. ECG was first dissolved in distilled water under moderate stirring; lanolin, previously softened by heating above its melting range, was incorporated into the aqueous phase, followed progressively by sweet almond oil, then by the SLN suspension and polyethylene glycol. The final vehicle therefore consisted of an aqueous phase containing the dissolved ECG, sweet almond oil as the oil phase, lanolin as a co-stabilising and consistency-enhancing agent, PEG as a consistency-modifying agent, and the SLN suspension as the colloidal component. Because neither the encapsulation

efficiency nor the distribution of ECG between the aqueous phase, the lipid nanoparticles and the oil–water interface was determined experimentally, the preparations are described throughout as extract-loaded rather than extract-encapsulated formulations. The formulation strategy was adapted from previously described topical preparations of *C. glutinosum* extracts and from established approaches for incorporating plant-derived active substances into lipid-based dermatological vehicles [7,13].

Table 3. Formulations containing the *C. glutinosum* extract (per 40 g).

Component	F0 (0 %)	F1 (0.2 %)	F2 (0.8 %)
ECG extract (mg)	0	71	313
Distilled water (mL)	3.5	3.5	3.5
Lanolin (mL)	3.5	3.5	3.5
Sweet almond oil (mL)	24	24	24
SLNs (mL)	20.5	20.5	20.5
PEG (g)	3	3	3

2.5. Physicochemical characterisation

2.5.1. Size and polydispersity index

The size of the SLNs and the polydispersity index (PDI) were determined by dynamic light scattering (DLS) using a Zetasizer Nano (Malvern Instruments, Malvern, United Kingdom). Before analysis, the SLN suspension was diluted 1:100 (v/v) in distilled water to minimise multiple scattering, and the diluted samples were allowed to equilibrate for 2 min at 25 °C. Measurements were performed at 25 ± 0.1 °C at a scattering angle of 173° (non-invasive backscatter mode), each sample being analysed in three independent measurements (n = 3). Results are expressed as the intensity-weighted hydrodynamic diameter and the PDI, the latter being a dimensionless measure of the width of the size distribution, with values below 0.3 being commonly regarded as indicative of a relatively narrow distribution [12].

2.5.2. Zeta potential

The electrostatic stability of the nanoparticles was assessed by micro-electrophoresis. The zeta potential of the SLNs was determined by electrophoretic light scattering on the same Zetasizer Nano. The suspension was diluted in distilled water and transferred into a disposable folded capillary cell, and measurements were performed at 25 °C in triplicate (n = 3). Absolute zeta-potential values of approximately 30 mV or greater are commonly regarded as indicative of electrostatic stabilisation sufficient to limit particle aggregation [15,21].

2.5.3. Morphology

The morphology of the SLNs was examined by transmission electron microscopy using a JEOL transmission electron microscope (JEOL Ltd., Tokyo, Japan). A drop of diluted SLN suspension was deposited onto a carbon-coated copper grid, negatively stained with uranyl acetate and allowed to dry before observation. Particle morphology and the presence of aggregation were assessed from the resulting micrographs.

2.5.4. pH and conductivity

The pH of formulations T1–T6 was determined at D0, D8, D15, D22 and D29 using a calibrated digital pH meter (Hanna Instruments HI 2211, Woonsocket, RI, USA) at room temperature, in triplicate (n = 3), results being expressed as mean ± SD. The physicochemical follow-up of the emulsions thus extended over 29 days, whereas the in vivo phase lasted 28 days; the two observation periods are distinct and are reported as such throughout. Electrical conductivity was determined at the same observation times, in triplicate, using a digital conductivity meter (Hanna Instruments HI 99300, Woonsocket, RI, USA) at room temperature, to identify the type of emulsion (oil-in-water, O/W, or water-in-oil, W/O).

2.5.5. Droplet size, macroscopic and microscopic examinations

Droplet morphology and size were assessed by optical microscopy using a ZEISS Primo Star microscope (Carl Zeiss Microscopy GmbH, Germany) equipped with a 40× objective and a digital image-acquisition system; micrographs were analysed with image-processing software to estimate droplet size. Macroscopic

examination was performed at D0, D8, D15, D22 and D29, that is, over 29 days; at each observation time the formulations were examined for changes in appearance, homogeneity, creaming, sedimentation, coalescence and macroscopic phase separation.

2.6. In vivo study of wound healing activity

2.6.1. Animals and housing conditions

A total of 15 adult male Wistar rats weighing approximately 180–250 g, naïve and free of visible skin lesions, were used and were housed individually at 25 ± 2 °C. The total of nine animals stated in the previous version covered only groups F0, F1 and F2 and did not include the untreated control group or the silver sulfadiazine reference group. The animals were reared under controlled conditions (12 h/12 h day/night cycle), fed a standard commercial rodent feed and given water *ad libitum*. Each rat was weighed, identified and housed individually. The experiments were conducted in accordance with the ethical principles governing animal experimentation and received the approval of the institutional research ethics committee of the host university (approval reference provided on the title page). The study is reported in accordance with the essential items of the ARRIVE 2.0 guidelines. Animals were allocated to the experimental groups by simple random allocation after initial weighing, with each group containing three animals (n = 3). The investigators were not blinded to treatment allocation during wound scoring, which is acknowledged as a potential source of observer bias and as a limitation of this exploratory study. Animals were monitored daily for general condition, food and water intake, mobility, wound appearance and signs of pain or distress. Humane endpoints included marked deterioration in general condition, persistent inability to eat or drink, severe reduction in mobility, uncontrolled bleeding or infection, severe wound deterioration, and signs of persistent pain or distress requiring veterinary intervention; animals reaching a predefined humane endpoint were to be withdrawn from the experiment and managed according to institutional animal-welfare procedures.

2.6.2. Experimental protocol

The 15 animals were randomly allocated to five experimental groups of three (n = 3 per group): an untreated control group; a positive-reference group treated with silver sulfadiazine; group F0, receiving the extract-free vehicle; group F1, receiving the formulation containing the lower ECG load; and group F2, receiving the formulation containing the higher ECG load. No group received the aqueous *C. glutinosum* extract alone; consequently, the design does not allow the intrinsic wound-healing effect of the extract to be distinguished from a possible contribution of the colloidal vehicle. The positive-reference group (n = 3) received 1 % (w/w) silver sulfadiazine cream (Flammazine®, Recipharm Parets S.L., Barcelona, Spain; batch No « Ch.-B.: 15436 »), applied topically to the entire burned area once daily under the same conditions as the experimental formulations.

The experimental burn model was adapted from the protocol previously described by our group [22]. Animals were anaesthetised by intraperitoneal administration of a 3 % chloral hydrate solution at 1 mL per 100 g body weight, and the dorsal region was carefully shaved and cleaned before burn induction. A cylindrical metal device 3 cm in diameter was heated for 5 min and applied to the shaved dorsal skin for 20 s under slight and constant pressure, producing a circular lesion of approximately 7.1 cm² consistent with a second-degree experimental burn [7,22]. Following burn induction and recovery from anaesthesia, the animals were monitored for signs of pain and distress. No additional post-operative systemic analgesic was administered, since analgesic treatment could interfere with the inflammatory component of the wound-healing response; this is acknowledged as a methodological and animal-welfare limitation of the study. Approximately 0.5 g of the corresponding topical preparation was gently spread over the entire burned area once daily for 28 consecutive days, the same nominal quantity and application procedure being used for F0, F1, F2 and the silver sulfadiazine reference, while the untreated control group received no topical preparation, according to a protocol adapted from previously described burn models [7,22].

2.6.3. Assessment of wound healing

Wound healing was assessed daily using the Kamoshida method (scores 0 to 5) [7,22], the scale of which is presented in Table 4. Three events were distinguished during follow-up: eschar detachment, macroscopic wound closure and complete re-epithelialization. Macroscopic wound closure was defined as the absence of a residual visible open wound on visual examination. Because histological examination was not performed, complete re-epithelialization could not be established. The primary endpoint was therefore the time to

macroscopic wound closure, expressed in days after burn induction, and is designated as such throughout. Individual wound scores were recorded for each animal over the 28-day observation period and used to calculate descriptive group means; they are provided in Supplementary Table S1.

Table 4. Wound healing scoring scale (Kamoshida).

Score	Clinical description
0	Complete healing – tissue repair achieved
1	Repair almost complete
2	Residual scab remnants – lesion size decreasing.
3	Necrotic tissue (scabs) removed – open wound with exudation.
4	Partially removed necrotic skin – ulceration and exudation
5	Necrotic skin entirely covering the burned area.

2.7. Statistical analysis

Data are expressed as individual values and as group means ± standard deviation (SD). Particle-size, zeta-potential, pH, conductivity and droplet-size measurements are summarised descriptively. The evolution of wound scores and the time to macroscopic wound closure were analysed graphically and descriptively. Given the exploratory nature of the *in vivo* study and the limited sample size (n = 3 animals per group), no inferential statistical test was applied to comparisons between treatment groups. At this sample size, the assumptions underlying conventional parametric analyses, in particular normality and homogeneity of variance, could not be reliably assessed, and inferential p-values would therefore have limited interpretability. All comparisons between groups are accordingly presented as descriptive observations rather than as statistically significant differences, and the in vivo component of the study should be considered a proof-of-concept experiment requiring confirmation in adequately powered studies.

3. Results

3.1. Characterisation of the SLNs

3.1.1. Size and polydispersity index

The SLNs formulated by hot spontaneous emulsification showed a unimodal, intensity-weighted size distribution with a modal diameter of 40 nm; the percentiles derived from the fitted distribution were D10 = 29 nm, D50 = 40 nm and D90 = 56 nm, with a full width at half maximum of 30–54 nm (Figure 1). The mean zeta potential was −35 ± 3 mV (n = 3), indicating a negatively charged particle surface and electrostaticrepulsion sufficient to contribute to colloidal stability.

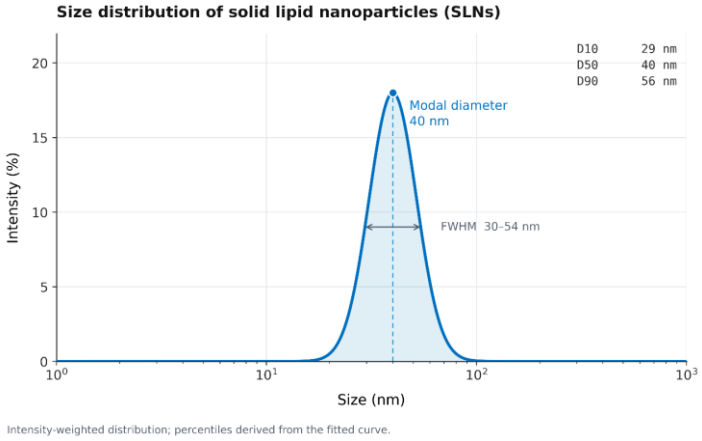


Figure 1. Intensity-weighted size distribution of the solid lipid nanoparticles measured by dynamic light scattering. The marker indicates the modal diameter (40 nm); D10, D50 and D90 percentiles and the full width at half maximum are derived from the fitted curve.

3.1.2. Morphology by transmission electron microscopy

The TEM images revealed particles of spherical morphology, well individualized and homogeneously dispersed, with a size consistent with the DLS data (≈ 40 nm) (Figure 2). No particle aggregation or deformation was observed, confirming the structural quality of the nanoparticles obtained.

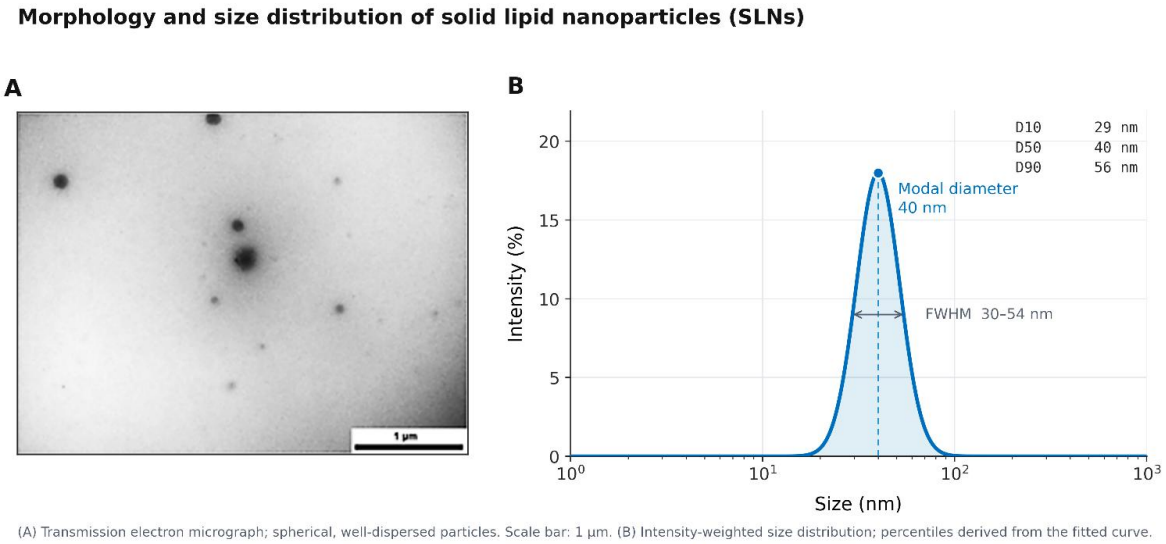


Figure 2. Transmission electron micrograph of the solid lipid nanoparticles; spherical, well-dispersed particles. Scale bar: 1 µm.

3.1.3. Conductometry and pH

Conductivity remained in the tens of mS/cm in all formulations throughout the follow-up, confirming that the external phase was aqueous and the emulsions were of the oil-in-water type (Figure 3). Conductivity was markedly higher in T1, the formulation with the lowest oil proportion, than in T2 to T6, which formed a homogeneous group. The pH of all six emulsions remained close to 4.5–4.6 from D0 to D22 (Figure 4). A marked rise was recorded at D29 in every formulation, most pronounced in T1, whose pH increased from 4.60 ± 0.05 at D0 to 7.10 ± 0.10 at D29; the rise was more limited in T2 (5.30 ± 0.09), T3 (5.10 ± 0.07), T4 and T6 (5.00 ± 0.06) and T5 (4.90 ± 0.06). This drift, which affects T1 far more than the formulations with a higher oil proportion, is consistent with hydrolysis of the ester functions of the lipid excipients, but no assay was performed to document it. The pH of the formulations is therefore not stable over the period studied, and the emulsion with the lowest oil content (T1) is the least stable in this respect.

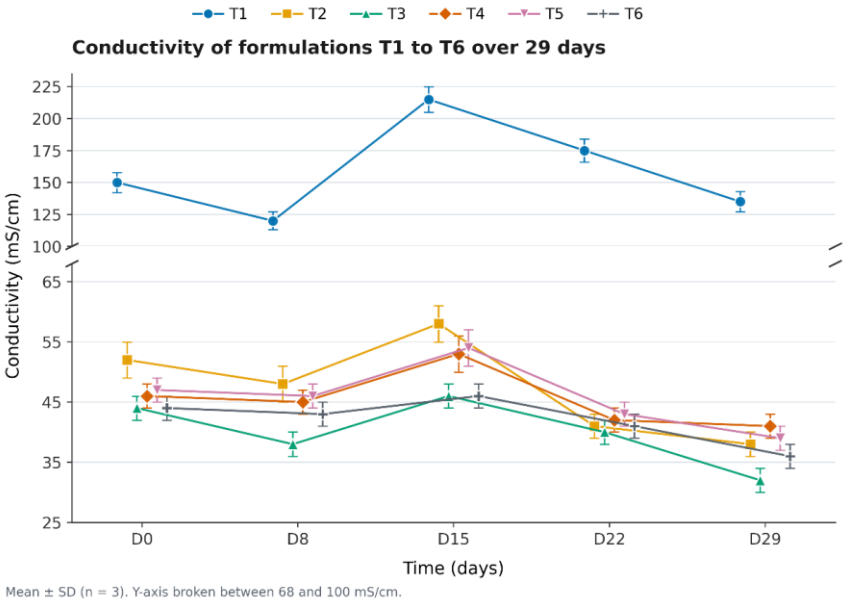


Figure 3. Change in the conductivity (mS/cm) of formulations T1 to T6 over 29 days (D0, D8, D15, D22, D29). Mean ± SD, n = 3; the y-axis is broken between 68 and 100 mS/cm.

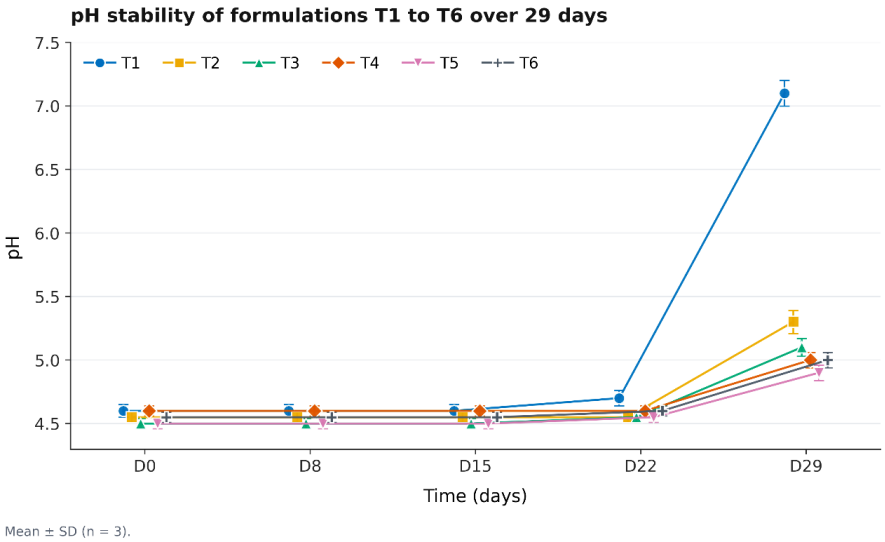


Figure 4. Change in the pH of formulations T1 to T6 over 29 days (D0, D8, D15, D22, D29). Mean ± SD, n = 3.

3.2. Characterisation of the Pickering emulsions

3.2.1. Droplet size

Droplet size was determined for the six formulations (T1–T6). Mean droplet size ranged from 91 ± 2 µm (T6) to 109 ± 3 µm (T3), with an overall mean of 98.7 µm across the six formulations (Figure 5; mean ± SD, n = 3). The values do not vary monotonically with the oil/SLN ratio: droplet size increased from T1 (95 ± 3 µm) to T3, then decreased again in T4 (104 ± 3 µm), T5 (97 ± 3 µm) and T6. The differences between formulations are of the same order as the standard deviations and were not tested statistically; no relationship between droplet size and oil proportion can therefore be established from these data. Optical microscopy confirmed that the droplets were spherical in every formulation.

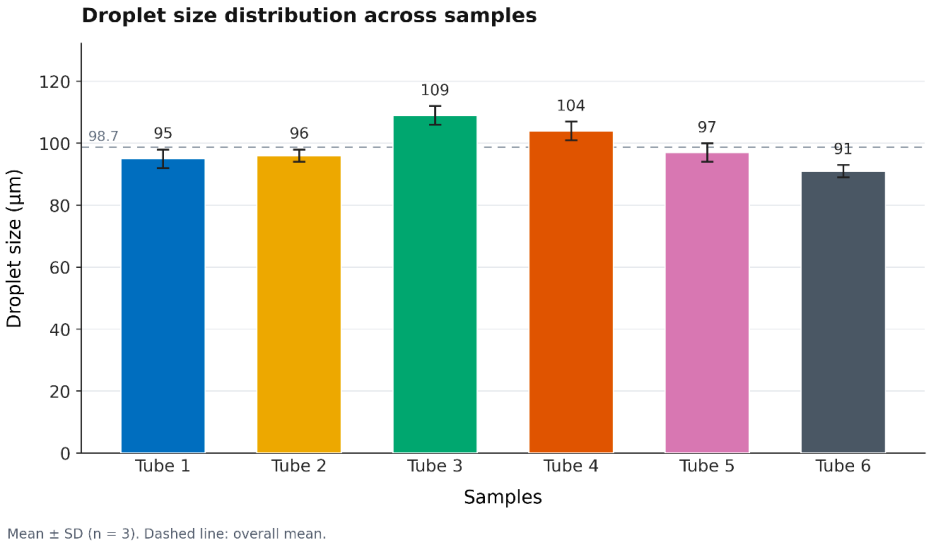


Figure 5. Mean droplet size (µm) of formulations T1 to T6. Mean ± SD, n = 3; the dashed line indicates the overall mean (98.7 µm).

3.2.2. Macroscopic examination

Immediate visual examination of the formulations revealed a cloudy to whitish appearance characteristic of oil-in-water emulsions. After 28 days of storage at room temperature, no macroscopic phase separation was detected in formulations T1 to T4. A slight sedimented layer was visible from D22 onwards in the tubes with the highest oil proportion (T5 and T6); no creaming, coalescence or complete phase separation was observed. These observations were made under non-controlled ambient conditions and therefore constitute preliminary macroscopic observations rather than a formal pharmaceutical stability study.

3.2.3. Microscopic examination

Observations under optical microscopy (40× objective) showed mostly spherical droplets dispersed in the continuous phase (Figure 6). A size distribution varying with the formulation was observed; the tubes with higher oil concentration showed larger droplets, in agreement with Taylor's law applicable to emulsified systems.

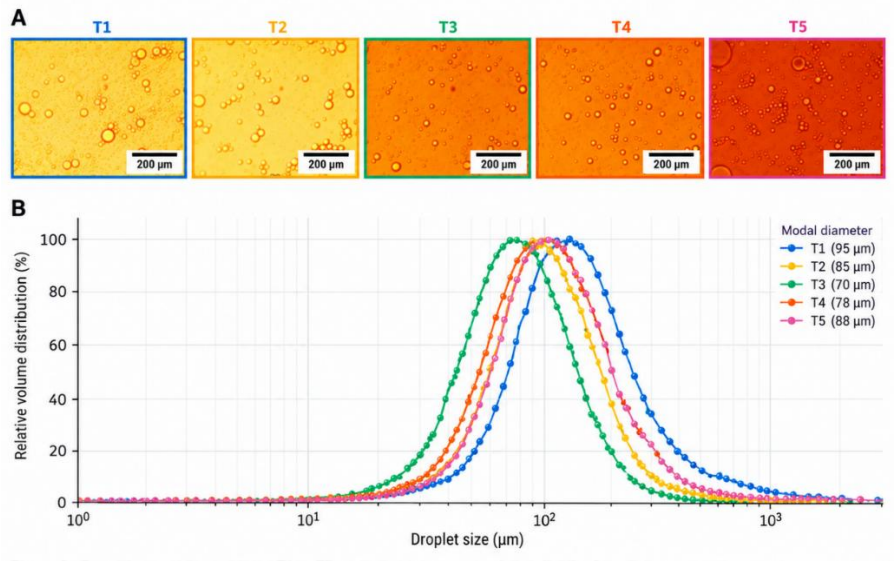


Figure 6. Droplet size of formulations T1 to T5: (A) representative optical micrographs; (B) relative volume-based size distribution, the markers indicating the modal diameter.

3.3. Assessment of in vivo wound healing activity

3.3.1. Change in burn scores in the absence of treatment

In the absence of treatment (untreated control), the burned area remained entirely covered with necrotic tissue until day 8 (score 5). Ulcerations with exudation were observed between D12 and D22 (scores 4–5). The mean score in this group fell from 5.00 at D0 to 4.35 at D12, 3.30 at D16, 2.30 at D20, 2.00 at D24 and 1.70 at D28 (Figure 7). Skin regeneration therefore became apparent from D16 onwards, and the mean score had not reached 0 by D28.

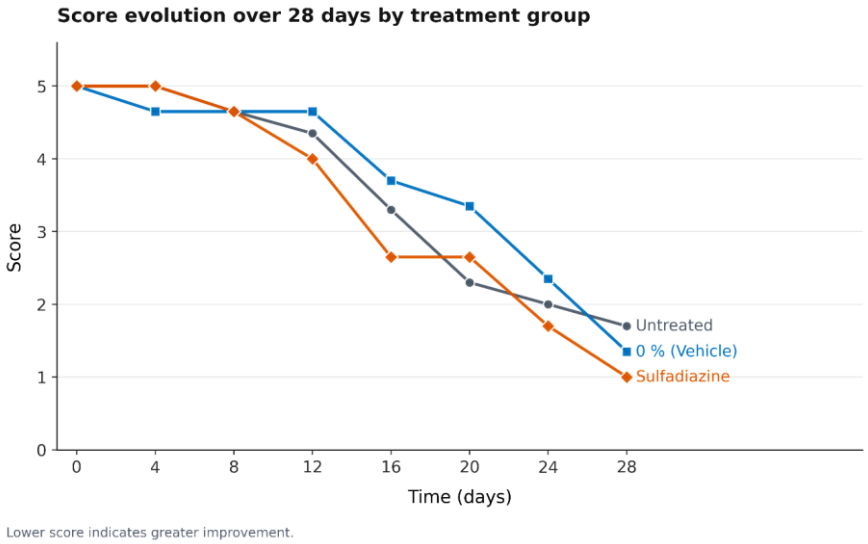


Figure 7. Change in mean burn scores over 28 days in the untreated group, in the silver sulfadiazine group and after daily application of the extract-free (0 % ECG) formulation (n = 3 per group). A lower score indicates a more advanced stage of repair.

3.3.2. Group treated with silver sulfadiazine (positive reference)

The change in scores in the group treated with silver sulfadiazine followed kinetics similar to those of the untreated group, but with lower scores from D12 onwards: 4.00 at D12, 2.65 at D16 and D20, 1.70 at D24 and 1.00 at D28, versus 1.70 at D28 in the untreated group.

3.3.3. SLN + ECG formulations

Daily application of the extract-free formulation (F0, 0 % ECG) gave mean scores of 4.65 at D12, 3.70 at D16, 3.35 at D20, 2.35 at D24 and 1.35 at D28. The mean scores at D28 were 1.65 for F1 (0.2 %) and 0.65 for F2 (0.8 %), the lowest of all groups, with corresponding values at D24 being 2.35 for F1 and 1.35 for F2. At D24 the mean wound scores were 2.35 for F1 and 1.35 for F2; at D28 they were 1.65 and 0.65 respectively, F2 showing the lowest mean score of all groups. The apparent discrepancy between the mean-score curves of Figure 8, none of which reaches 0 before D28, and the closure times of Table 5 follows from the different nature of the two measures: Figure 8 shows means calculated over the three animals of each group at each observation time, whereas Table 5 reports the individual time to macroscopic wound closure, so that closure in one animal as early as D20 is compatible with a group mean above 0 while the other two animals still present residual lesions. The two datasets are therefore not interchangeable. Individual closure times were D28, D24 and D28 for F0 (mean 26.7 days) and D24, D24 and D28 for F1 (mean 25.3 days). The F0 and F1 mean-score profiles largely overlapped up to D20, giving no descriptive evidence of an additional effect of the lower ECG load over the extract-free vehicle during the initial phase of healing; between D20 and D24 the two curves crossed, and at D28 the mean score was higher for F1 (1.65) than for F0 (1.35). F2 showed individual closure times of D28, D20 and D20; that is, the shortest mean closure time of the three formulations (22.7 days).

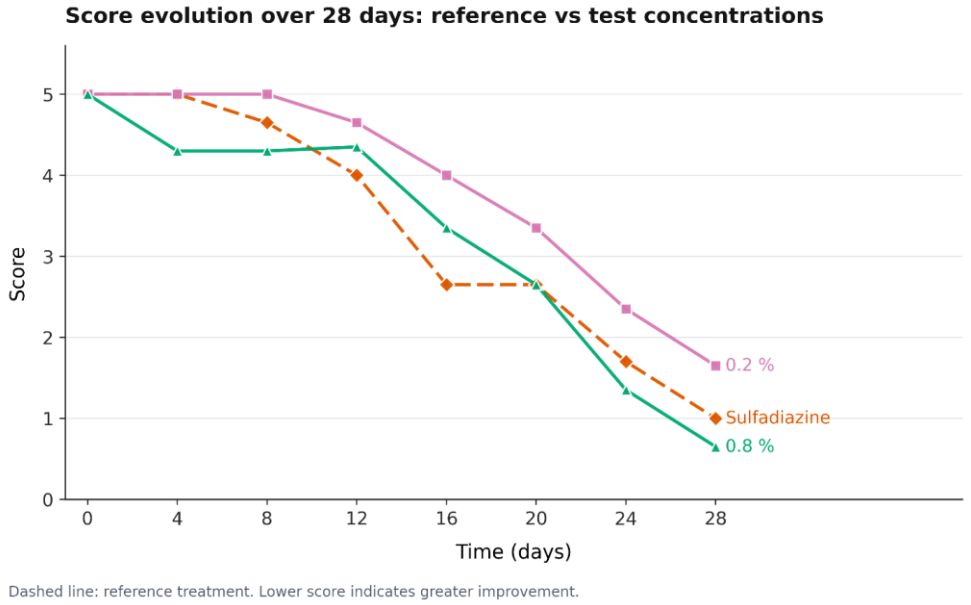


Figure 8. Change in mean burn scores over 28 days after daily application of the ECG-loaded formulations at 0.2 % and 0.8 %, compared with the silver sulfadiazine reference (dashed line); n = 3 per group. A lower score indicates a more advanced stage of repair.

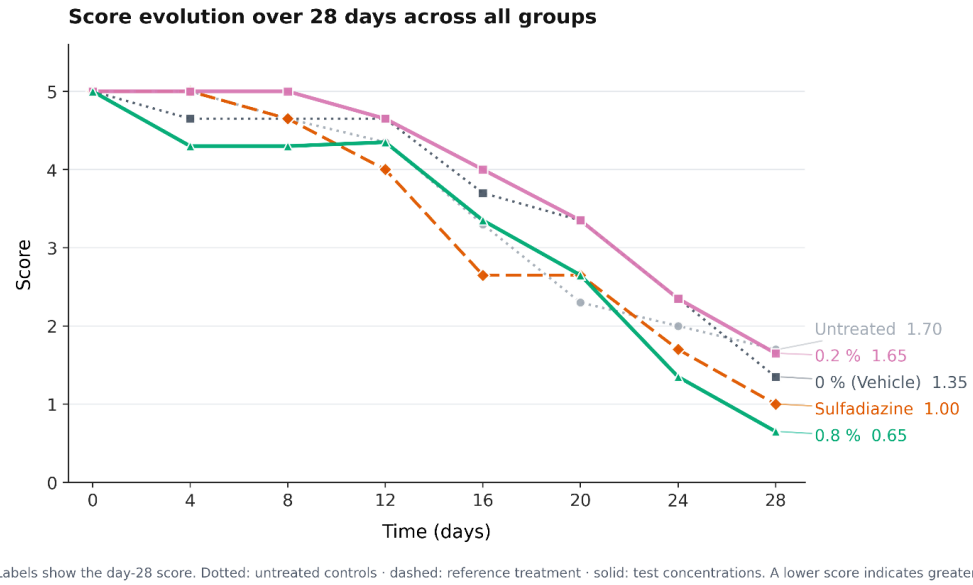


Figure 9. Summary of the change in mean burn scores after daily application of the different preparations (n = 3 per group).

Table 5. Time to macroscopic wound closure (in days) per rat and per group.

	F0 (0 %)	F1 (0.2 %)	F2 (0.8 %)
Rat 1	D28	D24	D28
Rat 2	D24	D24	D20
Rat 3	D28	D28	D20
Mean	26.7	25.3	22.7

Analysis of the individual closure times shows a more favourable descriptive profile for the higher load of the *C. glutinosum* extract (Table 5): F2 was associated with the shortest mean closure time, 22.7 days, versus 25.3 days for F1, 26.7 days for F0 and 28 days for the untreated group. Considerable inter-individual variability was observed within each group, and the means are strongly influenced by individual animals owing to the small group size (n = 3): one animal of the F2 group closed at D28, that is, later than one animal of the F0 group. No inferential statistical analysis was performed, and these differences should therefore not be interpreted as demonstrating statistical superiority of F2 over F0, F1 or the silver sulfadiazine reference. The macroscopic progression of the burn wounds under the three formulations is illustrated in Figure 10.

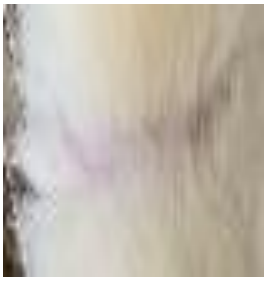
Day	Rat 2 – 0 %	Rat 2 – 0.2 %	Rat 3 – 0.8 %
D1			
D8			
D16			
D24			
D28			

Figure 10. Macroscopic progression of the experimental second-degree burn wounds in the rat, from D1 to D28. Each column corresponds to one group (untreated control, silver sulfadiazine, F0, F1, F2) and each row to one observation day; each image is identified by group, animal and day, and carries a scale bar.

4. Discussion

The present study demonstrates the feasibility of preparing a Pickering-type lipid emulsion loaded with a *C. glutinosum* extract and a solid lipid nanoparticle suspension for a topical wound healing application.

The preparation of the SLNs by hot spontaneous emulsification made it possible to obtain nanometric particles (≈ 40 nm), monodisperse (PDI < 0.3) and of spherical morphology, confirmed by TEM. These characteristics are favourable to skin penetration and to interaction with the cells involved in wound healing (keratinocytes, fibroblasts) [12,13]. The use of Suppocire® C, rich in saturated fatty acid triglycerides, allows a crystalline matrix to form on cooling, while Solutol® HS15 provides colloidal stabilisation, in accordance with the literature data [19,20]. It should be noted that the melting range of the batch used (33–36 °C) is close to skin surface temperature, so that the matrix is solid under storage conditions but may soften at the application site; the term solid lipid nanoparticle therefore refers to the state of the carrier as prepared and stored.

The hot spontaneous emulsification method has the advantage of not requiring sophisticated equipment such as high-pressure homogenisers, making the process simple and economically accessible in the context of resource-limited countries. The reproducibility and robustness of the process were not formally assessed.

Under the non-controlled ambient conditions of this study, no macroscopic phase separation was observed over 28 days in formulations T1 to T4, but the pH of every formulation rose at D29, markedly so in T1 (4.60 to 7.10). Since the lipid matrix and the co-stabiliser are ester-based, this drift is compatible with progressive hydrolysis; whatever its origin, it indicates that the formulations are not physicochemically stable over the period studied, and it argues against the formulations with the lowest oil content. These observations do not constitute a stability study: no protocol compliant with ICH Q1A(R2) (long-term and accelerated conditions, climatic zone IVb) was applied, temperature and relative humidity were neither controlled nor recorded, and no physicochemical assay of the extract was performed over time. The physical stability of the formulations therefore remains to be established. Adsorption of solid particles at the oil–water interface is the mechanism described for particle-stabilised emulsions [17], but the interfacial localization of the SLNs was not demonstrated here and no control emulsion free of SLNs or of soluble surfactant was prepared; the system is therefore described as a Pickering-type emulsion prepared with an SLN suspension, and the stabilization mechanism remains to be established. Application of Bancroft's rule made it possible to direct the type of emulsion formed, in agreement with the observations reported in the literature [18,23]. The two instabilities observed act at opposite ends of the composition range: the pH drift was most marked in T1, the formulation with the lowest oil content, whereas the sedimented layer appeared only in T5 and T6, the formulations with the highest oil content. The intermediate composition retained for the final vehicle (T4) therefore lies between the two.

From a pharmacological standpoint, the *in vivo* evaluation showed a trend towards shorter closure times at the higher extract concentration. The 0.8 % ECG formulation gave a mean time to macroscopic wound closure of 22 days, numerically similar to that of silver sulfadiazine, the reference drug in the treatment of burns; given the group size and the absence of inferential testing, no conclusion of equivalence can be drawn. The lower ECG load, by contrast, could not be distinguished from the extract-free vehicle: the F0 and F1 mean-score profiles overlapped up to D20 and the mean closure times differed by about one day. Any descriptive effect of the extract is therefore apparent only at the higher load. The trend observed at that load is consistent with the recognised antimicrobial, antioxidant and anti-inflammatory properties of *C. glutinosum*, which act synergistically in the tissue repair process [1,7].

It has been proposed that lipid nanoparticles may improve the local availability of bioactive compounds and prolong their release at the application site, and that a nanometric particle size facilitates skin penetration [13,14]. The present study provides no data in support of such a mechanism for this system: neither the release profile nor the skin penetration of the extract was assessed, and no group received the extract alone.

These results are part of the current momentum toward the pharmaceutical valorisation of African medicinal plants through advanced formulation technologies. They confirm the value of SLNs and Pickering emulsions as carriers of choice for plant extracts for dermatological use [1,8].

Certain limitations must nevertheless be highlighted. The *in vivo* phase was exploratory in nature: it involved three animals per group and the data were analysed descriptively, which precludes any inferential conclusion and requires confirmation in larger groups. The evaluation was moreover limited to the macroscopic closure of the wounds; histological analyses (quality of the regenerated epidermis, dermal organisation, neovascularization) would be necessary to validate the quality of the healing. In addition, the *in vitro* release profile of the active ingredient and skin toxicology studies were not carried out within the framework of this study. Four further limitations must be stated. First, the group size (n = 3) was not based on any sample size calculation, and the observed inter-individual variability is of the same order as the between-group differences, which precludes any conclusion on comparative efficacy. Second, neither the encapsulation efficiency nor the drug loading of the extract was determined, and the final extract-loaded formulations F0, F1 and F2 were not characterised in terms of particle or droplet size; the fate of the extract within the system, and any influence of the extract on the colloidal characteristics of the system, therefore remain unknown. Third, no phytochemical screening of the extract was performed: the bioactive markers are not identified, batch-to-batch reproducibility of the extract cannot be guaranteed, and the attribution of the observed activity to particular classes of metabolites rests solely on the literature. Fourth, no group receiving the aqueous extract alone was included, so that the intrinsic effect of the extract cannot be distinguished from any contribution of the delivery system. These points will have to be addressed in further work before pharmaceutical development can be considered.

5. Conclusion

This study demonstrates the feasibility of preparing a topical Pickering-type lipid emulsion loaded with a hydrophilic extract of *C. glutinosum* together with a solid lipid nanoparticle suspension. The SLNs obtained by hot spontaneous emulsification exhibit favourable physicochemical properties: nanometric size (≈ 40 nm), homogeneous distribution and spherical morphology. The emulsions showed no macroscopic phase separation over 28 days under non-controlled ambient conditions, which does not constitute a stability study. *In vivo*, the formulation containing 0.8 % *C. glutinosum* extract gave a mean time to macroscopic wound closure of 22 days in the rat, numerically shorter than that of the untreated group and in the same range as that of silver sulfadiazine. These differences are descriptive and were not tested statistically; in the absence of a group receiving the extract alone and of any determination of the encapsulation efficiency, no conclusion can be drawn as to whether the formulation enhances the activity of the extract. These results open promising prospects for the development of innovative, biocompatible and effective dermopharmaceutical formulations, integrating extracts from the traditional African pharmacopoeia into modern colloidal carriers. Confirmatory studies on adequately powered groups, including phytochemical characterisation of the extract, determination of the encapsulation efficiency, histology, the *in vitro* release profile and toxicological evaluation, are nevertheless necessary, on larger animal groups, before considering advanced pharmaceutical development.

Conflict of interest statement

The authors declare that they have no conflict of interest in connection with the present study.

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Supplementary Table S1. Individual wound-healing scores according to the Kamoshida scale during the 28-day follow-up (n = 3 per group).

Group	Animal	Time (days)							
		J0	**J4**	**J8**	**J12**	**J16**	**J20**	**J24**	**J28**
Untreated	Rat 1	5	5	5	4	3	3	2	2
	Rat 2	5	5	4	4	4	3	2	2
	Rat 3	5	5	4	5	4	2	2	1
	Mean ± SD	5.00 ± 0.00	5.00 ± 0.00	4.33 ± 0.58	4.33 ± 0.58	3.67 ± 0.58	2.67 ± 0.58	2.00 ± 0.00	1.67 ± 0.58
	Median [min–max]	5 [5–5]	5 [5–5]	4 [4–5]	4 [4–5]	4 [3–4]	3 [2–3]	2 [2–2]	2 [1–2]
0 % (Vehicle)	Rat 1	5	5	4	4	4	4	2	1
	Rat 2	5	5	4	5	4	3	2	1
	Rat 3	5	4	5	5	4	3	2	2
	Mean ± SD	5.00 ± 0.00	4.67 ± 0.58	4.33 ± 0.58	4.67 ± 0.58	4.00 ± 0.00	3.33 ± 0.58	2.00 ± 0.00	1.33 ± 0.58
	Median [min–max]	5 [5–5]	5 [4–5]	4 [4–5]	5 [4–5]	4 [4–4]	3 [3–4]	2 [2–2]	1 [1–2]
Sulfadiazine	Rat 1	5	5	4	4	3	3	2	1
	Rat 2	5	5	5	4	3	2	1	1
	Rat 3	5	5	5	4	2	2	2	1
	Mean ± SD	5.00 ± 0.00	5.00 ± 0.00	4.67 ± 0.58	4.00 ± 0.00	2.67 ± 0.58	2.33 ± 0.58	1.67 ± 0.58	1.00 ± 0.00
	Median [min–max]	5 [5–5]	5 [5–5]	5 [4–5]	4 [4–4]	3 [2–3]	2 [2–3]	2 [1–2]	1 [1–1]
0.2 %	Rat 1	5	5	5	5	4	3	2	2
	Rat 2	5	5	5	5	4	3	3	2
	Rat 3	5	5	5	4	4	4	3	2
	Mean ± SD	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	4.67 ± 0.58	4.00 ± 0.00	3.33 ± 0.58	2.67 ± 0.58	2.00 ± 0.00
	Median [min–max]	5 [5–5]	5 [5–5]	5 [5–5]	5 [4–5]	4 [4–4]	3 [3–4]	3 [2–3]	2 [2–2]
0.8 %	Rat 1	5	5	5	4	3	2	2	1
	Rat 2	5	5	4	4	3	3	2	1
	Rat 3	5	4	4	4	4	3	1	0
	Mean ± SD	5.00 ± 0.00	4.67 ± 0.58	4.33 ± 0.58	4.00 ± 0.00	3.33 ± 0.58	2.67 ± 0.58	1.67 ± 0.58	0.67 ± 0.58
	Median [min–max]	5 [5–5]	5 [4–5]	4 [4–5]	4 [4–4]	3 [3–4]	3 [2–3]	2 [1–2]	1 [0–1]