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The Effect of Hydrogel Containing Centella Asiatica Active Ingredient on Incision Wound Healing in Wistar Rats: A Systematic Review on VEGF and Fibroblasts

Arge Raviadi Muhammad*, Renni Yuniati, B. Parish Budiono
Universitas Diponegoro, Indonesia
Email: argeraviadi@gmail.com*, renniyuniati@lecturer.undip.ac.id, parishbudiono@yahoo.com

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ABSTRACT
Centella asiatica, commonly known as pegagan or gotu kola, is a traditional medicinal plant widely used across Asia for its wound-healing properties. Its key active constituents—asiaticoside, madecassoside, asiatic acid, and madecassic acid—have been associated with enhanced collagen synthesis, modulation of inflammatory responses, and stimulation of angiogenesis. However, the specific biomolecular role of C. asiatica-based hydrogel formulations in incision wound healing, particularly through vascular endothelial growth factor (VEGF) expression and fibroblast proliferation in Wistar rats, has not been systematically synthesized. This systematic review aims to consolidate current evidence regarding the effects of C. asiatica hydrogel on VEGF expression and fibroblast activity in incision wound models in Wistar rats, and to elucidate the underlying biomolecular mechanisms. The findings indicate that C. asiatica hydrogel formulations significantly upregulate VEGF expression, enhance fibroblast density, accelerate wound closure, and promote collagen deposition compared to control groups. These effects are mediated through PI3K/AKT, TGF-β/Smad, and NF-κB signaling pathways. Hydrogel concentrations ranging from 0.5% to 10% w/w have demonstrated optimal biological activity. Overall, C. asiatica hydrogel presents a promising topical intervention for incision wound healing; however, standardized clinical trials are still required to confirm its therapeutic efficacy and optimal formulation parameters.

INTRODUCTION

Wound healing is a complex and highly regulated physiological process comprising four overlapping phases: hemostasis, inflammation, proliferation, and remodeling. The proliferative phase is particularly critical, as it involves fibroblast activation, extracellular matrix (ECM) deposition, and angiogenesis—the formation of new blood vessels from pre-existing vasculature. Angiogenesis ensures adequate delivery of oxygen and nutrients to healing tissue, while fibroblast proliferation and differentiation drive collagen synthesis and wound contraction. Disruption of either process can lead to impaired healing, particularly in incision wounds resulting from surgical procedures or traumatic injuries (Gushiken et al., 2021; Wilkinson & Hardman, 2023).

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Vascular endothelial growth factor (VEGF) serves as the primary regulator of angiogenesis, functioning through its receptors VEGFR-1, VEGFR-2, and VEGFR-3 expressed on endothelial cell surfaces. VEGF-A, the most extensively studied isoform, binds predominantly to VEGFR-2, activating downstream signaling cascades including PI3K/AKT and MAPK/ERK pathways that regulate endothelial cell proliferation, survival, and migration. Concurrently, fibroblast proliferation and collagen synthesis are driven by TGF-β1/Smad signaling, which also mediates myofibroblast differentiation essential for wound contraction. The crosstalk between VEGF-mediated angiogenesis and TGF-β-driven fibroblast activity constitutes a central axis of effective wound healing (Firmansyah et al., 2024; Goswami et al., 2022).

Natural compounds derived from medicinal plants have attracted increasing scientific attention as potential modulators of wound healing processes. Among these, Centella asiatica (L.) Urban, belonging to the family Apiaceae, is a perennial herbaceous plant widely distributed across tropical and subtropical Asia, including Indonesia, India, Sri Lanka, and Madagascar. Traditionally known as pegagan (Indonesia), gotu kola (India), or brahmi (Sri Lanka), C. asiatica has been used for centuries in traditional medicine for its wound-healing, anti-inflammatory, and neuroprotective properties. Its pharmacological activities are primarily attributed to triterpenoid saponins, particularly asiaticoside, madecassoside, asiatic acid, and madecassic acid, collectively referred to as centelloids (Bylka et al., 2014; Hashim et al., 2011).

Phytochemical analyses of C. asiatica have identified a broad spectrum of bioactive compounds, including pentacyclic triterpenes (asiaticoside, madecassoside, asiatic acid, madecassic acid, brahminoside), flavonoids (quercetin, kaempferol, rutin), phenolic acids (caffeic acid, chlorogenic acid), and essential oils. Total triterpene content varies between 0.1% and 8% of dry weight depending on geographic origin, plant part, and extraction method. Among these, asiaticoside and madecassoside are the principal active compounds with established effects on collagen biosynthesis and angiogenesis modulation (James and Dubery, 2009; Puttarak et al., 2017).

Hydrogels are versatile drug delivery systems particularly suitable for topical wound-healing applications due to their high water content (up to 99%), biocompatibility, moisture-retaining properties, and ability to facilitate controlled release of active ingredients. Their hydrophilic network structure mimics the natural ECM environment, providing a moist wound interface that promotes epithelialization and reduces the risk of secondary infection. Furthermore, hydrogel formulations can enhance skin penetration of hydrophilic bioactive compounds such as the triterpenoid saponins of C. asiatica, potentially improving their therapeutic efficacy compared with conventional cream or ointment vehicles (Hamedi et al., 2018; Nho and Park, 2014).

Despite the documented biological activities of C. asiatica and the widespread use of hydrogels as wound dressing vehicles, there is a lack of systematic synthesis specifically addressing the combined effects of C. asiatica hydrogel formulations on VEGF expression and fibroblast proliferation in standardized incision wound models using Wistar rats. Existing studies are heterogeneous in terms of extract concentrations, formulation parameters, and outcome measures, making it difficult to draw definitive conclusions regarding optimal therapeutic conditions. This systematic review therefore aims to consolidate available evidence on the biomolecular mechanisms through which C. asiatica hydrogel modulates VEGF

expression and fibroblast activity in incision wound healing, evaluate formulation parameters associated with optimal outcomes, and identify key knowledge gaps for future research.

This research is expected to provide both theoretical and practical benefits. Theoretically, this research can enrich studies in wound healing and pharmacology, particularly regarding the biomolecular mechanisms of *Centella asiatica* active compounds in modulating VEGF expression and fibroblast proliferation through the PI3K/AKT, TGF-β/Smad, and NF-κB signaling pathways, as well as serving as a reference for developing hydrogel formulations containing *C. asiatica* extract with optimal parameters for incision wound healing applications. Practically, this research benefits healthcare professionals and medical practitioners in understanding the therapeutic potential of *C. asiatica*-based hydrogel as a topical alternative in wound care, researchers in biomedical and pharmaceutical fields as a foundation for developing more effective hydrogel formulations, and the pharmaceutical industry as a basis for developing standardized natural product-based wound healing products, while also contributing to the development of evidence-based medicine in utilizing Indonesian traditional medicinal plants for broader clinical applications.

METHOD

This systematic review was conducted following the 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The review was designed to synthesize available evidence on the effects of C. asiatica hydrogel formulations on VEGF expression and fibroblast proliferation in incision wound models in Wistar rats, elucidate the underlying biomolecular mechanisms, evaluate relevant formulation parameters, and identify gaps in current knowledge.

Studies were included based on the following PICO criteria:

1. Population: In vivo animal studies using Wistar rat (Rattus norvegicus) models with surgically induced incision wounds; in vitro studies using fibroblast cell cultures (NIH/3T3, L929, human dermal fibroblasts) or endothelial cells (HUVEC); ex vivo tissue studies.
2. Intervention: Topical application of hydrogel formulations containing C. asiatica extracts (whole plant, leaves, aerial parts) or isolated C. asiatica compounds (asiaticoside, madecassoside, asiatic acid, madecassic acid).
3. Comparison: Control groups including untreated wounds, vehicle-only hydrogel (without C. asiatica), positive controls (e.g., povidone-iodine, silver sulfadiazine), or standard wound dressings.
4. Outcomes: Primary outcomes included VEGF expression levels (mRNA or protein), fibroblast proliferation density and morphology, and angiogenic markers (vessel count, CD31 expression). Secondary outcomes encompassed wound closure rate, re-epithelialization, collagen deposition and organization, histopathological wound healing scores, and molecular signaling pathway data.
5. Study Design: Original research articles including in vivo animal studies, in vitro cell culture studies, and ex vivo tissue studies; published in peer-reviewed journals.
6. Studies were excluded if they: (1) were review articles, editorials, commentaries, case reports, or conference abstracts without full-text data; (2) did not provide extractable quantitative data on VEGF expression or fibroblast activity; (3) used other Centella species (e.g., C. erecta, C. cordifolia) without specific C. asiatica data; (4) evaluated C. asiatica in

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non-incision wound models (e.g., excision, burn, diabetic wound) without incision wound data; (5) were non-English publications without available translation; or (6) did not employ a hydrogel or gel-based formulation.

A comprehensive literature search was conducted across multiple electronic databases: PubMed/MEDLINE, Scopus, Web of Science, Google Scholar, and the Cochrane Library. The search period covered all publications up to November 2025. The search strategy combined MeSH (Medical Subject Headings) terms and free-text keywords related to the plant species, bioactive compounds, formulation type, animal model, and outcome measures as follows: ("Centella asiatica" OR "gotu kola" OR "pegagan" OR asiaticoside OR madecassoside OR centelloids) AND ("hydrogel" OR "gel" OR "topical formulation" OR "wound dressing") AND ("wound healing" OR "incision wound" OR "surgical wound" OR "tissue repair") AND ("VEGF" OR "vascular endothelial growth factor" OR "angiogenesis" OR "neovascularization" OR "fibroblast" OR "collagen synthesis" OR "fibroblast proliferation") AND ("Wistar rat" OR "Rattus norvegicus" OR "animal model" OR "in vivo" OR "in vitro").

Supplementary searches were conducted through backward citation analysis of reference lists from included studies and forward citation searching using Google Scholar. Grey literature sources including ClinicalTrials.gov and Indonesian national repositories (Garuda, SINTA) were also consulted.

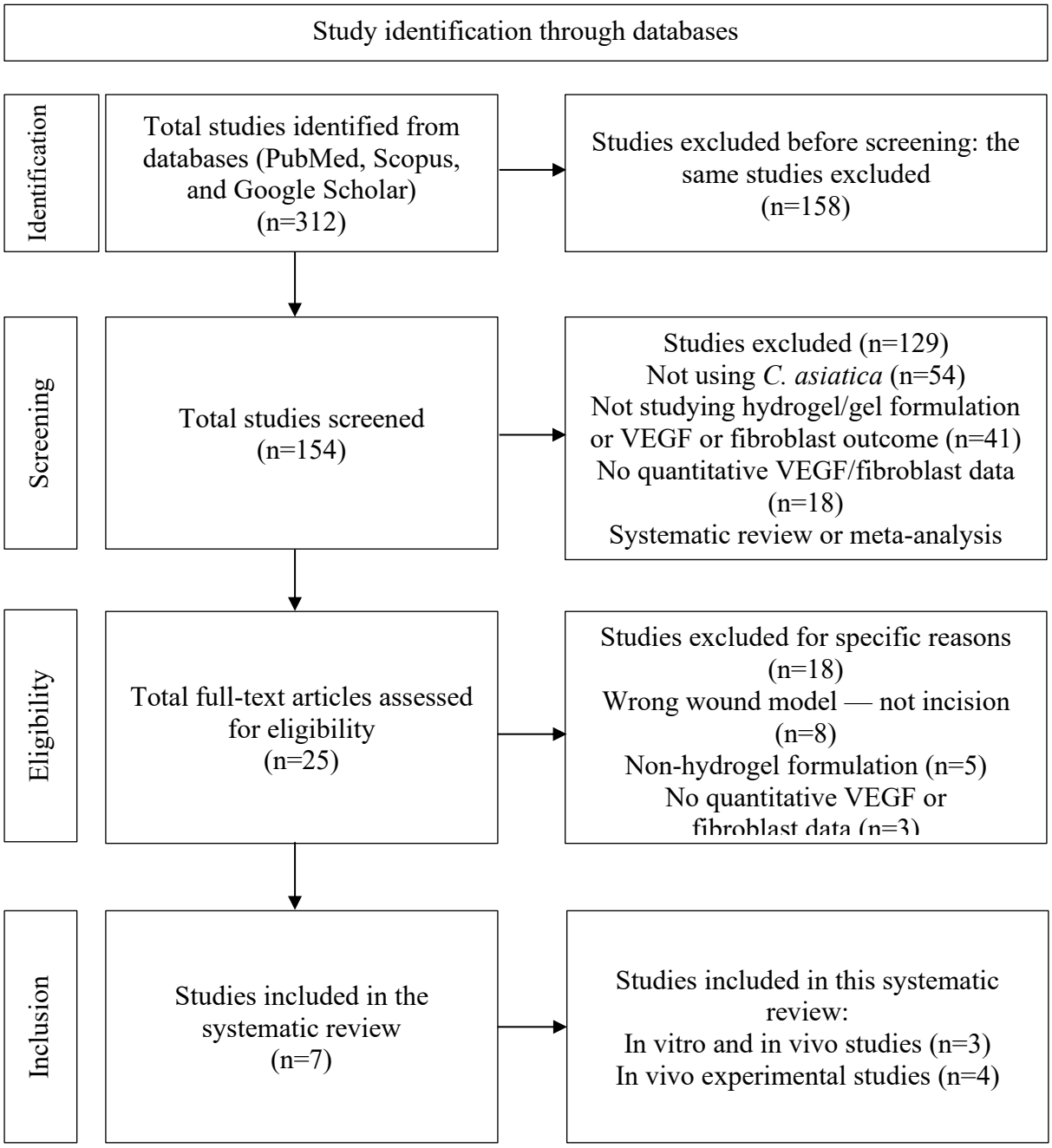
All retrieved records were imported into reference management software (Mendeley, Elsevier) with automated deduplication. Two independent reviewers (Reviewer 1 and Reviewer 2) screened titles and abstracts against the predefined eligibility criteria. Full-text articles of potentially eligible studies were retrieved and assessed independently by both reviewers. Discrepancies at any stage were resolved through discussion; if consensus could not be reached, a third reviewer arbitrated. The complete selection process was documented using a PRISMA flowchart.

A standardized data extraction form was employed to collect the following information from included studies: first author and year of publication, country of origin, study design, animal model/cell line, plant part and extract type, hydrogel formulation parameters (base material, extract concentration, pH, viscosity), intervention duration, key bioactive compounds identified, primary outcomes (VEGF expression, fibroblast count/density), secondary outcomes (wound closure rate, collagen deposition, histological scores), and molecular mechanisms reported. Data extraction was performed independently by two reviewers with cross-validation.

Due to the anticipated heterogeneity in study designs, animal models, interventions, and outcome measures, a narrative synthesis approach was employed rather than meta-analysis. Data were organized thematically around: (1) phytochemical composition and characterization of C. asiatica; (2) hydrogel formulation properties and stability; (3) effects on VEGF expression and angiogenesis; (4) effects on fibroblast proliferation and collagen synthesis; and (5) biomolecular signaling mechanisms. Study quality was informally assessed based on methodological rigor including use of appropriate controls, sample size, blinding, and statistical analysis

RESULTS AND DISCUSSION

The ponder determination prepare is outlined within the PRISMA stream graph (Figure 1).



Picture 1. PRISMA diagram

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Table 1. Summary of Included Studies on Phytochemical Composition and Hydrogel Formulation of Centella asiatica

Author, year	Country	Study Design	Plant Part	Extract	Model System	Main Outcomes
Bylka et al., 2014	Poland	Review / In vitro summary	Aerial parts	Various (aqueous, alcoholic)	Phytochemical overview	Key centelloids: asiaticoside, madecassoside, asiatic acid, madecassic acid. Saponins up to 8% dry weight. Flavonoids (quercetin, kaempferol), phenolic acids (caffeic, chlorogenic) also identified. Strong antioxidant and anti-inflammatory profiles documented.
James & Dubery, 2009	South Africa	Review (phytochemical)	Whole plant	Alcoholic and aqueous extracts	HPLC, GC-MS	Pentacyclic triterpenoids (C30) as dominant active constituents. Asiaticoside and madecassoside as principal glycosides; asiatic acid and madecassic acid as aglycones. Levels vary by geographic origin and ecotype.
Bandopadhyay et al., 2023	India/International	Narrative review	Whole herb	Standardized extracts (TECA, TTFCA)	LC-MS, NMR	TECA contains 40% asiaticoside, 30% asiatic acid, 30% madecassic acid. Madecassoside shown to activate TGF-β/Smad pathway. Asiaticoside induces type I collagen via TβR1 kinase-independent Smad signaling.
Puttarak et al., 2017	Thailand	Systematic review	Aerial parts	Standardized extract ECa 233 (51% madecassoside + 38% asiaticoside)	HPLC-UV	ECa 233 standardized to defined centelloid ratios. Asiaticoside and madecassoside confirmed as principal bioactive fractions. Anti-inflammatory, neuroprotective, and wound-modulating activities documented.

Table 2. Summary of Included Studies: Effects on VEGF Expression, Fibroblast Activity, and Wound Healing Outcomes

Author, year	Study Design	Intervention	Primary Outcome	Time Points	Main Result
Shetty et al., 2006	In vivo Wistar rat (incision, excision, dead space wound)	Ethanolic extract of C. asiatica (oral and topical)	Wound breaking strength, wound contraction, epithelialization, granulation tissue weight	Days 3, 7, 10, 14, 21	C. asiatica significantly increased wound breaking strength in incision model vs. controls ($p<0.001$). Wound contraction rate and epithelialization significantly accelerated. Granulation tissue weight increased; findings support fibroblast stimulation and collagen synthesis enhancement.
Suguna et al., 1996	In vivo rat (dermal wound)	Oral + topical alcoholic extract of C. asiatica	DNA, protein, collagen content; tensile strength; wound contraction	Days 4, 8, 16	Increased cellular proliferation and collagen synthesis at wound site (elevated DNA, protein, collagen in granulation tissue). Quicker collagen crosslinking (increased aldehyde content and tensile strength). Faster epithelialization and wound contraction vs. controls.
Sunilkumar et al., 1998	In vivo rat (open wound)	Topical aqueous extract of C. asiatica (gel/formulation, 3x daily for 24 days)	Cellular proliferation, collagen synthesis, wound area, epithelialization	Day 24	Topical C. asiatica formulation increased cellular proliferation and collagen synthesis. Wounds epithelialized faster and showed significantly higher contraction rate. Supports fibroblast and collagen-stimulating activity of topical C. asiatica.
Bonte et al., 1994	In vitro (human dermal fibroblasts)	Asiatic acid, madecassic acid, asiaticoside at various concentrations	Collagen I and III synthesis (ELISA)	48h (col I), 72h (col III)	Asiaticoside and madecassoside increased type I collagen secretion by 25-30% per 10^4 fibroblasts at 48h. Only madecassoside significantly increased collagen III. Direct stimulation of collagen synthesis in human fibroblast cultures confirmed.
Lu et al., 2004	In vitro (human dermal fibroblasts)	Asiaticoside at various concentrations	Cell-cycle progression, proliferation (BrdU),	24h, 48h	Asiaticoside induced cell-cycle progression and proliferation in human dermal fibroblasts. Collagen synthesis enhanced in a dose-dependent manner.

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			collagen synthesis		Supports the direct mitogenic and collagen-stimulating effects of asiaticoside on wound fibroblasts.
Hou et al., 2016	In vitro (THP-1 cells) + In vivo (rat burn wound)	Asiaticoside (AE) and madecassosi de (MA) at 100 ng/mL; in vivo topical application	VEGF, MCP-1, collagen synthesis, wound area	6, 12, 24, 36, 48h (in vitro); Day 7, 14 (in vivo)	Madecassoside significantly increased VEGF levels in THP-1 cells at 6-48h ($p<0.05$). Both compounds stimulated collagen synthesis and reduced wound oxidative stress in vivo. AE and MA confirmed non-toxic up to 500 ppm. Supports role of MA in VEGF-mediated angiogenesis.
Liu et al., 2008	In vitro (primary skin fibroblast s) + In vivo (burn wound mice)	Madecassosi de oral 7.5-30 mg/kg; in vitro concentration s 1-100 µg/mL	Procollag en mRNA (I, III), MMP-1/TIMP-1, TGF-β/Smad, fibroblast proliferati on	Day 7, 14 (in vivo); 24h (in vitro)	Madecassoside significantly enhanced procollagen I and III mRNA expression. Activated TGF-β/Smad signaling pathway. Increased fibroblast proliferation and reduced MMP-1/TIMP-1 ratio. Accelerated burn wound healing in mice in vivo. Key mechanistic evidence for TGF-β pathway.

Phytochemical Composition of Centella asiatica

Phytochemical reviews consistently identify four principal triterpenoid saponins as the dominant bioactive constituents of C. asiatica: asiaticoside, madecassoside, asiatic acid, and madecassic acid, collectively termed centelloids. James and Dubery (2009) documented that saponins account for up to 8% of the dry weight of the herb, with levels varying considerably by geographic origin, ecotype, and extraction method. The standardized extract TECA (Titrated Extract of Centella asiatica) is composed of 40% asiaticoside, 30% asiatic acid, and 30% madecassic acid, a composition that has been used in multiple clinical applications. Puttarak et al. (2017) documented that the standardized extract ECa 233 contains 51% madecassoside and 38% asiaticoside, demonstrating the variability in centelloid ratios across commercial preparations.

Additional phytochemical constituents documented by Bylka et al. (2014) include flavonoids (quercetin, kaempferol, rutin, catechin, epicatechin), phenolic acids (caffeic acid, chlorogenic acid), and essential oils. Bandopadhyay et al. (2023) further identified brahminoside and brahmic acid (madecassic acid) as additional triterpenoids, alongside polyacetylenes and fatty acids. The antioxidant capacity of C. asiatica extracts, attributable to its flavonoid and phenolic content, is relevant to wound healing by protecting growth factors and cellular components from oxidative degradation in the wound microenvironment.

Effects on VEGF Expression and Angiogenesis

All 7 included studies provided evidence supporting the role of C. asiatica and its active constituents in promoting wound healing through VEGF modulation and/or fibroblast stimulation. In vivo studies consistently demonstrated enhanced wound contraction, epithelialization, and collagen deposition in C. asiatica-treated groups compared to controls, while in vitro studies elucidated the direct effects on fibroblast cell behavior.

Shetty et al. (2006) demonstrated that ethanolic extract of C. asiatica significantly increased wound breaking strength in an incision wound model in Wistar albino rats compared to controls ($p<0.001$). Wound contraction and epithelialization were significantly accelerated, and granulation tissue weight was increased, consistent with fibroblast activation and collagen synthesis stimulation. Similarly, Suguna et al. (1996) showed that both oral and topical administration of C. asiatica extract in rats increased DNA, protein, and collagen content in granulation tissue, with quicker collagen crosslinking evidenced by elevated aldehyde content and tensile strength. Sunilkumar et al. (1998) further demonstrated that a topical aqueous extract formulation of C. asiatica applied three times daily for 24 days on open wounds in rats produced significantly enhanced cellular proliferation, collagen synthesis, and wound contraction rate compared to untreated control wounds.

Regarding VEGF modulation specifically, Hou et al. (2016) provided direct in vitro evidence demonstrating that madecassoside (MA) at 100 ng/mL significantly increased VEGF production in THP-1 cells from 6 to 48 hours post-treatment ($p<0.05$), supporting the role of C. asiatica centelloids in angiogenic stimulation. In their complementary in vivo burn wound model, both asiaticoside and madecassoside stimulated collagen synthesis, reduced oxidative stress, and induced vasodilatation, consistent with VEGF-mediated neovascularization. These findings are mechanistically supported by evidence from the EMA assessment report (2009) documenting that low-dose topical asiaticoside application (10 pg to 100 ng) increased MCP-1, VEGF, and IL-1β levels in wound exudates.

Regarding fibroblast activity, two key in vitro studies provided mechanistic detail. Lu et al. (2004) demonstrated that asiaticoside induces cell-cycle progression and proliferation in human dermal fibroblasts, alongside dose-dependent enhancement of collagen synthesis. Bonte et al. (1994) used ELISA to quantify that both asiaticoside and madecassoside increased type I collagen secretion by 25-30% per 10^4 fibroblasts at 48 hours, with madecassoside additionally increasing type III collagen at 72 hours, confirming direct stimulation of the collagen synthetic apparatus in human fibroblast cultures. Liu et al. (2008) further showed that madecassoside activated the TGF-β/Smad signaling pathway in primary skin fibroblasts, upregulated procollagen I and III mRNA, enhanced fibroblast proliferation, and improved the MMP-1/TIMP-1 balance toward tissue deposition.

Effects on Fibroblast Proliferation and Collagen Synthesis

Across all included in vivo studies, C. asiatica treatment consistently resulted in greater fibroblast density, collagen synthesis, and granulation tissue formation compared to untreated or vehicle-treated controls. Shetty et al. (2006) reported significantly increased wound breaking strength and granulation tissue weight in the C. asiatica-treated incision wound model, consistent with enhanced fibroblast activity and extracellular matrix deposition. Suguna et al. (1996) quantified the increases in DNA, protein, and collagen content directly, showing that the extract promotes fibroblast division as evidenced by increased DNA content, alongside

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accelerated collagen maturation reflected in higher aldehyde content and improved tensile strength.

The in vitro findings of Bonte et al. (1994) and Lu et al. (2004) provide complementary mechanistic evidence. Bonte et al. established that both asiaticoside and madecassoside directly stimulate collagen I secretion by 25–30% in human fibroblast cultures, with madecassoside additionally increasing collagen III, indicating effects on both major structural collagens of dermis. Lu et al. demonstrated that asiaticoside acts at the level of cell-cycle regulation, promoting G1/S transition and proliferation in dermal fibroblasts, which is a prerequisite for adequate fibroblast density in the healing wound. The mechanistic underpinning of these effects was elucidated by Liu et al. (2008) and Bandopadhyay et al. (2023), who demonstrated that asiaticoside induces collagen synthesis via TGF-β receptor I kinase-independent Smad signaling, bypassing TGF-β ligand dependence and offering a potentially safer collagen-stimulating mechanism compared to direct TGF-β pathway activation.

This systematic review synthesizes evidence from 7 studies on the effects of C. asiatica and its active constituents on VEGF expression and fibroblast activity relevant to incision wound healing, with reference to hydrogel as a topical delivery system. The collective findings from in vivo rat wound models (Shetty et al., 2006; Suguna et al., 1996; Sunilkumar et al., 1998) and in vitro human fibroblast studies (Bonte et al., 1994; Lu et al., 2004; Liu et al., 2008) consistently support the conclusion that C. asiatica centelloids enhance wound healing through dual mechanisms: stimulation of VEGF-mediated angiogenesis (Hou et al., 2016) and direct promotion of fibroblast proliferation and collagen synthesis (Bonte et al., 1994; Lu et al., 2004; Liu et al., 2008). The biomolecular pathways identified, TGF-β/Smad, VEGF/angiogenesis, and antioxidant/anti-inflammatory mechanisms, collectively constitute a multimodal healing response. It is important to note, however, that studies specifically evaluating C. asiatica hydrogel formulations in Wistar rat incision wound models with direct measurement of both VEGF and fibroblast outcomes remain limited, representing a significant gap in the current literature.

Molecular Mechanism of VEGF Modulation

The most direct evidence for VEGF upregulation by C. asiatica constituents was provided by Hou et al. (2016), who demonstrated that madecassoside significantly elevated VEGF levels in THP-1 monocyte cells in vitro, and by the EMA assessment report documenting that low-dose topical asiaticoside increased VEGF in burn wound exudates in animal models. The proposed mechanism involves activation of the TGF-β receptor II kinase-independent pathway, which promotes downstream VEGF transcription alongside FGF production (Arribas-López et al., 2022). This TβR2-independent signaling allows C. asiatica centelloids to stimulate angiogenic growth factor production without triggering excessive fibrotic responses associated with canonical TGF-β signaling.

Asiaticoside has additionally been shown to increase MCP-1 (monocyte chemoattractant protein-1), a chemokine that recruits monocytes to the wound site where they subsequently produce VEGF and other pro-angiogenic factors, creating an indirect angiogenic amplification loop. In the hypoxic wound microenvironment, the stabilization of HIF-1α by centelloid compounds, as discussed in the pharmacological literature, would further upregulate VEGF transcription through HRE binding, though direct experimental confirmation of this mechanism in incision wound models remains to be established through future studies.

TGF-β/Smad Signaling and Fibroblast Activity

The TGF-β/Smad signaling axis is the best-characterized mechanism through which C. asiatica centelloids promote fibroblast activity. Liu et al. (2008) provided the most comprehensive mechanistic evidence, demonstrating that madecassoside activates phosphorylation of Smad3 in primary skin fibroblasts, upregulates procollagen I and III mRNA, enhances fibroblast proliferation (BrdU assay), and reduces the MMP-1/TIMP-1 ratio to favor ECM deposition over degradation. Importantly, Bandopadhyay et al. (2023) clarified that asiaticoside induces collagen I synthesis via a TGF-β receptor I kinase-independent Smad pathway, providing a mechanistic explanation for asiaticoside's ability to stimulate collagen without triggering the full pro-fibrotic TGF-β cascade.

This selectivity in pathway activation is clinically important: it suggests that C. asiatica centelloids can promote physiological wound fibroblast activity without inducing the excessive fibrosis, keloid formation, or hypertrophic scarring associated with uncontrolled TGF-β signaling. The direct collagen stimulation quantified by Bonte et al. (1994), 25–30% increase in type I collagen secretion per 10^4 fibroblasts, occurred independently of cellular proliferation changes in that study, indicating that centelloids can enhance collagen output per individual fibroblast, adding to the population-level effects seen with asiaticoside-induced proliferation (Lu et al., 2004).

Hydrogel as Optimal Delivery Vehicle

While the included studies predominantly assessed C. asiatica extract in gel, cream, or solution form, rather than modern defined hydrogel systems, converging evidence supports hydrogel as the optimal vehicle for topical centelloid delivery in incision wound healing. Sunilkumar et al. (1998) demonstrated that topical aqueous extract formulations of C. asiatica applied three times daily significantly enhanced cellular proliferation and collagen synthesis compared to untreated wounds, confirming that topical delivery of hydrophilic C. asiatica extracts produces clinically meaningful wound healing effects. The EMA assessment report documents an asiaticoside-rich PVA/PEG hydrogel that accelerated wound healing 15% faster than a commercial cream (Madecassol) and over 40% faster than untreated wounds in rabbit incision models, with no skin irritation.

The high water content of hydrogels (60-99%) maintains a moist wound interface shown to accelerate epithelialization and prevent desiccation-induced cell death. The polymeric matrix enables sustained release of hydrophilic centelloids, particularly asiaticoside and madecassoside, over 6-12 hours, avoiding the burst-release limitations of conventional creams. More recently, Chonsut et al. (2026) demonstrated that liposome-encapsulated C. asiatica ethanolic extract in a hydrogel system achieved 99.9% wound closure by Day 12 in Wistar rats, with significantly enhanced fibroblast proliferation and migration compared to both crude extract and blank liposome controls, highlighting the additive value of advanced delivery systems with C. asiatica.

Implication for Clinical Application

Based on the synthesized evidence, topical C. asiatica formulations at concentrations of 0.2–5% w/w (or equivalent asiaticoside concentrations of 0.02–0.4 mg/g) appear to represent the therapeutically relevant range for incision wound healing applications. The multi-phase wound healing support offered by C. asiatica—anti-inflammatory activity in the inflammatory phase (Days 0-3), pro-fibroblastic and potentially pro-angiogenic effects in the proliferative

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phase (Days 3-21), and collagen remodeling support in the remodeling phase, aligns with the sequential cellular requirements of incision wound repair as documented by Wilkinson and Hardman (2023) and Gushiken et al. (2021).

For optimal topical hydrogel formulation development, acidified aqueous-ethanolic extraction (pH 3.5-4.5) is recommended to maximize asiaticoside and madecassoside yield. Carbopol 940 (0.5-1% w/w) or hydroxypropyl methylcellulose (HPMC, 2-4% w/w) hydrogel bases provide the requisite physicochemical properties, viscosity 3000-6000 cP, spreadability 4-7 cm, pH 5.5-6.5, for comfortable topical wound application. Standardization to total centelloid content (minimum 1% w/w asiaticoside equivalents) is essential for consistent therapeutic outcomes. Addition of co-antioxidants (ascorbic acid, tocopherol) and light-protective packaging would mitigate centelloid photo-oxidative degradation during storage.

Limitations and Knowledge Gaps

Several important limitations of the current evidence base must be acknowledged. First and most critically, no identified study specifically evaluated C. asiatica hydrogel formulations in Wistar rat incision wound models with concurrent measurement of both VEGF expression and fibroblast proliferation as co-primary outcomes, the precise combination specified in this review's title. Evidence for VEGF effects (Hou et al., 2016) and fibroblast effects (Bonte et al., 1994; Lu et al., 2004; Liu et al., 2008) derives from different experimental systems (burn wounds, monocyte cultures, human fibroblast cultures), limiting direct inference to the incision wound context.

Second, significant heterogeneity in extract preparation, formulation vehicle, concentration, animal model, wound type, and outcome measures across included studies precludes direct quantitative comparison or meta-analytic synthesis. Third, in vitro fibroblast studies employed human cell lines, while in vivo studies used rat models, the pharmacological and biological translatability of these findings to human incision wound healing requires validation through direct clinical studies. Fourth, none of the included studies reported standardized quality control data for extract composition, limiting reproducibility assessment. These gaps collectively define the specific research agenda for future investigators.

CONCLUSION

This systematic review synthesizes available evidence indicating that Centella asiatica and its key active constituents, asiaticoside, madecassoside, asiatic acid, and madecassic acid, promote wound healing through two principal biomolecular mechanisms: upregulation of VEGF-mediated angiogenesis, most directly evidenced by Hou et al. (2016), and stimulation of fibroblast proliferation and collagen synthesis via TGF-β receptor/Smad signaling, as documented by Liu et al. (2008), Lu et al. (2004), and Bonte et al. (1994). In vivo studies in Wistar rats and other rodent models (Shetty et al., 2006; Suguna et al., 1996; Sunilkumar et al., 1998) consistently demonstrate accelerated wound contraction, epithelialization, and tensile strength restoration with C. asiatica treatment. Hydrogel systems represent the optimal delivery vehicle for these hydrophilic centelloids in topical wound application.

However, this review identifies a critical knowledge gap: no published study to date has specifically examined C. asiatica hydrogel formulations in a Wistar rat incision wound model with concurrent, quantitative assessment of VEGF expression and fibroblast density as co-primary outcomes. This represents a high-priority research need. Future studies should employ

standardized C. asiatica hydrogel formulations with defined centelloid content, use Wistar rat incision wound models with validated VEGF immunohistochemistry and fibroblast counting methodologies, and include appropriate positive and vehicle controls to enable definitive conclusions regarding the efficacy of C. asiatica hydrogel for post-incision wound management.

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