

## Optimized *Teucrium polium* topical cream improves wound closure in a rat excisional model: A D-optimal design and RBF modelling approach

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**Abstract:** Chronic wounds require topical formulations that combine favorable physicochemical properties with measurable biological activity. In this study, a bio-based, oil-in-water cream containing *Teucrium polium* methanolic extract was developed and optimized using a D-optimal design and a radial basis function (RBF) modeling approach. Three formulation variables, extract content (X1), cetyl alcohol (X2), and shea butter (X3), were evaluated in relation to two responses: spreadability (Y1) and wound closure on Day 7 (Y2). Predictive modeling was performed using an RBF network with leave-one-out cross-validation (LOOCV), which provided an internal estimate of predictive performance ( $R^2$  training = 0.98-0.99;  $R^2$  test = 0.95-0.96). Multi-response optimization identified an optimal formulation containing 8.0% extract, 1.5% cetyl alcohol, and 1.0% shea butter. Experimental confirmation yielded a spreadability of  $54.8 \pm 0.9$  cm<sup>2</sup> and  $84.1 \pm 1.2\%$  wound closure on Day 7. In the confirmatory *in vivo* comparison, a vehicle control, a positive control (Madecassol 1%), and selected formulations were followed through Day 14. F6, F11, and F18 showed the most favorable macroscopic wound-closure profiles, whereas F2 displayed a more moderate effect. These findings support the use of a multivariate optimization strategy for the rational development of bio-based topical creams, while the biological interpretation remains limited to wound closure under the tested conditions.

**Keywords:** wound healing; *Teucrium polium*; topical cream; D-optimal design; radial basis function (RBF) network

### INTRODUCTION

Skin wound healing is a highly coordinated biological process involving overlapping phases of hemostasis, inflammation, proliferation, and remodeling. Effective repair depends on the synchronized regulation of immune responses, fibroblast activation, extracellular matrix deposition, angiogenesis, and re-epithelialization. Disruption of these events may result in delayed healing, chronic wounds, or excessive scar formation [1-3].

Plant-derived bioactive compounds continue to attract considerable attention in dermatological and pharmaceutical research because of their structural diversity and potential to modulate multiple pathways involved in tissue repair. In addition to providing

symptomatic relief, phytochemicals may influence inflammatory mediators, fibroblast activity, collagen organization, and epithelial regeneration, all of which are critical determinants of wound-healing outcomes [4,5]. Within this context, the genus *Teucrium* (Lamiaceae) has received growing interest in both traditional medicine and modern pharmacological research for its dermatological relevance [6].

*Teucrium polium* L. is a Mediterranean and North African aromatic plant widely used in folk medicine. Available evidence indicates that *T. polium* contains flavonoids, phenolics, and other bioactive constituents supporting its relevance for topical pharmaceutical development [6,7]. Experimental studies have provided direct evidence of its wound-oriented biological activity,

including improvement of wound healing in diabetic rats and additional support from phytochemical and toxicity investigations [8,9].

Although clinical evidence remains limited, translational data are beginning to emerge. A randomized triple-blind clinical trial reported improved episiotomy wound healing with *T. polium* ointment compared with control treatment, reinforcing the therapeutic promise of this species and justifying further formulation-oriented investigations [10]. However, despite this encouraging pharmacological background, important gaps remain in the pharmaceutical development of standardized and stable semisolid formulations containing *T. polium*.

From a pharmaceutical development perspective, semisolid topical formulations benefit from quality-by-design approaches in which formulation variables are systematically related to critical quality attributes and intended product performance [11,12]. Recent work has emphasized that the rational design of semisolid dosage forms should integrate composition, microstructure, spreadability, texture, rheological behavior, and cutaneous performance rather than rely solely on empirical adjustment [13].

Accordingly, the present study aimed to develop and optimize a bio-based, oil-in-water topical cream containing *Teucrium polium* methanolic extract and to evaluate its main pharmaceutical characteristics together with its exploratory wound-healing performance in a rat excisional wound model. We hypothesized that a rationally optimized formulation could improve wound closure while maintaining acceptable spreadability and physical quality attributes. At the same time, mechanistic interpretation was deliberately kept cautious because macroscopic wound closure alone does not fully establish the quality or mechanism of tissue repair without complementary endpoints such as histology, collagen remodeling, inflammatory profiling, angiogenesis-related markers, and oxidative stress assessment [2,14,15].

## MATERIALS AND METHODS

### Ethics statement

All animal procedures were conducted in accordance with the ARRIVE 2.0 guidelines and the Guide for the

Care and Use of Laboratory Animals. Ethical approval was obtained from the Ethics Committee of the Pasteur Institute, Algiers, Algeria, under approval voucher number VB09887.

### Plant material

Aerial parts of *Teucrium polium* L. were collected from the Timgad area (Batna Province, northeastern Algeria). The plant material was authenticated by a botanist from the Department of Organism Biology, University of Batna (Batna, Algeria). A voucher specimen was deposited in the herbarium of the same institution under reference number UB-HB-TP-2024-001. The harvested aerial parts were shade-dried at ambient temperature, ground into a fine powder, passed through a 1.0 mm sieve, and stored in sealed bags at 4°C until extraction.

### Extraction of *Teucrium polium*

The extraction procedure was adapted from previously published methods for *Teucrium polium* [19]. Briefly, 5 g of dried powdered aerial parts were mixed with 50 mL of methanol (1:10, w/v) and extracted by magnetic stirring for 10 h at room temperature (25±1°C). The mixture was filtered through Whatman No. 1 filter paper, and the solvent was removed under reduced pressure using a rotary evaporator to obtain the crude dry extract. The dried extract was stored in airtight containers at 4°C until phytochemical analysis and formulation.

### Phytochemical characterization of the extract

The methanolic extract was characterized by determining total phenolic content (TPC) and total flavonoid content (TFC). Total phenolic content was measured using the Folin-Ciocalteu colorimetric method, with gallic acid as the reference standard, and the results were expressed as mg gallic acid equivalents per gram of extract (mg GAE/g extract). Total flavonoid content was determined by the aluminum chloride colorimetric method, using quercetin as the calibration standard, and the results were expressed as mg quercetin equivalents per gram of extract (mg QE/g extract) [20-22]. All measurements were carried out in triplicate and are reported as mean±standard deviation.

## Formulation of the bio-based wound-healing cream

The composition of the bio-based, oil-in-water (O/W) wound-healing cream is summarized in Table 1. Within the experimental design, three formulation constituents were treated as independent variables: X1, methanolic *T. polium* extract; X2, plant-derived cetyl alcohol; and X3, organic shea butter. All other ingredients were kept constant across the experimental runs so that the measured responses could be attributed specifically to variations in X1-X3. The constant ingredients were organic Aloe vera gel (5.0%), organic extra virgin olive oil (4.0%), plant-derived stearic acid (3.0%), glyceryl stearate citrate (2.0%), organic vegetable glycerin (3.0%), tocopherol (0.5%), a COSMOS-compatible preservative system (1.0%), and purified water *quantum satis* to 100% (w/w).

**Table 1.** Phytochemical characterization of the methanolic extract of *Teucrium polium*.

Parameter	Value
Total phenolic content (TPC)	88.6±3.1 mg GAE/g extract
Total flavonoid content (TFC)	39.8±2.4 mg QE/g extract

TPC – total phenolic content; TFC – total flavonoid content; GAE – gallic acid equivalent; QE – quercetin equivalent; SD – standard deviation.

## Experimental design and optimization

A D-optimal design was used to evaluate the effects of the three independent variables on formulation performance. A total of 19 experimental runs (F1-F19) were generated to investigate the influence of extract concentration, cetyl alcohol, and shea butter on the selected responses [23,24]. The investigated factor ranges were as follows: X1, *T. polium* extract (4-8%); X2, cetyl alcohol (1.5-2.5%); and X3, shea butter (1.0-2.0%). Two dependent responses were evaluated: Y1, spreadability (cm<sup>2</sup>), and Y2, wound closure at Day 7 (%).

### Determination of spreadability (Y1)

Spreadability was measured using the parallel-plate method [25,26]. Briefly, 0.5 g of cream was placed at the center of a glass plate, covered with a second plate, and subjected to a 250 g load for 1 min. The diameter of the spread circle (d, cm) was measured, and the spreadability area (S) was calculated according to Equation (1):

$$S = \pi d^2 / 4 \quad (1)$$

where S is expressed in cm<sup>2</sup>.

## Animals and housing

Adult male Wistar rats (*Rattus norvegicus*), weighing 180-220 g, were obtained from the animal facility of the University of Batna 2 (Batna, Algeria). Animals were acclimatized for at least 7 days before the experiment and housed under standard laboratory conditions (22±2°C, 12 h light/dark cycle, controlled humidity) with free access to standard diet and water *ad libitum*.

## Animal study design and excisional wound model

The in vivo biological evaluation was organized as a two-stage study. In accordance with the prespecified outcome structure, Day 7 wound closure was used as the primary optimization endpoint for the D-optimal/RBF workflow. In the confirmatory stage, six experimental groups were compared: vehicle control (base cream), positive control (Madecassol 1%), and four selected formulations (F2, F6, F11, and F18). Each group contained six animals (n = 6), giving a total of 36 rats for the confirmatory comparison. The selected formulations were chosen to represent informative regions of the formulation space: F2 as a lower-extract comparator, F6 as the predicted optimum, and F11 and F18 as high-extract formulations with different structuring-exipient balances. Animals were allocated to groups by simple randomization before wound induction, and wound-area measurements were performed by an assessor blinded to treatment allocation. Wound closure was evaluated using a full-thickness excisional wound model. Wounds were photographed on Day 0, Day 3, Day 7, and Day 14 using a standardized imaging setup with fixed camera-to-wound distance, perpendicular orientation, constant illumination, and a calibration scale placed in the same plane as the wound. Wound area (WA, mm<sup>2</sup>) was quantified by digital planimetry using ImageJ after scale calibration. Each wound area was measured at least in duplicate, and the mean value was retained for analysis [27].

The percentage of wound closure was calculated according to Equation (2):

$$\% \text{ Wound closure} = ((WA_0 - WA_t)/WA_0) \times 100 \quad (2)$$

where  $WA_0$  is the wound area at baseline, and  $WA_t$  is the wound area at time  $t$  [27].

### Model development and validation

Model development and optimization were performed using an RBF network applied to the D-optimal dataset (F1-F19). LOOCV was used to assess predictive performance on the available dataset. This approach was used to maximize the use of the available observations and to obtain an internal estimate of predictive performance [24,28]. The predictive performance of the RBF model was evaluated using the coefficient of determination ( $R^2$ ), root mean square error (RMSE), and mean absolute error (MAE) for both training and test conditions. Residual analysis was also used to assess model adequacy. For validation of the optimized formulation, the predicted response values were compared with experimentally observed means obtained from triplicate preparations. RBF networks were selected because they are well-suited for modeling nonlinear relationships in relatively small experimental datasets. Compared with traditional polynomial regression models commonly used in response surface methodology, RBF models can capture complex factor response interactions without requiring explicit specification of the functional form. This flexibility makes them particularly appropriate for formulation optimization problems involving multivariate and potentially nonlinear relationships.

### Statistical analysis

All data are presented as mean  $\pm$  standard deviation (SD). Statistical analyses were performed using IBM SPSS Statistics 26, with the significance threshold set at  $\alpha = 0.05$ . For validation of the optimized formulation, experimentally observed values were compared with predicted values using a two-sided one-sample  $t$ -test. For the confirmatory wound-closure experiment, one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was used to compare treatment groups at each selected time point. Before ANOVA, normality and homogeneity of variance were assessed using the Shapiro-Wilk and Levene tests, respectively. Day-7 wound closure was treated as the predefined primary optimization endpoint, whereas

Day-14 wound closure was interpreted as confirmatory follow-up in the selected groups.

## RESULTS

### Phytochemical characterization

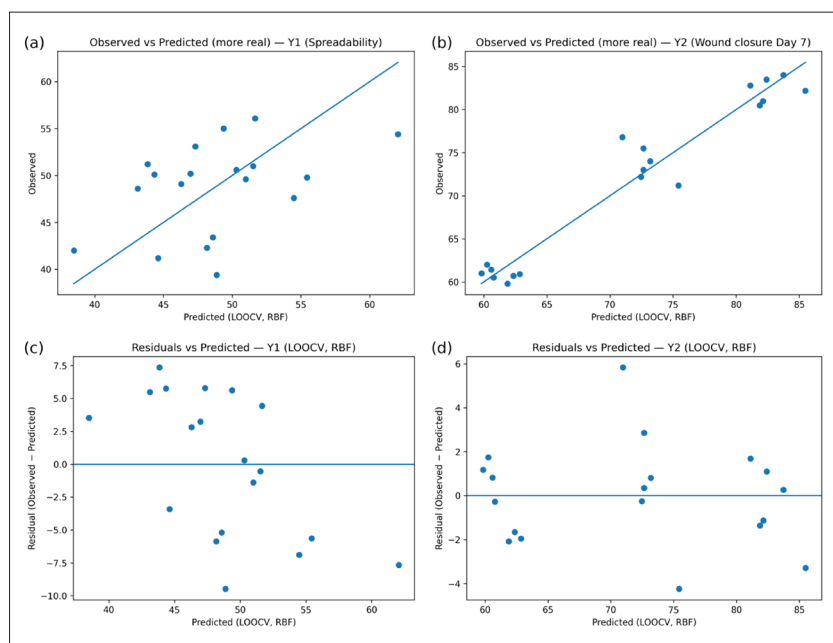
The methanolic extract of *T. polium* showed relatively high levels of phenolic and flavonoid compounds. The total phenolic content was estimated at  $88.6 \pm 3.1$  mg GAE/g extract, whereas the total flavonoid content reached  $39.8 \pm 2.4$  mg QE/g extract. These data are presented in Table 1.

### RBF model performance and predictive ability

The RBF model showed high explanatory and predictive performance for both responses within the studied design space. For Y1 (spreadability), the error metrics remained low and comparable between the training and test sets (RMSE: 0.67 vs. 0.95  $\text{cm}^2$ ; MAE: 0.53 vs. 0.76  $\text{cm}^2$ ). For Y2 (wound closure at Day 7), the increase in test error (RMSE: 0.90 vs. 2.02%; MAE: 0.72 vs. 1.61%) remained limited in absolute terms. The detailed predictive performance metrics are provided in Supplementary Table S2. Residual plots (Fig. 1) showed an approximately random scatter around zero, particularly for Y2, with no marked funnel-shaped pattern, indicating a satisfactory model fit for prediction within the investigated formulation domain.

### Response-surface analysis

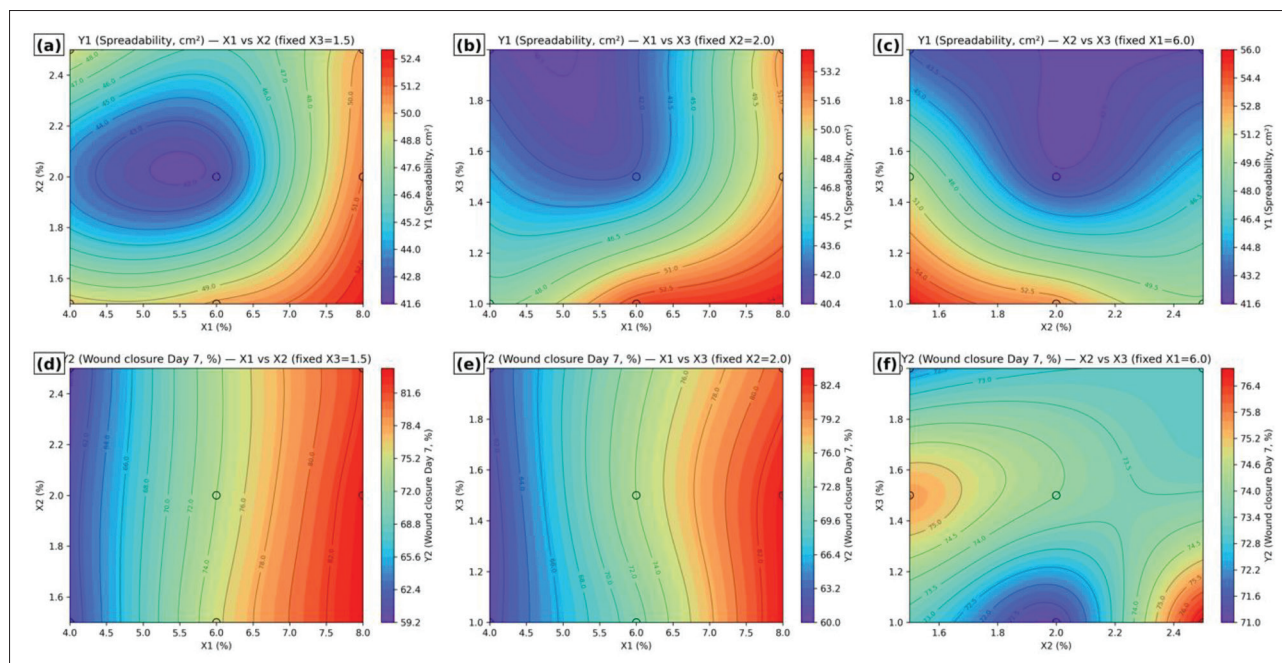
The response-surface contour plots (Fig. 2) illustrate the combined effects of the three formulation factors on Y1 and Y2. For Y1 (Fig. 2a, c), the highest spreadability values were observed at higher extract levels (X1) combined with lower levels of the structuring/lipidic components (X2 and/or X3). For Y2 (Fig. 2d, f), the main response gradient was associated with X1 across the explored range, whereas X2 and X3 provided a secondary non-linear modulation, particularly in the X2-X3 interaction map (Fig. 2f). These patterns indicate that both responses were influenced by the combined variation of the formulation variables.



**Fig. 1.** Predictive performance and residual diagnostics of the RBF model for Y1 (spreadability) and Y2 (wound closure on day 7). Panels (a-b) show observed versus predicted values with the identity line; panels (c-d) show residuals versus predicted values.

## Experimental confirmation of the optimum

The optimized formulation (X1=8.0%, X2=1.5%, X3=1.0%) was prepared in triplicate (n=3) and experimentally evaluated for Y1 and Y2. The experimental means were in close agreement with the RBF predictions. For Y1, the predicted value (54.6 cm<sup>2</sup>) was close to the observed value (54.8±0.9 cm<sup>2</sup>; p=0.88), corresponding to a relative error of approximately 0.37%. For Y2, the predicted value (84.4%) was close to the observed value (84.1±1.2%; P=0.91), corresponding to a relative error of approximately 0.36%. The detailed predicted-versus-observed comparison is provided in Supplementary Table S3. Taken together, these findings support the reliability of the RBF-based optimization within the investigated factor ranges.

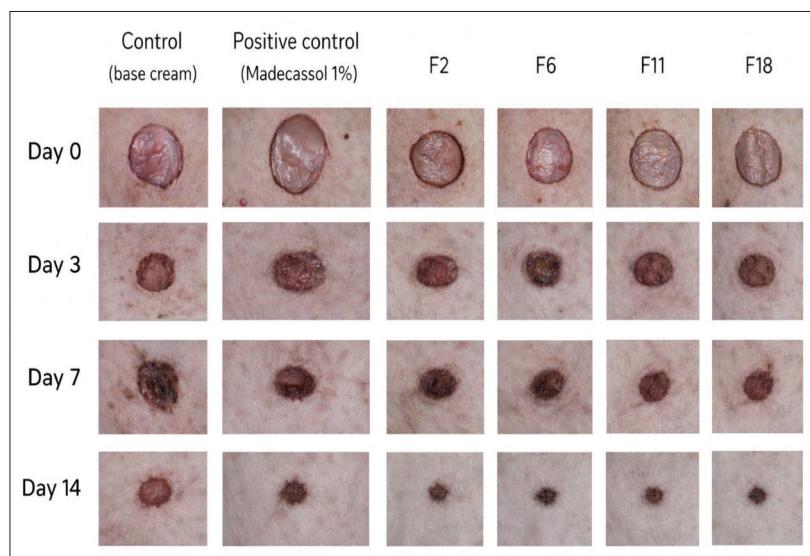


**Fig. 2.** Response surface contour plots of (Y1) spreadability and (Y2) wound closure at day 7 as functions of formulation factors. Panels (a-c) show Y1 as a function of the factor pairs (X1, X2), (X1, X3), and (X2, X3) while holding the remaining factor constant; panels (d-f) show the corresponding plots for Y2 (fixed-factor values indicated on each panel).

**Table 2.** Wound closure percentages on Days 3, 7, and 14 in the control, positive control, and selected *Teucrium polium* cream groups.

Formulation	Day 3 (%)	Day 7 (%)	Day 14 (%)
Control (base cream)	28.5±4.2 <sup>c</sup>	52.0±5.1 <sup>d</sup>	80.0±4.0 <sup>c</sup>
Positive control (Madecassol 1%)	38.5±3.5 <sup>b</sup>	74.0±2.8 <sup>b</sup>	94.0±1.6 <sup>a</sup>
F2 (4% extract)	31.0±4.0 <sup>c</sup>	60.5±3.6 <sup>c</sup>	86.0±3.5 <sup>b</sup>
F6 (8% extract)	45.5±3.2 <sup>a</sup>	84.0±2.1 <sup>a</sup>	97.0±1.1 <sup>a</sup>
F11 (8% extract)	43.0±3.6 <sup>ab</sup>	83.5±2.4 <sup>a</sup>	96.5±1.2 <sup>a</sup>
F18 (8% extract)	41.0±3.8 <sup>ab</sup>	80.5±2.6 <sup>a</sup>	95.0±1.5 <sup>a</sup>

Values are expressed as mean±SD (n=6 per group). Values with different superscript letters within the same column are significantly different at  $p < 0.05$  according to one-way ANOVA followed by Tukey's post hoc test.



**Fig. 3.** Representative macroscopic photographs illustrating wound healing progression across different treatment groups at days 0, 3, 7, and 14. Rows correspond to time points, while columns represent experimental groups (control, positive control, F2, F6, F11, and F18). A progressive reduction in wound area is observed over time; the treated groups, particularly the positive control (Madecassol 1%) and extract-based formulations show enhanced wound contraction and tissue regeneration compared to the base cream control. The most pronounced healing effects are evident at days 7 and 14.

### Wound-healing kinetics and formulation ranking

Wound closure percentages for the vehicle control, positive control, and selected formulations are presented in Table 2. Day-7 wound closure was retained as the predefined primary optimization endpoint, whereas Day-14 measurements were collected as confirmatory follow-up in the selected groups. At Day 3, F6 showed the highest wound closure (45.5±3.2%, a), whereas F11 (43.0±3.6%, ab) and F18 (41.0±3.8%, ab) occupied an intermediate position. The positive control (Madecassol 1%) reached 38.5±3.5% (b), while F2 (31.0±4.0%, c) remained close to the vehicle control

**Table 3.** Final product specifications of the optimized cream.

Parameter	Result
Appearance	Homogeneous cream
Odor	Mild herbal odor
pH	5.6±0.1
Spreadability	54.8±0.9 cm <sup>2</sup>
Physical stability (centrifugation, 24 h)	Compliant
Wound closure (Day 7)	84.1±1.2 %

(28.5±4.2%, c). By Day 7, the ranking became more pronounced: F6 (84.0±2.1%), F11 (83.5±2.4%), and F18 (80.5±2.6%) formed the highest-performing group (a), exceeding the positive control (74.0±2.8%, b), F2 (60.5±3.6%, c), and the vehicle control (52.0±5.1%, d). By Day 14, F6, F11, F18, and the positive control reached similarly high wound-closure values (group a), whereas F2 remained intermediate (86.0±3.5%, b) and the vehicle control remained the least effective (80.0±4.0%, c). Overall, these data indicate that the optimized high-extract formulations, particularly F6 and F11, provided the most favorable macroscopic wound-closure response under the tested conditions

These results show that wound closure remained formulation-dependent throughout the confirmatory follow-up. Among the tested creams, F6 and F11 consistently showed the strongest performance, whereas F18 remained intermediate, and F2 showed only moderate activity despite containing the botanical extract. Representative photographs of wound appearance at Days 0, 3, 7, and 14 are presented in Fig. 3 to complement the planimetric assessment.

### Final product specifications of the optimized cream

The optimized cream was homogeneous, had a mild herbal odor, and met the centrifugation-based physical stability criterion over 24 h. Its pH (5.6±0.1), spreadability (54.8±0.9 cm<sup>2</sup>), and Day 7 wound closure (84.1±1.2%) were consistent with the experimental confirmation of the optimized formulation. The final

physicochemical characteristics of the optimized cream are presented in Table 3.

## DISCUSSION

This study supports a structured and resource-efficient strategy for optimizing a bio-based *T. polium* topical cream by jointly targeting a user-relevant attribute, spreadability, and an efficacy-oriented endpoint, wound closure at Day 7. This development logic is consistent with quality-by-design principles applied to topical semisolid products, in which formulation variables are linked to critical quality attributes and intended in-use performance rather than adjusted empirically [15-17].

From a formulation perspective, the simultaneous optimization of spreadability and wound closure is justified because the performance of semisolid systems depends strongly on microstructure, internal consistency, yield behavior, film persistence, and physical stability after application. These properties are influenced by the balance between the dispersed and continuous phases as well as by structuring excipients such as cetyl alcohol and shea butter, which can modify rheological behavior and practical spreading characteristics. In topical emulsion-based semisolids, even relatively small changes in excipient composition may alter stability, sensorial properties, and in-use performance, which supports their interpretation as critical material attributes within a QbD framework [15,16,29].

Spreadability measured by parallel-plate methods remains widely used in topical formulation studies because it provides a simple approximation of application behavior. However, meaningful interpretation requires careful reporting of protocol variables, including load, time, plate geometry, and testing conditions, since these parameters can substantially affect the measured response. Accordingly, the spreadability values reported in the present study should be interpreted within the limits of the selected protocol rather than as universal formulation constants [24,25].

The phytochemical characterization of the methanolic extract showed appreciable total phenolic and flavonoid contents, indicating that the selected botanical material was chemically rich in classes of compounds commonly associated with antioxidant and bioactive potential. This observation is consistent with previous

reports describing *T. polium* as a source of phenolics, flavonoids, and related secondary metabolites with possible pharmacological relevance [9,10]. At the same time, these measurements provide only a preliminary level of extract standardization. Because no chromatographic profiling of the tested batch was performed, the individual compounds responsible for the observed activity remain undefined, and batch-to-batch reproducibility cannot yet be discussed in greater chemical detail [9,10].

Biologically, the improved wound closure observed for the optimized and high-extract formulations is plausible in light of prior work on *T. polium*. In particular, topical methanolic extract-based formulations have previously been associated with improved wound-oriented outcomes in experimental models, and at least one study combined biological assessment with polyphenolic profiling, thereby strengthening the link between composition and activity [11,12]. Complementary evidence also exists for other *T. polium*-based topical systems, including honey-based formulations that improved wound repair-related parameters in rats [13]. In the present study, the inclusion of both a vehicle control and a positive control improved the interpretability of the confirmatory *in vivo* comparison.

The optimization results also suggest that biological performance was not governed by extract concentration alone. Although the highest-performing formulations contained 8% extract, differences among F6, F11, and F18 indicate that excipient balance contributed to the final wound-closure response. This interpretation is supported by the response-surface analysis, which showed that X2 and X3 exerted a secondary but non-negligible modulation of Y2. Such behavior is expected in semisolid formulations, where the vehicle does not merely carry the active substance but also influences residence time, film formation, hydration behavior, and local availability at the wound surface [16,17]. The observed superiority of F6 and F11, therefore, supports a multivariate optimization logic rather than a simple single-factor maximization strategy [22,23].

Methodologically, the use of an RBF model with LOOCV is reasonable for a relatively small DoE dataset because it permits efficient use of the available observations while providing an internal estimate of predictive performance [23,27]. The close agreement between predicted and experimentally observed values

at the optimum further supports the utility of the optimization workflow within the investigated factor ranges. However, internal validation should not be interpreted as equivalent to independent external validation. From a modeling standpoint, the robustness of the optimization could be further strengthened by confirming results on additional nearby formulations or independent batches, ideally with uncertainty estimates reported alongside predicted responses [23,27].

Despite these strengths, the biological interpretation of the present findings should be approached with caution. Wound closure is a useful screening endpoint, but in rodent excisional models, it may partly reflect contraction rather than complete regenerative repair [26]. Current wound-healing literature emphasizes that a more informative biological assessment should include re-epithelialization, collagen deposition and remodeling, inflammatory cell infiltration, angiogenesis-related parameters, and oxidative stress markers [30-34]. Without such complementary endpoints, the present study supports improved wound closure under the tested conditions, but does not fully establish the quality or mechanism of tissue repair [26,32,33].

This point is important because current wound-care research increasingly evaluates not only whether wounds close, but also how they close. Biomaterial-based and advanced wound-healing studies now place considerable emphasis on immune regulation, redox balance, vascularization, and matrix remodeling as complementary indicators of repair quality. In that broader context, the *T. polium* cream may be viewed as a promising optimized prototype, but not yet as a fully characterized mechanistic wound-healing system [31,34,37].

Another limitation concerns the scope of the *in vivo* design. The confirmatory arm was intentionally focused on a selected subset of formulations and used Day 14 as an extended follow-up endpoint rather than as a second optimization target. This staged strategy is appropriate for exploratory formulation optimization because it allows the primary screening endpoint to remain anchored to Day 7 while providing additional biological confirmation in the most informative groups. Even so, the *in vivo* study remains limited by the absence of histological analysis, mechanistic biomarkers, and a broader external validation framework for the predictive model.

From a translational standpoint, the present formulation strategy also fits within a broader movement toward multifunctional wound-care systems that combine bioactive plant-derived compounds with rational carrier design. Recent reviews on plant extracts, natural biomaterials, and smart wound platforms suggest that future formulations will increasingly be expected to integrate not only application quality and efficacy, but also stability, responsiveness, and mechanistic traceability [35,38-40]. In that sense, the present study may serve as a useful intermediate step between empirical herbal formulations and more advanced evidence-based topical systems.

Overall, the workflow adopted in this study is consistent with established multivariate optimization practice in formulation development, where DoE supports efficient mapping of factor-response relationships and facilitates multi-response decision making under practical experimental constraints [22,23]. Within the limits of the measured endpoints, the optimized *T. polium* cream combined acceptable physicochemical characteristics with improved wound-closure performance *in vivo*. The study, therefore, provides a useful formulation-development framework while also identifying the phytochemical and biological extensions needed for stronger mechanistic and translational positioning [12,15,35].

## CONCLUSIONS

The present study demonstrates that a bio-based, oil-in-water topical cream containing *Teucrium polium* extract can be successfully developed and optimized using a data-driven strategy combining D-optimal design and radial basis function modelling. The optimized formulation showed satisfactory physicochemical characteristics, good spreadability, and improved wound closure in a rat excisional wound model. The confirmatory comparison against both a vehicle control and a positive control strengthened the interpretation of the selected formulations, and the Day-14 follow-up supported the persistence of the wound-closure response in the best-performing creams. The preliminary phytochemical characterization of the extract indicated appreciable levels of phenolic and flavonoid compounds, but no chromatographic profiling was performed. Because the biological assessment was limited mainly to macroscopic wound closure, further investigations,

including histological evaluation, collagen remodeling, inflammatory markers, angiogenesis-related parameters, and oxidative-stress assessment, are required to better elucidate the mechanisms underlying the observed effects. Overall, the proposed multivariate optimization workflow provides a practical framework for the rational development of plant-based semisolid topical formulations.

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**Author Contributions:** Conceptualization, MMA and MSE; methodology, MMA, MSE, and KN; software, MSE; validation, MMA, MSE, KN, and BI; formal analysis, MSE and KN; investigation, MMA, MSE, KN, and BI; resources, MMA; data curation, MSE and BI; writing original draft preparation, MSE; writing review and editing, MMA, KN, and BI; visualization, MSE and BI; supervision, MMA; project administration, MMA; funding acquisition, MMA All authors have read and agreed to the published version of the manuscript.

**Conflict of interest disclosure:** The authors declare no conflict of interest.

**Data availability:** The data presented in this study are openly available in Zenodo at DOI: <https://doi.org/10.5281/zenodo.18971814>

## REFERENCES

- Guo S, DiPietro LA. Factors affecting wound healing. *J Dent Res.* 2010;89(3):219-29. <https://doi.org/10.1177/0022034509359125>
- Rodrigues M, Kosaric N, Bonham CA, Gurtner GC. Wound healing: a cellular perspective. *Physiol Rev.* 2019;99(1):665-706. <https://doi.org/10.1152/physrev.00067.2017>
- Velnar T, Bailey T, Smrkolj V. The wound healing process: an overview of the cellular and molecular mechanisms. *J. Int. Med. Res.* 2009;37(5):1528-42. <https://doi.org/10.1177/147323000903700531>
- Wilkinson HN, Hardman MJ. Wound healing: cellular mechanisms and pathological outcomes. *Open Biol.* 2020;10(9):200223. <https://doi.org/10.1098/rsob.200223>
- Mamun AA, Shao C, Geng P, Wang S, Xiao J. Recent advances in molecular mechanisms of skin wound healing and its treatments. *Front Immunol.* 2024;15:1395479. <https://doi.org/10.3389/fimmu.2024.1395479>
- Patenall BL, Carter KA, Ramsey MR. Kick-Starting Wound Healing: A Review of Pro-Healing Drugs. *Int J Mol Sci.* 2024;25(2):1304. <https://doi.org/10.3390/ijms25021304>
- Flynn K, Mahmoud NN, Sharifi S, Gould LJ, Mahmoudi M. Chronic wound healing models. *ACS Pharmacol Transl Sci.* 2023;6(5):783-801. <https://doi.org/10.1021/acspsci.3c00030>
- Sarian MN, Zulkefli N, Zain SMC, Maniam S, Fakurazi S. A review with updated perspectives on in vitro and in vivo wound healing models. *Turk J Biol.* 2023;47(4):236-46. <https://doi.org/10.55730/1300-0152.2659>
- Bahramikia S, Hemmati Hassan Gavyar P, Yazdanparast R. *Teucrium polium* L: an updated review of phytochemicals and biological activities. *Avicenna J Phytomed.* 2022;12(3):224-40. <https://doi.org/10.22038/AJP.2021.19155>
- Sharifi-Rad M, Pohl P, Epifano F, Zengin G, Jaradat N, Messaoudi M. *Teucrium polium* (L.): phytochemical screening and biological activities at different phenological stages. *Molecules.* 2022;27(5):1561. <https://doi.org/10.3390/molecules27051561>
- Fallah Huseini H, Abdolghaffari AH, Ahwazi M, Jasemi E, Yaghoobi M, Ziaee M. Topical application of *Teucrium polium* can improve wound healing in diabetic rats. *Int J Low Extrem Wounds.* 2020;19(2):132-8. <https://doi.org/10.1177/1534734619868629>
- Chabane S, Boudjelal A, Keller M, Doubakh S, Potterat O. *Teucrium polium* wound healing potential, toxicity and polyphenolic profile. *S Afr J Bot.* 2021;137:228-35. <https://doi.org/10.1016/j.sajb.2020.10.017>
- Alizadeh AM, Sohanaki H, Khaniki M, Mohaghheghi MA, Ghmami G, Mosavi M. The effect of *Teucrium polium* honey on the wound healing and tensile strength in rat. *Iran J Basic Med Sci.* 2011;14(6):499-505. <https://doi.org/10.22038/ijbms.2011.5048>
- Rouzbahani AM, Dolatian M, Jahedbozorgan T, Mojab F, Tajik M. Evaluation of the effect of *Teucrium polium* ointment on the episiotomy wound healing in primiparous women. *Immunopathol Persa.* 2021;7(2):e33. <https://doi.org/10.34172/ipp.2021.33>
- Namjoshi S, Dabbaghi M, Roberts MS, Grice JE, Mohammed Y. Quality by design: development of the quality target product profile for semisolid topical products. *Pharmaceutics.* 2020;12(3):287. <https://doi.org/10.3390/pharmaceutics12030287>
- Herbig ME, Evers D-H, Gorissen S, Köllmer M. Rational design of topical semi-solid dosage forms-how far are we? *Pharmaceutics.* 2023;15(7):1822. <https://doi.org/10.3390/pharmaceutics15071822>
- Mohammed YH, Namjoshi SN, Jung N, Windbergs M, Benson HAE, Grice JE, Raney SG, Roberts MS. Topical semi-solid drug product critical quality attributes with relevance to cutaneous bioavailability and pharmacokinetics: Part I-bioequivalence of acyclovir topical creams. *Pharm Res.* 2024;41(7):1507-20. <https://doi.org/10.1007/s11095-024-03736-9>
- Garg A, Aggarwal D, Garg S, Singla AK. Spreading of semisolid formulations: an update. *Pharm Technol.* 2002;26(9):84-105.
- Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* 1999;299:152-78. [https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1)
- Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nat Protoc.* 2007;2(4):875-7. <https://doi.org/10.1038/nprot.2007.102>
- Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary

- colorimetric methods. *J Food Drug Anal.* 2002;10(3):178-82. <https://doi.org/10.38212/2224-6614.2748>
22. Bezerra MA, Santelli RE, Oliveira EP, Villar LS, Escalera LA. Response surface methodology as a tool for optimization in analytical chemistry. *Talanta.* 2008;76(5):965-77. <https://doi.org/10.1016/j.talanta.2008.05.019>
  23. Fukuda IM, Pinto CFF, Moreira CS, Saviano AM, Lourenço FR. Design of experiments applied to pharmaceutical and analytical quality by design. *Braz J Pharm Sci.* 2018;54(Special):e01006. <https://doi.org/10.1590/S2175-97902018000001006>
  24. Jelvehgari M, Rashidi MR, Mirza Mohammadi SH. Adhesive and spreading properties of a pharmaceutical gel composed of cellulose polymer. *Jundishapur J Nat Pharm Prod.* 2007;2(1):45-58.
  25. Al-Barghouthy EY, Hamed SH, Mehyar GF, Alkhatib HS. Comparative evaluation of spreadability measurement methods for topical semisolid formulations: a scoping review. *Gels.* 2025;11(12):1006. <https://doi.org/10.3390/gels11121006>
  26. Chen L, Mirza R, Kwon Y, DiPietro LA, Koh TJ. The murine excisional wound model: contraction revisited. *Wound Repair Regen.* 2015;23(6):874-7. <https://doi.org/10.1111/wrr.12338>
  27. Lv L, Song X, Sun W. Modify leave-one-out cross validation by moving validation samples around random normal distributions: move-one-away cross validation. *Appl Sci.* 2020;10(7):2448. <https://doi.org/10.3390/app10072448>
  28. Ansari M, Darvishi A. A review of the current state of natural biomaterials in wound healing applications. *Front Bioeng Biotechnol.* 2024;12:1309541. <https://doi.org/10.3389/fbioe.2024.1309541>
  29. Las Heras K, Garcia-Orue I, Rancan F, Igartua M, Santos-Vizcaino E, Hernandez RM. Modulating the immune system towards a functional chronic wound healing: a biomaterials and nanomedicine perspective. *Adv Drug Deliv Rev.* 2024;210:115342. <https://doi.org/10.1016/j.addr.2024.115342>
  30. Pastar I, Stojadinovic O, Yin NC, Ramirez H, Nusbaum AG, Sawaya A, et al. Epithelialization in wound healing: a comprehensive review. *Adv Wound Care (New Rochelle).* 2014;3(7):445-64. <https://doi.org/10.1089/wound.2013.0473>
  31. Mathew-Steiner SS, Roy S, Sen CK. Collagen in wound healing. *Bioengineering (Basel).* 2021;8(5):63. <https://doi.org/10.3390/bioengineering8050063>
  32. Lopes FB, Sarandy MM, Novaes RD, Valacchi G, Gonçalves RV. OxInflammatory Responses in the Wound Healing Process: A Systematic Review. *Antioxidants (Basel).* 2024;13(7):823. <https://doi.org/10.3390/antiox13070823>
  33. Herman A, Leska A, Wińska P, Herman AP. Plant extracts as modulators of the wound healing process – preliminary study. *Int J Mol Sci.* 2025;26(15):7490. <https://doi.org/10.3390/ijms26157490>
  34. Sabarudin NS, Ab Ghani N, Ahmat N, Harlin EW, Hao LQ, Handajani J, Nor FM, Md Fadilah NI, Maarof M, Fauzi MB. Harnessing plant bioactive compounds in biomaterial scaffolds for advanced wound healing: a comprehensive review. *Biomedicines.* 2025;13(10):2414. <https://doi.org/10.3390/biomedicines13102414>
  35. Song H, Hao D, Zhou J, Farmer D, Wang A. Development of pro-angiogenic skin substitutes for wound healing. *Wound Repair Regen.* 2024;32(3):208-16. <https://doi.org/10.1111/wrr.13154>
  36. Joorabloo A, Liu T. Smart theranostics for wound monitoring and therapy. *Adv Colloid Interface Sci.* 2024;330:103207. <https://doi.org/10.1016/j.cis.2024.103207>
  37. Wang X, Yang Y, Zhao W, et al. Recent advances of hydrogels as smart dressings for diabetic wounds. *J Mater Chem B.* 2024;12(5):1126-48. <https://doi.org/10.1039/D3TB02355A>
  38. Wang F, Yao J, Zuo H, Jiao Y, Wu J, Meng Z. Diverse-origin exosomes therapeutic strategies for diabetic wound healing. *Int J Nanomedicine.* 2025;20:7375-402. <https://doi.org/10.2147/IJN.S519379>
  39. Farahani M, Shafiee A. Wound healing: from passive to smart dressings. *Adv Healthc Mater.* 2021;10(16):2100477. <https://doi.org/10.1002/adhm.202100477>
  40. Duarte J, Mascarenhas-Melo F, Pires PC, Veiga F, Paiva-Santos AC. Multifunctional hydrogel-based therapies for chronic diabetic wound healing. *Eur Polym J.* 2024;211:113026. <https://doi.org/10.1016/j.eurpolymj.2024.113026>

## SUPPLEMENTARY MATERIAL

**Supplementary Table S1.** Composition of the wound-healing cream used as the base formulation for the experimental design (w/w). X1–X3 were varied as design factors; all other components were fixed; purified water was added q.s. to 100%.

No.	Ingredient (INCI/common)	Function	Content (% w/w)
1	Teucrium polium extract (methanolic)	Wound-healing active	Variable (X1)
2	Aloe vera gel (organic)	Soothing; healing support	5.0
3	Extra virgin olive oil (organic)	Emollient; occlusive	4.0
4	Cetyl alcohol (plant origin)	Consistency agent	Variable (X2)
5	Stearic acid (plant origin)	Co-emulsifier	3.0
6	Shea butter (organic)	Barrier repair; occlusive	Variable (X3)
7	Glyceryl stearate citrate	Natural O/W emulsifier	2.0
8	Vegetable glycerin (organic)	Humectant	3.0
9	Tocopherol (vitamin E)	Antioxidant (oil phase)	0.5
10	COSMOS-compatible preservative system	Microbiological safety	1.0
11	Purified water	q.s. to 100%	q.s.

**Supplementary Table S2.** Experimental variables and objectives

Independent variables	Level 1	Level 2	Level 3
X1: Teucrium polium extract (%)	4	6	8
X2: Cetyl alcohol (%)	1.5	2.0	2.5
X3: Shea butter (%)	1.0	1.5	2.0
Dependent variables	Constraints		
Y1: Spreadability (cm <sup>2</sup> )	Maximum		
Y2: Wound closure at Day 7 (%)	Maximum		

**Supplementary Table S3.** Experimental matrix and responses (F1–F19)

	X1	X2	X3	Y1 (cm <sup>2</sup> )	Y2 (% Day 7)
F1	4.0	2.0	2.0	41.2	62.0
F2	4.0	1.5	2.0	48.6	60.5
F3	6.0	2.0	1.0	53.1	71.2
F4	8.0	2.5	2.0	39.4	82.8
F5	6.0	2.0	1.5	42.3	74.0
F6	8.0	1.5	1.0	54.4	84.0
F7	6.0	1.5	1.5	50.2	75.5
F8	6.0	2.5	2.0	42.0	73.0
F9	6.0	2.5	1.0	49.8	76.8
F10	6.0	1.5	2.0	43.4	72.2

	X1	X2	X3	Y1 (cm <sup>2</sup> )	Y2 (% Day 7)
F11	8.0	2.0	1.5	51.0	83.5
F12	4.0	1.5	1.0	56.1	61.4
F13	4.0	2.5	1.5	49.1	59.8
F14	4.0	2.5	1.0	55.0	60.9
F15	4.0	2.0	1.0	47.6	61.0
F16	4.0	1.5	1.5	49.6	60.7
F17	8.0	2.0	2.0	51.2	81.0
F18	8.0	1.5	2.0	50.6	80.5
F19	8.0	2.5	1.5	50.1	82.2

**Supplementary Table S4.** RBF model performance indicators

Response	R <sup>2</sup> (training)	R <sup>2</sup> (test)	RMSE (training)	RMSE (test)	MAE (training)	MAE (test)
Y1 (Spreadability, cm <sup>2</sup> )	0.98	0.96	0.67 cm <sup>2</sup>	0.95 cm <sup>2</sup>	0.53 cm <sup>2</sup>	0.76 cm <sup>2</sup>
Y2 (Wound closure, Day 7, %)	0.99	0.95	0.90 %	2.02 %	0.72 %	1.61 %

**Supplementary Table S5.** RBF-predicted versus observed values for the optimized formulation

Response	Predicted (RBF)	Observed (n=3)	P
Y1 (cm <sup>2</sup> )	54.6	54.8±0.9	0.88
Y2 (% Day 7)	84.4	84.1±1.2	0.91