

Integrated In Vivo and In Silico Evaluation of *Centella asiatica* Transfersomes and *Rosmarinus officinalis* Nanoemulsions on VEGF Expression During Caesarean Wound Healing in Rats

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Abstract

Caesarean section wound healing requires coordinated angiogenesis, tissue regeneration, and extracellular matrix remodeling. This study evaluated the effects of a combination of *Centella asiatica* transfersomes and *Rosmarinus officinalis* nanoemulsions on vascular endothelial growth factor (VEGF) expression during Caesarean wound healing using integrated *in vivo* and *in silico* approaches. Archived formalin-fixed paraffin-embedded (FFPE) wound tissue samples from a previous study involving 25 female Wistar rats (*Rattus norvegicus*) allocated into five experimental groups were analyzed for VEGF expression using immunohistochemistry on day 14. Molecular docking against VEGFR2 was performed using YASARA Structure, and ADMET properties were predicted using pkCSM. The treatment groups showed improved wound healing and modulation of VEGF expression compared with the controls, with T3 producing the most favorable response. Molecular docking demonstrated that *C. asiatica* phytochemicals generally exhibited stronger binding affinities toward VEGFR2 than *R. officinalis* compounds. Kaempferol showed the highest binding affinity (9.7960 kcal/mol), while Asiaticoside formed stable interactions with key VEGFR2 residues. ADMET analysis indicated favorable pharmacokinetic and safety profiles for most compounds. These findings suggest that *C. asiatica* transfersomes combined with *R. officinalis* nanoemulsions may accelerate Caesarean wound healing by enhancing VEGF-associated angiogenesis and improving local delivery of bioactive phytochemicals.

Keywords: Angiogenesis; *Centella asiatica*; Molecular docking; *Rosmarinus officinalis*; VEGF; Wound healing.

INTRODUCTION

The global rate of Caesarean section (CS) has increased substantially over recent decades, reaching approximately 21.1% of births worldwide and projected to rise to 28.5% by 2030 (Betran et al., 2021). Although CS is an essential obstetric procedure that significantly reduces maternal and neonatal morbidity and mortality when medically indicated, its increasing prevalence has been accompanied by a growing burden of postoperative complications, particularly impaired wound healing and surgical site infections (SSIs) (Chien, 2021). SSIs remain among the most common postoperative complications, affecting approximately 3–15% of surgical procedures (Hayasaka et al., 2026). These complications adversely affect maternal physical and psychological well-being and impose substantial economic burdens on healthcare systems through prolonged hospitalization, increased readmission rates, and additional treatment requirements (Guo et al., 2022; Hayasaka et al., 2026). Consequently,

improving wound healing outcomes following CS has become an important clinical priority in contemporary maternal healthcare.

Wound healing is a complex and highly regulated biological process that restores tissue integrity after injury. This process consists of four overlapping phases: hemostasis, inflammation, proliferation, and remodeling (Jin et al., 2025). Following hemostatic clot formation and inflammatory cell recruitment, the proliferative phase initiates tissue regeneration through extracellular matrix synthesis, fibroblast activation, and angiogenesis (Jin et al., 2025). Among these events, angiogenesis is particularly critical because newly formed blood vessels provide oxygen, nutrients, and immune mediators required for tissue repair (Chandra et al., 2026). Impaired angiogenesis can result in inadequate tissue perfusion, delayed wound closure, chronic inflammation, and increased susceptibility to infection, thereby compromising healing outcomes (Jin et al., 2025).

Vascular Endothelial Growth Factor (VEGF) is a key regulator of angiogenesis and plays a central role in wound repair. VEGF stimulates endothelial cell proliferation, migration, differentiation, and survival, thereby promoting the formation of new microvascular networks within damaged tissues (Jin et al., 2025). Increased VEGF expression has been associated with enhanced granulation tissue formation, accelerated wound closure, and improved tissue regeneration (Chandra et al., 2026; Palomeque Chávez et al., 2025). Conversely, insufficient VEGF activity may impair neovascularization and contribute to delayed healing and chronic wound development (Chandra et al., 2026). Owing to its crucial role in angiogenesis, VEGF is widely recognized as an important biomarker and therapeutic target in wound-healing research (Jin et al., 2025).

Natural products have attracted considerable attention as alternative therapeutic agents for wound management. Among these, *Centella asiatica* (L.) Urb. is one of the most extensively studied medicinal plants due to its long-standing use in traditional medicine and its demonstrated wound-healing properties. The biological activity of *C. asiatica* is primarily attributed to pentacyclic triterpenoid compounds, including asiaticoside, madecassoside, asiatic acid, and madecassic acid (Bandopadhyay et al., 2023). Previous studies have shown that these compounds promote fibroblast proliferation, collagen synthesis, extracellular matrix remodeling, and angiogenesis while exerting anti-inflammatory and antioxidant effects (Arribas-López et al., 2022; Witkowska et al., 2024). Furthermore, *C. asiatica* has been reported to stimulate the expression of growth factors, including VEGF, thereby facilitating vascular development and tissue regeneration (Arribas-López et al., 2022). These effects are mediated through multiple signaling pathways, including TGF- β /Smad and NF- κ B, which contribute to coordinated wound repair (Handayani et al., 2025).

Similarly, *Rosmarinus officinalis* L. has demonstrated considerable therapeutic potential in regenerative medicine. Its major bioactive constituents include carnosic acid, carnosol, rosmarinic acid, and ursolic acid, compounds known for their antioxidant, anti-inflammatory, and antimicrobial activities (Aly et al., 2026; Ibrahim et al., 2022). These phytochemicals help reduce oxidative stress and microbial contamination, both of which are critical factors influencing wound healing (Li Pomi et al., 2023; J. Xu et al., 2025). In addition, rosemary-derived compounds have been shown to enhance fibroblast migration and tissue regeneration through mechanisms involving activation of cutaneous TRPA1 receptors and modulation of inflammatory mediators such as IL-6 and TNF- α (Aly et al., 2026; Rapp et al., 2025). The multifaceted biological activities of *R. officinalis* suggest its potential to complement the

angiogenic and regenerative effects of *C. asiatica* during wound repair.

Despite their promising pharmacological properties, the therapeutic application of plant-derived compounds is often limited by poor aqueous solubility, low stability, and insufficient penetration through the skin barrier. Nanotechnology-based drug delivery systems have emerged as effective strategies to overcome these limitations and improve the bioavailability of phytochemicals. Transfersomes are highly deformable lipid vesicles capable of penetrating the stratum corneum and delivering active compounds into deeper skin layers (Matei et al., 2021). Nanoemulsions, in contrast, provide stable colloidal systems that enhance solubility, protect bioactive molecules from degradation, and facilitate sustained release at the target site (Umekar et al., 2025). Previous studies have demonstrated that these nanocarriers improve wound-healing outcomes by enhancing local drug delivery, accelerating re-epithelialization, and promoting tissue regeneration (Matei et al., 2021; Umekar et al., 2025).

Although the wound-healing activities of *C. asiatica* and *R. officinalis* have been investigated individually, evidence regarding their combined application in advanced nanoformulations remains limited. Furthermore, no study has comprehensively evaluated the effects of *C. asiatica* transfersomes and *R. officinalis* nanoemulsions on VEGF expression during Caesarean wound healing. The integration of in vivo experimentation with in silico approaches may provide valuable mechanistic insights into the molecular interactions underlying the therapeutic effects of these phytochemicals. Therefore, this study aimed to evaluate the effects of combined *Centella asiatica* transfersomes and *Rosmarinus officinalis* nanoemulsions on VEGF expression during Caesarean wound healing in rats using integrated in vivo and in silico approaches.

MATERIALS AND METHODS

Study Design

This was a true experimental study using a post-test only control group design. This study utilized archived formalin-fixed paraffin-embedded (FFPE) tissue samples obtained from a previously conducted animal experiment (Khotimah et al., 2026).

Experimental Animals and Grouping

The original animal experiment was conducted from June to October 2022 at the Pharmacology Laboratory, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia, while the formulations were prepared at the Pharmacy Laboratory, Universitas Muhammadiyah Malang. Twenty-five healthy adult female Wistar rats (*Rattus norvegicus*) were used and randomly divided into five groups (n = 5 per group) as follows:

Table 1. Experimental groups and treatment protocols.

Code	Group	Formulation Details
NC	Negative Control.	Gel base only (no active compound)
PC	Positive Control.	3% <i>C. asiatica</i> extract + 1% <i>R. officinalis</i> oil
T1	Treatment 1 (low-dose nanoformulation).	0.15% <i>C. asiatica</i> transfersomes + 0.1% <i>R. officinalis</i> nanoemulsions
T2	Treatment 2 (mid-dose nano-formulation)	0.3% <i>C. asiatica</i> transfersomes + 0.1% <i>R. officinalis</i> nanoemulsions
T3	Treatment 3 (high-dose nano-formulation)	0.6% <i>C. asiatica</i> transfersomes + 0.1% <i>R. officinalis</i> nanoemulsions

Note. NC = negative control; PC = positive control; T1-T3 = treatment groups 1-3. Doses were based on Khotimah et al. (2026).

Animal and group allocation

Twenty-five healthy adult female Wistar rats (*Rattus norvegicus*) were randomly allocated into five groups (n = 5 per group). The negative control (NC) received gel base without active compounds, while the positive control (PC) received 3% *Centella asiatica* extract combined with 1% *Rosmarinus officinalis* oil. The treatment groups received *C. asiatica* transfersomes combined with 0.1% *R. officinalis* nanoemulsion at concentrations of 0.15% (T1), 0.30% (T2), and 0.60% (T3), respectively (Khotimah et al., 2026).

Formulation of treatment

The topical formulations were prepared at the Pharmacy Laboratory, Universitas Muhammadiyah Malang. The standard gel was prepared by incorporating crude *C. asiatica* extract (Materia Medica Batu) and pure *R. officinalis* oil (Young Living®) into a neutral gel base. The transfersomes were prepared by dissolving phospholipids and the specified concentration of *C. asiatica* extract in methanol to obtain a concentrated suspension. The *R. officinalis* nanoemulsion was prepared by high-speed homogenization of rosemary essential oil with emulsifying agents for 20 min. The resulting transfersome and nanoemulsion preparations were subsequently incorporated into the gel matrix to obtain the final topical formulations (Khotimah et al., 2026).

Wound creation and treatment application

Following anesthesia and aseptic preparation, a caesarean abdominal incision was created using the Bowers technique. The incision was closed using absorbable 4.0 catgut sutures and covered with sterile gauze and Tegaderm® film dressing. Wound care was performed for 14 days. The wound was cleaned with physiological saline, dried using sterile gauze, and the assigned gel formulation was applied topically once daily according to the experimental group (Khotimah et al., 2026).

Immunohistochemical Analysis

On day 14, archived FFPE tissue sections were deparaffinized, rehydrated, and subjected to antigen retrieval. Endogenous peroxidase was blocked with 3% hydrogen peroxide. Non-specific binding was minimized using Background Sniper (Biocare Medical). Sections were incubated with primary antibodies against VEGF (1:50 dilution; Starr Trek HRP Det Kit, US) for 2 h at

room temperature. Detection was performed using TrekAvidin Universal Link, TrekAvidin-HRP Label, and DAB chromogen, followed by hematoxylin counterstaining.

Digital images were captured under a light microscope (400× magnification) and analyzed using QuPath software for automated quantification of staining intensity and percentage of positive area.

In Silico Molecular Docking

Pharmacokinetic and Toxicity Prediction (ADMET)

The pharmacokinetic and toxicity properties of the selected phytochemicals were predicted using the pkCSM online platform (<https://biosig.lab.uq.edu.au/pkcsm/prediction>). Canonical SMILES structures were obtained from the PubChem database and submitted to the pkCSM server for ADMET analysis. The evaluated parameters included water solubility, human intestinal absorption (HIA), skin permeability (log Kp), blood–brain barrier permeability (logBB), total clearance, AMES toxicity, and hepatotoxicity. The generated predictions were used to assess the pharmacokinetic suitability and safety profiles of the compounds for topical wound-healing applications (Pires et al., 2015).

Protein Selection and Preparation

The crystal structure of Vascular Endothelial Growth Factor Receptor 2 (VEGFR2) kinase domain (PDB ID: 4AG8) was retrieved from the Protein Data Bank (PDB; <https://www.rcsb.org/>). Protein preparation was performed using YASARA Structure version 19.9.17 (YASARA Biosciences GmbH, Vienna, Austria). Water molecules and non-essential heteroatoms were removed, hydrogen atoms were added, and the protein structure was optimized using the AMBER14 force field at physiological pH (7.4). Energy minimization was subsequently performed to obtain a stable receptor conformation prior to molecular docking (Safithri et al., 2025).

Ligand Preparation

The phytochemical compounds identified from *Centella asiatica* and *Rosmarinus officinalis* were obtained from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) in SDF format. The selected compounds included Asiaticoside, Madecassoside, Asiatic acid, Madecassic acid, Quercetin,

Kaempferol, Catechin, Luteolin, Gallic acid, Stigmasterol, Rosmarinic acid, Carnosic acid, Caffeic acid, Ursolic acid, and Carnosol. Ligands were prepared using YASARA Structure through the addition of hydrogen atoms and energy minimization under the AMBER14 force field. The optimized ligands were saved in SDF format for subsequent docking simulations (Gholam et al., 2025).

Binding Pocket Analysis

The binding cavity of VEGFR2 was identified using the CavityPlus web server (<http://www.pkumdl.cn:8000/cavityplus>). Predicted cavities were evaluated according to cavity volume, surface area, DrugScore, and druggability score. The cavity exhibiting the highest druggability score and containing the catalytic ATP-binding region of VEGFR2 was selected as the docking site. The selected cavity showed a DrugScore of 2919.00 and was classified as druggable. The cavity possessed a surface area of 1614.75 Å² and a volume of 2731.88 Å³, indicating its suitability for ligand accommodation and molecular interaction analysis (Y. Xu et al., 2018).

Molecular Docking

Molecular docking simulations were performed using the docking protocol implemented in YASARA Structure. The prepared VEGFR2 structure (PDB ID: 4AG8) was treated as a rigid receptor, while all phytochemical ligands were treated as flexible molecules under the AMBER14 force field. For each ligand, 100 independent docking runs were conducted, and the pose exhibiting the highest binding affinity was selected for further analysis. The docking grid was defined as a cubic box centered on the active-site cavity predicted by CavityPlus, with dimensions of 67.18 × 67.18 × 67.18 Å. Binding affinity scores generated by YASARA were used to rank ligand-receptor interactions, where higher positive binding energy values indicate stronger predicted binding affinity. The resulting protein-ligand complexes were subsequently analyzed using BIOVIA Discovery Studio Visualizer to identify hydrogen bonds, hydrophobic interactions, electrostatic interactions, and other non-covalent interactions contributing to VEGFR2 binding (Safithri et al., 2025).

Statistical Analysis

All experimental data obtained from the in vivo study were analyzed using SPSS version 25 (IBM Corp., Armonk, NY, USA). Data normality and homogeneity

were evaluated prior to analysis. Differences among experimental groups were assessed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range post hoc test. Results were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Effect of Combined *Centella asiatica* Transfersomes and *Rosmarinus officinalis* Nanoemulsion on VEGF Expression on Day 14

Immunohistochemical analysis demonstrated significant differences in VEGF expression among the experimental groups ($p < 0.001$). As shown in Figure 2, the highest VEGF expression was observed in the positive control (PC) group (60.8 ± 3.0 AU), followed by T1 (54.6 ± 18.3 AU), T2 (36.6 ± 3.0 AU), negative control (NC) (34.8 ± 13.8 AU), and T3 (25.5 ± 3.4 AU), which exhibited the lowest VEGF expression level. Figure 2 illustrates a decreasing trend in VEGF expression from the PC and T1 groups toward the T3 group, with the T3 group showing the lowest expression. Different superscript letters indicate statistically significant differences among groups, whereas groups sharing the same letter are not significantly different. These findings indicate that the higher-dose formulation in T3 was associated with lower VEGF expression on day 14.

VEGF is a key angiogenic growth factor that regulates endothelial cell proliferation, migration, and neovascularization during wound healing. Under normal physiological conditions, VEGF expression increases during the proliferative phase to support angiogenesis and gradually declines as the wound enters the remodeling phase (da Silva et al., 2024; Elbially et al., 2020). On day 14, VEGF expression was analyzed using IHC staining. Images of the stained skin tissues (Figure 1) were examined under a light microscope and quantified using QuPath software. The expression is represented as arbitrary units (AU) of staining intensity. The significantly lower VEGF expression observed in the T3 group on day 14 suggests a more advanced stage of wound healing characterized by reduced angiogenic demand and progression toward tissue remodeling. Conversely, the higher VEGF expression detected in the PC and T1 groups indicates that angiogenic activity remained active, suggesting that the healing process was still predominantly within the proliferative phase (da Silva et al., 2024).

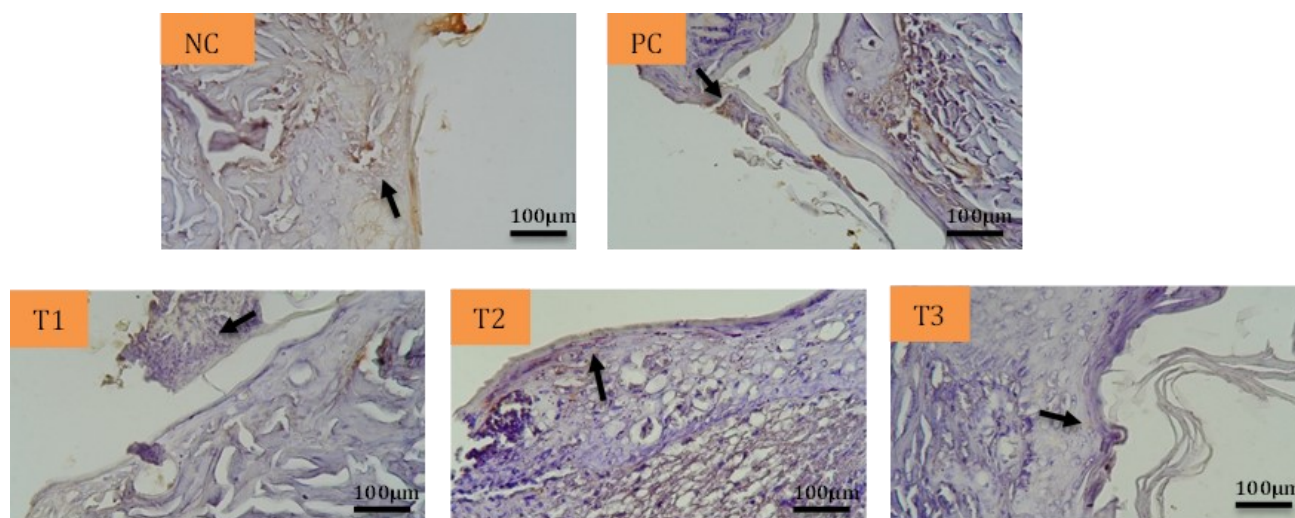


Figure 1. Immunohistochemical staining of VEGF expression in representative skin sections from all experimental groups on day 14 after caesarean incision. The arrows indicate areas of positive VEGF immunohistochemical expression, represented by brown staining. Stronger brown staining indicates higher VEGF expression. NC, negative control; PC, positive control; T1, *Centella asiatica* transfersome + rosemary nanoemulsion (0.15%/0.1%); T2, *Centella asiatica* transfersome + rosemary nanoemulsion (0.3%/0.1%); T3, *Centella asiatica* transfersome + rosemary nanoemulsion (0.6%/0.1%).

Immunohistochemical staining of VEGF expression in representative skin sections from all experimental groups on day 14 after caesarean incision. NC, negative control; PC, positive control; T1, *Centella asiatica* transfersome + rosemary nanoemulsion (0.15%); T2, *Centella asiatica* transfersome (0.3%) + rosemary nanoemulsion (0.1%); T3, *Centella asiatica* transfersome (0.6%) + rosemary nanoemulsion (0.1%).

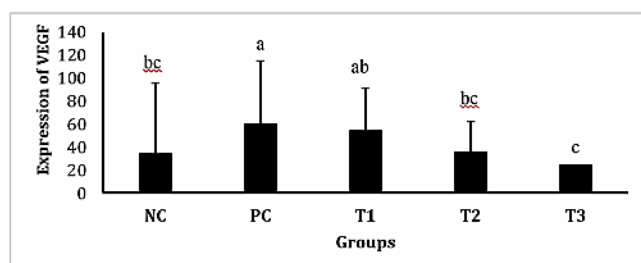


Figure 2. Quantification of VEGF staining intensity (AU) among experimental groups on day 14 after caesarean incision.

The accelerated downregulation of VEGF in T3 may be attributed to the synergistic effects of *Centella asiatica* and *Rosmarinus officinalis*. Bioactive compounds from *C. asiatica*, particularly asiaticoside and asiatic acid, have been reported to promote tissue regeneration and extracellular matrix formation, thereby accelerating wound repair and enabling earlier transition to the remodeling phase (Arribas-López et al., 2022; Diniz et al., 2023). Meanwhile, rosemary-derived compounds such as rosmarinic acid and carnosic acid possess potent antioxidant and anti-inflammatory properties that facilitate resolution of inflammation and prevent prolonged angiogenic stimulation (Arajyan et al., 2025; Gutierrez-Albanchez et al., 2025). Furthermore, the use of transfersomes and nanoemulsions may enhance the bioavailability and dermal penetration of these

phytochemicals, improving their therapeutic efficacy within the wound environment (Handayani et al., 2025).

Therefore, the lower VEGF expression observed in T3 should not be interpreted as impaired healing but rather as evidence of accelerated wound maturation and successful progression from angiogenesis to tissue remodeling. These findings suggest that the combination of *Centella asiatica* transfersomes and *Rosmarinus officinalis* nanoemulsions effectively modulates VEGF expression, promoting a more favorable healing trajectory in cesarean wound repair.

Pharmacokinetics Analysis and Discussion

The predicted pharmacokinetics properties of the selected phytochemicals from *Centella asiatica* and *Rosmarinus officinalis* are presented in (Table 2). Overall, the compounds exhibited diverse pharmacokinetic profiles but generally favorable safety characteristics, as all compounds were predicted to be non-mutagenic (AMES negative), while only ursolic acid showed a potential hepatotoxicity signal. Among the major *Centella asiatica* constituents, Asiaticoside and Madecassoside demonstrated relatively high-water solubility (-2.874 and -2.832 log mol/L, respectively) but low predicted human intestinal absorption (29.201% and 23.756%). These findings are consistent with their glycosylated structures, high molecular weights, and increased polarity, which limit passive membrane diffusion (Bandopadhyay et al., 2023; Witkowska et al., 2024). In contrast, their corresponding aglycones, Asiatic acid and Madecassic acid, exhibited markedly higher intestinal absorption values (62.855% and 57.113%, respectively), reflecting improved membrane permeability and more favorable physicochemical properties. Previous studies have reported that triterpenoid glycosides may undergo biotransformation

into their aglycone forms, which subsequently contribute to their biological activity during tissue repair (Arribas-López et al., 2022; Bandopadhyay et al., 2023).

Among the compounds derived from rosemary, Rosmarinic acid demonstrated moderate absorption in the intestines at 32.516%, while Carnosic acid showed a very high predicted absorption rate of 99.030%. The excellent absorption characteristics of Carnosic acid align with its lipophilic diterpene structure and favorable membrane permeability properties (Aly et al., 2026; J. Xu et al., 2025). Similar patterns were noted for other lipophilic rosemary components, such as Carnosol and Ursolic acid, which exhibited absorption rates above 90%. These results indicate that rosemary diterpenes have strong membrane affinity but may need formulation strategies to address their relatively low solubility in water. Although oral absorption varied significantly among the compounds, skin permeability values were fairly consistent, ranging from -2.735 to -2.887 log Kp for most phytochemicals. This suggests that dermal delivery might be more affected by solubility and formulation characteristics than by inherent skin permeability alone. Hydrophilic glycosides like Asiaticoside and Madecassoside may face limited penetration through the lipid-rich stratum corneum due to their large molecular size and polarity, whereas lipophilic compounds such as Carnosic acid, Carnosol, and Ursolic acid might experience poor aqueous dissolution on the skin surface (Dixena et al., 2025; Jacob & Nair, 2026).

These complementary physicochemical limitations provide a strong rationale for the combined use of transfersomes and nanoemulsions in the present study. Transfersomes are highly deformable phospholipid vesicles capable of enhancing the dermal penetration of hydrophilic molecules such as Asiaticoside and Madecassoside, whereas nanoemulsions improve the solubilization, stability, and skin delivery of lipophilic compounds such as Carnosic acid, Carnosol, and Ursolic

acid (Dixena et al., 2025; Jacob & Nair, 2026; Lapmanee et al., 2025). Consequently, the combination of these nanocarrier systems may maximize local bioavailability within the wound environment while overcoming the inherent pharmacokinetic limitations of individual phytochemicals.

The predicted blood–brain barrier permeability values were generally low for most compounds, indicating limited central nervous system exposure. This characteristic is desirable for topical wound-healing formulations because the therapeutic target is localized within the skin and underlying tissues rather than systemic organs. Furthermore, all compounds were predicted to be non-mutagenic and non-hepatotoxic, except for ursolic acid, which displayed a potential hepatotoxicity signal. However, because the present formulation is intended for localized topical administration, systemic exposure is expected to be minimal, thereby reducing the clinical significance of this prediction (Jacob & Nair, 2026; Lapmanee et al., 2025).

Collectively, the pharmacokinetics analysis suggests that the major phytochemicals of *Centella asiatica* and *Rosmarinus officinalis* possess complementary pharmacokinetic characteristics that support their combined use in topical wound-healing formulations. The hydrophilic triterpenoid glycosides (Asiaticoside and Madecassoside) provide well-documented wound-healing activity, whereas the more lipophilic aglycones and rosemary diterpenes exhibit favorable permeability and absorption characteristics. When incorporated into transfersome and nanoemulsion delivery systems, these compounds are expected to achieve enhanced local bioavailability, thereby supporting VEGF-mediated angiogenesis, fibroblast proliferation, collagen synthesis, extracellular matrix remodeling, and ultimately accelerated Caesarean wound healing (Kim et al., 2025; Witkowska et al., 2024; J. Xu et al., 2025).

Table 2. Predicted Pharmacokinetics properties of selected bioactive compounds from *Centella asiatica* and *Rosmarinus officinalis* using pkCSM.

Compound	WS	HIA	SP	BBB	CYP3A4-I	TC	AMES	Hepatotox.	SS
<i>Centella asiatica</i>									
a	-2.87	29.20	-2.74	-1.88	No	0.23	No	No	No
b	-2.83	23.76	-2.74	-1.98	No	0.23	No	No	No
c	-3.01	62.86	-2.74	-0.65	No	0.20	No	No	No
d	-3.04	57.11	-2.74	-0.74	No	0.21	No	No	No
e	-2.93	77.21	-2.74	-1.10	No	0.41	No	No	No
f	-3.04	74.29	-2.74	-0.94	No	0.48	No	No	No
g	-3.12	68.83	-2.74	-1.05	No	0.18	No	No	No
h	-2.56	43.37	-2.74	-1.10	No	0.52	No	No	No
i	-2.89	23.45	-2.74	-1.90	No	-0.37	No	No	No
j	-3.09	81.13	-2.74	-0.91	No	0.50	No	No	No
k	-2.92	25.80	-2.74	-1.60	No	0.32	No	No	No
l	-6.68	94.97	-2.78	0.77	No	0.62	No	No	No

Compound	WS	HIA	SP	BBB	CYP3A4-I	TC	AMES	Hepatotox.	SS
<i>Rosmarinus officinalis</i>									
m	-3.06	32.52	-2.74	-1.38	No	0.25	No	No	No
n	-3.22	99.03	-2.74	-0.55	No	0.38	No	No	No
o	-2.33	69.41	-2.72	-0.65	No	0.51	No	No	No
p	-3.09	81.13	-2.74	-0.91	No	0.50	No	No	No
q	-3.33	93.25	-2.74	-0.73	No	0.57	No	No	No
r	-3.07	100.00	-2.74	-0.14	No	0.08	No	Yes	No
s	-4.12	91.21	-2.89	-0.10	No	0.28	No	No	No
t	-3.75	95.58	-1.81	0.80	No	0.03	No	No	No

Note: Compound codes: a = Asiaticoside; b = Madecassoside; c = Asiatic acid; d = Madecassic acid; e = Quercetin; f = Kaempferol; g = Catechin; h = Gallic acid; i = Rutin; j = Luteolin; k = Naringin; l = Stigmasterol; m = Rosmarinic acid; n = Carnosic acid; o = Caffeic acid; p = Luteolin; q = Apigenin; r = Ursolic acid; s = Carnosol; t = α -Pinene. WS = Water Solubility (log mol/L); HIA = Human Intestinal Absorption (%); SP = Skin Permeability (log Kp); BBB = Blood–Brain Barrier Permeability (logBB); CYP3A4-I = CYP3A4 Inhibitor; TC = Total Clearance (log mL/min/kg); AMES = AMES Toxicity; HT = Hepatotoxicity; SS = Skin Sensitization. Numerical values were rounded to two decimal places.

Molecular Docking

The docking results revealed considerable variation in binding energies among the investigated compounds. Kaempferol exhibited the highest binding energy (9.7960 kcal/mol), followed by Luteolin (9.6880 kcal/mol), Quercetin (9.3800 kcal/mol), Ursolic acid (9.3010 kcal/mol), and Catechin (9.0240 kcal/mol) (Table 3). Overall, compounds derived from *Centella asiatica* demonstrated stronger binding affinities toward VEGFR2 than most compounds from *Rosmarinus officinalis*, suggesting a greater potential for receptor engagement (Lee et al., 2025; Yim et al., 2026). Although flavonoids achieved the highest docking scores, particular attention should be given to the principal triterpenoid constituents of *Centella asiatica*, namely Asiaticoside, Asiatic acid, and Madecassic acid, because these compounds are widely recognized as the major bioactive markers responsible for the wound-healing activity of the plant (Bandopadhyay et al., 2023; Witkowska et al., 2024). Asiaticoside demonstrated a binding energy of 7.7520 kcal/mol and formed the largest hydrogen-bonding network among all compounds, involving ASN923, LEU840, ARG1051, ARG842, ASP1052, GLU850, THR926, and ARG1032 (Figure 3).

The formation of multiple hydrogen bonds indicates strong receptor recognition and favorable

accommodation within the VEGFR2 binding cavity despite its moderate docking score. Similarly, Madecassic acid exhibited a binding energy of 7.6540 kcal/mol and interacted with HIS1026, ARG1027, and VAL899, while Asiatic acid formed hydrogen bonds with LYS1055 and ASN923. These interactions suggest that the major triterpenoids of *C. asiatica* possess favorable binding characteristics that may contribute to VEGFR2-associated angiogenic signaling. The biological significance of Asiaticoside, Asiatic acid, and Madecassic acid extends beyond their docking scores. Previous studies have demonstrated that these compounds stimulate fibroblast proliferation, collagen synthesis, extracellular matrix remodeling, and angiogenesis, thereby accelerating tissue regeneration and wound closure (Arribas-López et al., 2022; Bandopadhyay et al., 2023; Witkowska et al., 2024). Furthermore, transdermal delivery of *Centella asiatica* has been reported to enhance fibroblast activity and collagen deposition, resulting in improved wound-healing outcomes (Lapmanee et al., 2025). Therefore, their contribution to wound healing should be interpreted not only through receptor-binding affinity but also through their established biological functions.

Table 3. Molecular Docking Interaction Profile of Selected Phytochemicals Against VEGFR2.

Compound	Binding Energy (kcal/mol)	Conventional H-Bond	Carbon H-Bond	Hydrophobic Interaction	Unfavorable Interaction	Other Interactions
Asiaticoside	7.7520	ASN923, LEU840, ARG1051, ARG842, ASP1052, GLU850	THR926, ARG1032	LYS838	None	None
Madecassoside	7.3800	THR926, ARG842, ASN923, ARG1051	GLU850, LEU840, ASP1056	LYS838	ARG929	None

Compound	Binding Energy (kcal/mol)	Conventional H-Bond	Carbon H-Bond	Hydrophobic Interaction	Unfavorable Interaction	Other Interactions
Asiatic acid	7.1370	LYS1055, ASN923	ARG842	PRO1057	None	None
Madecassic acid	7.6540	HIS1026, ARG1027, VAL899	None	ALA881	None	None
Quercetin	9.3800	LYS868, CYS919	PHE918	PHE1047, CYS1045, VAL848, ALA866, LEU1035, LEU840	None	None
Kaempferol	9.7960	GLU917, CYS919, LEU840	None	LEU1035, ALA866, VAL848, CYS1045, PHE1047, LEU840	None	(LEU840)
Catechin	9.0240	GLU917, LEU840	PHE1047	LEU1035, VAL848, ALA866, CYS1045	None	None
Luteolin	9.6880	LYS868, ASP1046, CYS919	PHE918	VAL916, VAL848, LEU1035, LEU840, VAL899, ALA866, PHE1047	None	(CYS1045)
Gallic acid	6.4810	VAL914, LYS868	None	VAL848, VAL916, ALA866	None	None
Stigmasterol	8.6010	None	None	ALA881, ILE888, CYS817, CYS1024, ILE892, LEU1019, HIS1026	None	None
Rosmarinic acid	8.6010	ILE1025	CYS1045	LYS868, VAL916	None	GLU885, ASP1046
Carnosic acid	8.7650	ILE1025, HIS1026	None	CYS817, ILE888, LEU889	None	None
Caffeic acid	7.3870	CYS919	None	ALA866, CYS1045, VAL848, PHE1047	None	None
Ursolic acid	9.3010	ILE1044, ARG1027, HIS1026	None	LEU889, VAL898, LEU1019, ILE892	None	None
Carnosol	8.1800	None	GLU885	ARG1027, LEU889	None	ASP1046 (Pi-Anion)

Among the highest-scoring compounds, Kaempferol, Luteolin, and Quercetin demonstrated favorable interaction profiles with important VEGFR2 residues. Kaempferol formed conventional hydrogen bonds with GLU917, CYS919, and LEU840, while Luteolin interacted with LYS868, ASP1046, and CYS919. Quercetin established hydrogen bonds with LYS868 and CYS919. Notably, the repeated interaction with CYS919 observed for these flavonoids suggests favorable receptor recognition because this residue is located within the hinge region of the VEGFR2 kinase domain and

contributes significantly to ligand binding (Şandor et al., 2024). Likewise, the interaction of Luteolin and Rosmarinic acid with ASP1046 indicates strong structural complementarity with an important regulatory region of VEGFR2. Although molecular docking cannot directly confirm receptor activation or conformational changes, these interactions suggest the potential involvement of these compounds in VEGFR2-associated signaling pathways related to angiogenesis and tissue repair (Lee et al., 2025; Modi & Kulkarni, 2022).

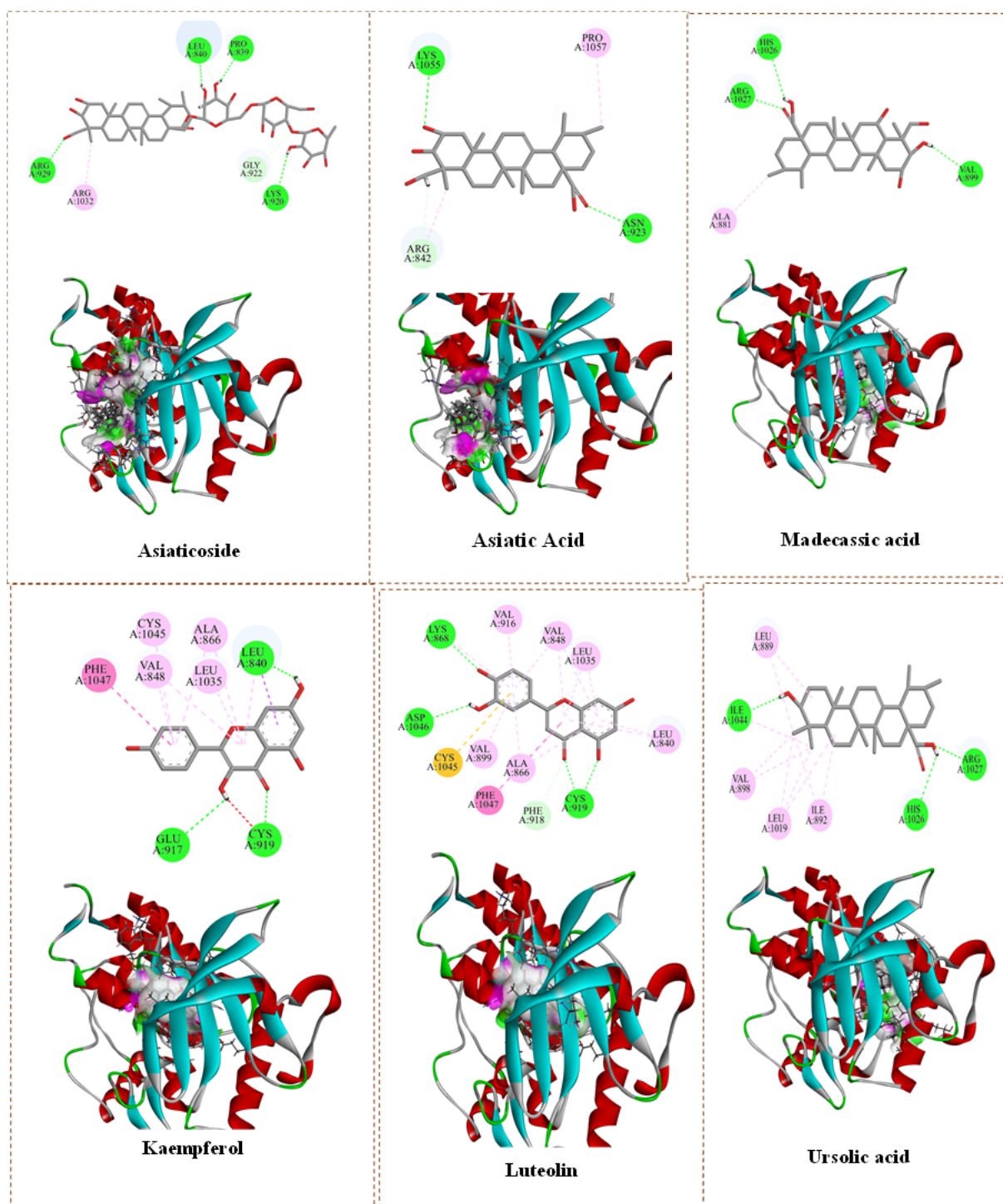


Figure 3. 2D interaction maps and 3D binding poses of selected *Centella asiatica* and *Rosmarinus officinalis* phytochemicals within the VEGFR2 active site.

Among the rosemary-derived compounds, Ursolic acid demonstrated the strongest binding affinity (9.3010 kcal/mol), followed by Carnosic acid (8.7650 kcal/mol) and Rosmarinic acid (8.6010 kcal/mol). Ursolic acid formed hydrogen bonds with ILE1044, ARG1027, and HIS1026, whereas Carnosic acid interacted with ILE1025 and HIS1026. Rosmarinic acid exhibited a distinctive interaction profile involving ILE1025, CYS1045, GLU885, and ASP1046. These findings are

consistent with previous reports demonstrating the antioxidant, anti-inflammatory, and regenerative properties of rosemary phytochemicals during wound healing (Aly et al., 2026; J. Xu et al., 2025).

The integration of the *in silico* and *in vivo* findings provides a plausible mechanistic explanation for the biological effects observed in the present study. Immunohistochemical analysis demonstrated increased VEGF expression in the treated groups, indicating

enhanced angiogenic activity within the wound tissue. Simultaneously, molecular docking analysis revealed favorable interactions between several phytochemicals and VEGFR2, suggesting their potential involvement in VEGF-associated signaling pathways (Lee et al., 2025). Collectively, these findings indicate that the wound-healing effects of *Centella asiatica* transfersomes and *Rosmarinus officinalis* nanoemulsions may result from the combined actions of bioactive triterpenoids, flavonoids, and phenolic compounds that support angiogenesis, granulation tissue formation, extracellular matrix remodeling, and tissue regeneration during Caesarean wound healing (Lee et al., 2025; Mendonce et al., 2026; Witkowska et al., 2024).

CONCLUSIONS

The findings of this study demonstrated that the bioactive compounds derived from *Centella asiatica* and *Rosmarinus officinalis* possess complementary pharmacokinetic properties that support their combined application in topical wound-healing formulations. Molecular docking analysis revealed favorable binding affinities between several phytochemicals and VEGFR2, suggesting their potential involvement in VEGF-mediated angiogenic signaling pathways. Furthermore, immunohistochemical analysis showed significant differences in VEGF expression among the experimental groups, with the T3 group exhibiting the lowest VEGF expression on day 14, indicating a more advanced transition from the proliferative phase to the tissue remodeling phase of wound healing. Collectively, these findings suggest that the combination of *Centella asiatica* transfersomes and *Rosmarinus officinalis* nanoemulsions may enhance Caesarean wound healing by improving local bioavailability, modulating angiogenesis, promoting tissue regeneration, and facilitating extracellular matrix remodeling.

Competing Interests: The authors declare that there are no competing interests.

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