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REVIEW

Centella asiatica in Dermatology: An Overview

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Centella asiatica **is a medicinal plant that was already used as a 'panacea' 3000 years ago. The active compounds include pentacyclic triterpenes, mainly asiaticoside, madecasosside, asiatic acid and madecassic acid. We have conducted an overview to summarize current knowledge on the results of scientific *in vitro* and *in vivo* experiments focused on the improvement of the healing process of small wounds, hypertrophic scars and burns by *C. asiatica*. In this paper, we discuss the data on constituents, recommended preparations and the potential side effects of *C. asiatica*.**

Keywords: Centella asiatica; triterpenes; review; dermatology.

INTRODUCTION

Some of herbal remedies may be particularly helpful in treating and relieving symptoms of skin diseases, due to the presence of various compounds responsible for their activity.

One of the plants used in dermatology is *Centella asiatica* (L.) Urban., synonym *Hydrocotyle asiatica* L. from the family *Apiaceae*, also known by the common name *Gotu kola* or Indian pennywort. It grows in the tropical regions of Asia, Oceania, Africa and America.

C. asiatica herb is recommended in the treatment of dermatoses and skin lesions such as excoriations, burns, hypertrophic scars or eczema as well as in non-dermatological diseases like gastric ulcers, gastric mucosal lesions (Shinomol and Muralidhara, 2011), anxiety (Wijeweeraa *et al*., 2006) and for improving cognition in neurodegenerative disorders (Subathra *et al*., 2005). *C. asiatica* has also been found beneficial in chronic venous insufficiency, mainly by improvement of microcirculation (Chong and Aziz, 2013). *C. asiatica* extract (International Nomenclature of Cosmetic Ingredients, INCI) is used also as an ingredient of cosmetics (Bylka *et al*., 2013).

Many studies present activity of *C. asiatica*, but until now there have been no reviews presenting the scientific information about the usage of *H. asiatica* in dermatological diseases. For this reason, this study provides an overview of the current knowledge on the *in vitro* and *in vivo* experiments, focused on the activity of *C. asiatica* extracts and individual compounds in facilitating the process of healing wounds, psoriasis and scleroderma lesions. The mechanisms of the above-mentioned activities as well as the potential side effects are discussed.

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METHODS

The following electronic English databases were searched: Ovid Medline, Pubmed and The Cochrane Library, from 1988 up to March 2013. They have been searched by the title and abstract using the following search terms: *Centella asiatica*, *Hydrocotyle asiatica, Gotu kola*, Indian pennywort, centelloids, asiaticoside, madecasosside, asiatic acid, madecassic acid, wounds, wound healing, burn wounds, scleroderma, psoriasis and toxicity. Hand searches were also conducted for publications not stored in the databases (e.g. webpages). Reference lists of all articles were searched for further publications.

For the selection of the manuscripts, three independent investigators (PZA, ESS and MB) assessed at first all the titles and abstracts and then through the full-text analysis of the publications, against pre-defined inclusion criteria. Disagreements over a study's inclusion were resolved by discussion between them and the consensus, arbitrated by authors WB and ADP.

CHEMICAL CONSTITUENTS

Ursane type pentacyclic triterpenoids known as centelloids, mainly: asiaticoside, madecasosside (brahminoside), asiatic acid and madecassic acid (brahmic acid) (Fig. 1) were the most important constituents isolated from *C. asiatica*. Other triterpenoids in *Gotu kola* include: asiaticoside C, D, E, F; centellasaponin B, C; isothankunic acid and oleanane type saponins, e.g. terminolic acid; centellasaponin D. *C. asiatica* contains about 0.1% essential oils with α-humulene, germacrene B/D, β-caryphyllene, flavonoids, sesquiterpenes, steroids (Brinkhaus *et al*., 2000; James and Duebery, 2009; James and Dubery, 2011; Nhiem *et al*., 2011). Saponins may account for 1% to 8%, according to the *European Pharmacopoeia*, not less than 6.0% (Ph.Eur. 2011).

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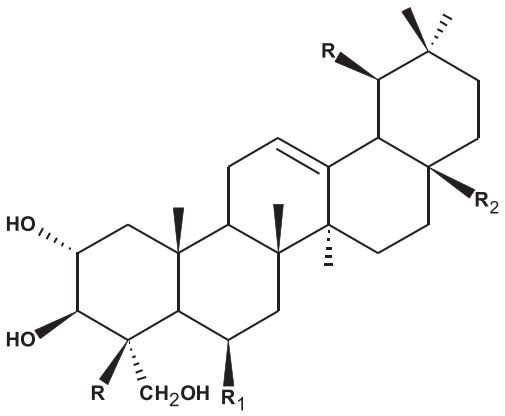

Figure 1. Triterpenes in *Centella asiatica*.
Asiatic acid R=CH_3; R_1=H; R_2 =COOH
Asiaticoside R=CH_3; R_1=H; R_2 =COO-glc(1→6)glc(1→4)rha
Madecassic acid R=CH_3; R_1=OH; R_2=COOH
Madecassoside R=CH_3; R_1=OH; R_2 =COO-glc(1→6)glc(1→4)rha

HERBAL PREPARATIONS

Pharmacological and clinical studies were carried out on the defined extracts as well as undefined aqueous or alcohol extracts (Table 1). However, information on the medicinal products suggests that all extracts: titrated extract of *C. asiatica* (TECA), total triterpenoid fraction of *C. asiatica* (TTFCA), total triterpenic fraction (TTF), as well as *C. asiatica* total triterpenic fraction (CATTF) and estratto titolato di *C. asiatica* (ETCA) are different acronyms of the same extract, contained in the used preparations: Madecassol®, Centellase® or Blastoestimulina®. These extracts include 40% of asiaticoside and a 60% mixture of asiatic and madecassic acids (Brinkhaus *et al.*, 2000; EMEA (European Medicines Agency), 2012).

One to two tablets (10 mg/tabl.) three times a day for adults and a half of this dose for children under 3 years of age are recommended by the European Medicines Agency (EMEA) in the case of non-healing wounds, hypertrophic scars or keloids in the active phase. For external use, to support the local treatment and to improve the granulation phase of non-healing ulcers and wounds, 1% cream is recommended. Disinfection of the wound/ulcer is required before treatment with TTFCA. Moreover, 1% ointment and 2% powder are available for the treatment of non-healing wounds. Two to three applications of ointment and 1–3 of powder per day are recommended. The ointment may also be used to cover skin on radiotherapy (EMEA, 2012).

Table 1. Investigated extracts of *C. asiatica*

Extract	Composition of extract
TECA	Asiatic acid (30%), madecassic acid (29–30%), asiaticoside (40%)
Titrated extract of *C. asiatica*	
TTFCA	Asiatic acid (30%), madecassic acid (30%), asiaticoside (40%)
Total triterpenoid fraction of *C. asiatica*	
TTF	Asiatic acid and madecassic acid (60%), asiaticoside (40%)
Total triterpenic fraction	
CATTF	Undefined
C. asiatica total triterpenic fraction	
ETCA	Undefined
Estratto titolato di *C. asiatica*	

RESULTS

This review identified 31 studies on facilitating the process of healing wounds, psoriasis and scleroderma lesions by *C. asiatica* extracts and its individual compounds such as: asiaticoside, madecassoside, asiatic acid and madecassic acid. Studies include 19 *in vitro*, ten *in vivo* and two clinical studies with different methodologies and importance. Twenty three citations were published after 2000, eight between 1988 and 2000. Results from the included studies are presented below and also summarized in chronological order in Table 2.

In vitro experiments

Wound healing. Wound healing is a complex biological process involving coagulation, inflammation, cytokine production, cell migration, proliferation and differentiation, angiogenesis, synthesis and remodeling of extracellular matrix (including collagen production and deposition). Type I and III collagen are the major components of the skin extracellular matrix. Both types play an important role in the wound healing process. As a result, proliferation of epithelial cells and wound contraction occur (Lu *et al.*, 2004a, 2004b; Liu *et al.*, 2008).

C. asiatica extracts, individual triterpene compounds and the mixture of triterpenoids from *C. asiatica* have been proven to support wound healing in a large number of scientific reports.

A statistically significant increase in the percentage of collagen and cell layer fibronectin in cultures of human skin fibroblasts, after application of TTFCA extract (25 μg/mL), was detected (Tenni *et al.*, 1988).

The TECA and its components including asiatic acid, madecassic acid and asiaticoside have been studied on human foreskin fibroblast monolayer cultures. TECA increased the collagen synthesis in a dose-dependent manner. In addition, TECA and all terpenes increased the intracellular free proline level, but this effect was independent of the stimulation of collagen synthesis (Maquart *et al.*, 1990).

The influence of asiatic acid, madecassic acid and asiaticoside on human skin fibroblast type I collagen synthesis was investigated *in vitro* separately for each agent and in combination. Additionally, the culture was or was not stimulated with ascorbic acid. In the presence of ascorbic acid, secretion of type I collagen was higher for each individual component and for the mixture, than in the absence of ascorbic acid (Bonté *et al.*, 1994).

To determine secretion of type I and III collagen in human fibroblast culture with or without stimulation with asiaticoside and madecassoside, the enzyme-linked immunosorbent assay (ELISA) was performed. The secretion of type I collagen was increased for 25–30% with asiaticoside and madecassoside. Authors concluded that *C. asiatica* extracts may facilitate maturity of a scar by increasing the amount of type I collagen and thus increasing the type I:III collagen ratio (Bonté *et al.*, 1995).

The activity of *C. asiatica* triterpenes (asiatic acid, madecassic acid asiaticoside and madecassoside) and

Table 2. Studies of the extracts and constituents of *C. asiatica* in chronological order

Extract/compound	Model/effect/route of application[a]	Reference
***IN VITRO* MODELS**		
WOUND HEALING		
TTFCA	Human skin fibroblast/↑ collagen and fibronectin synthesis	Tenni *et al.*, 1988
TECA, asiatic acid, madecassic acid and asiaticoside	Human foreskin fibroblast monolayer cultures/↑ proline level, collagen synthesis	Maquart *et al.*, 1990
Asiatic acid, madecassic acid, asiaticoside	Human skin fibroblast, stimulated or not stimulated with ascorbic acid/↑ type I collagen synthesis	Bonté *et al.*, 1994
Asiaticoside, madecassoside	Human fibroblast culture/↑ type I and III collagen synthesis	Bonté *et al.*, 1995
TECA, asiatic acid, madecassic acid asiaticoside and madecassoside	Human fibroblasts, DNA microarrays analysis/changes of genes expression involved in angiogenesis and wound healing	Coldren *et al.*, 2003
Asiaticoside	Human dermal fibroblasts, DNA microarray analysis/changes of genes expression responsible for cell proliferation, cell cycle, extracellular matrix	Lu *et al.*, 2004a, 2004b
Asiaticoside	Human dermal fibroblasts/↑ type I collagen synthesis, activation of Smad pathway	Lee *et al.*, 2006
Ethanolic extract	Human fibroblast cells/↑ collagen synthesis	Hashim *et al.*, 2011
Methanolic extract, six triterpenoid compounds	LPS-stimulated RAW 264.7 cells/↓ NO production, TNF-α secretion	Nhiem *et al.*, 2011
Asiaticoside	Keloid-derived fibroblasts/↑ collagen synthesis, normalization of healing process	Tang *et al.*, 2011
Asiaticoside	Human skin fibroblasts/↑ migration and proliferation of the fibroblasts, ↑ ECM synthesis	Lee *et al.*, 2012
Aqueous extract	Rabbit corneal epithelial cells wound healing model/↑ cell migration, changes of proliferation and cell cycle	Ruszymah *et al.*, 2012
Asiaticoside	Human periodontal ligament cells/↑ mRNA and proteins of fibronectin and type I collagen,↓ metalloproteinase-I mRNA expression	Nowwarote *et al.*, 2013
ANTIMICROBIAL ACTIVITY		
Hexane, carbon tetrachloride, chloroform fractions from methanolic extract	Disc diffusion method/Antimicrobial activity	Ullah *et al.*, 2009
ANTIOXIDANT ACTIVITY		
Ethanolic extract	Human dermal fibroblasts/↑ collagen synthesis DPPH assay/antioxidant effect	Hashim *et al.*, 2011
ANTI-PSORIATIC ACTIVITY		
Water extracts, asiaticoside, madecasosside	SVK-14 keratinocytes/Inhibition of growth of SVK-14 keratinocytes	Sampson *et al.*, 2001
***IN VIVO* MODELS**		
WOUND HEALING		
Ointment, cream, gel with 1% of aqueous extract	Wounds/↑ cellular proliferation, collagen synthesis, tensile strength/topical application in rats	Sunilkumar *et al.*, 1998
Asiaticoside (0.2% solution)	Wounds/↑ levels of enzymatic and non- enzymatic antioxidants/ topical application in rats	Shukla *et al.*, 1999a, 1999b
Asiaticoside (0.2% solution)	Normal and delayed wound, hydroxyproline content and tensile strength/topical and oral application in guinea pigs	Shukla *et al.*, 1999a
TECA, asiatic acid, madecassic acid and asiaticoside	Wound chamber model implanted under the skin of rats/↑ dry weight, DNA, protein, hydroxyproline, collagen synthesis/injections	Maquart *et al.*, 1999
Asiatic and madecassic acids (mixture)	Influence on the connective tissue of rats/↑ collagen synthesis, tensile strength, ↓ scar tissue/oral or subcutaneous administration in rats	Brinkhaus *et al.*, 2000
Ethanolic extract	Normal and dexamethasone suppressed wound/↑ wound healing (epithelization, contraction, tensile strength)/topical application in rats	Shetty *et al.*, 2006
Madecassoside	Burn wound, ↑ antioxidative activity, collagen synthesis, angiogenesis/ oral administration in mice	Liu *et al.*, 2008
Asiaticoside	Burn wound/influence on the level of cytokines, ↑ angiogenesis, stimulation VEGF production, MCP-1, IL-1/topical application on the backs of mice	Kimura *et al.*, 2008

(Continues)

Table 2. (Continued)

Extract/compound	Model/effect/route of application[a]	Reference
Hexane, methanolic, ethyl acetate and water extracts	Incision and burn wounds/↑ wound healing/topical application in rats	Somboonwong *et al.*, 2012
Asiaticoside	LPS-treated rats/anti-inflammatory, antipyretic activity (↑ TNF-α, IL-6, COX-2, PGE2, liver myeloperoxidase, ↓ IL-10, up-regulation heme oxygenase-1)/oral administration in rats	Wan *et al.*, 2013
CLINICAL TRIALS		
Madecassol® tablets Madecassol® ointment	Patients with systemic and localized scleroderma/positive effect of treatment	Guseva *et al.*, 1998
Extract of *C. asiatica* (50 mg asiaticoside/capsule)	Wound healing in diabetic patients; 200 patients, randomized study/shorten healing than in placebo group	Paocharoen, 2010

[a]only in *in vivo* studies; ECM - extracellular matrix; DPPH - 2,2-diphenyl-1-picrylhydrazyl; COX-2 - prostaglandin-endoperoxide synthase 2 (cyclooxygenase-2); IL-1 - interleukin 1; IL-6 - interleukin 6; IL-10 - interleukin 10; LPS - lipopolysaccharide; VEGF - vascular endothelial growth factor; MCP-1 - monocyte chemotactic protein-1; TNF-α - tumor necrosis factor alpha; PGE2 - prostaglandin E2; TTFCA - total triterpenoid fraction *of C. asiatica*; TECA - titrated extract of *C. asiatica*.

TECA depends on the modulation of the expression of genes involved in angiogenesis and wound healing. TECA was demonstrated to carry out changes in hyaladherin and cytokine expression, which may cause a decrease of proteolysis in the extracellular matrix, and therefore support the accumulation of collagen and fibronectin. Proangiogenic changes in the expression of a number of growth factors were detected (Coldren *et al.*, 2003).

Asiaticoside influences the wound healing even in infected wounds. The *in vitro* studies by Lu *et al.* (2004a, 2004b) on human dermal fibroblasts with DNA microarray analysis proved that in the presence of asiaticoside (30 μg/mL) changes of the genes expression are observed. These genes were responsible for cell proliferation, cell cycle process and extracellular matrix synthesis. Furthermore, type I and type III procollagen mRNA level and proteins level increased in response to asiaticoside.

Lee *et al.* (2006) have shown that asiaticoside significantly induced type I collagen synthesis in human dermal fibroblast. Type I collagen synthesis is stimulated by transforming growth factor β (TGF-β). The Smad proteins transmit the signal downstream from the TGF-β receptor into the nucleus. Following the binding of TGF-β to its receptors, the receptor-regulated Smads (so called R-Smads, which include Smad 1, 2, 3, 5 and 8) are phosphorylated and then translocated to the nucleus, where they act as regulators of the target genes expression, e.g. type I collagen gene. Asiaticoside induced phosphorylation of Smad2 and Smad3. Interactions between Smad3 and Smad4 after stimulation with asiaticoside were also observed. It was proved that asiaticoside induced translocation of Smad3–Smad4 complex into the nucleus. Moreover, Smad2 phosphorylation and synthesis of type I collagen induced by asiaticoside were not inhibited by SB431542 (TGF-β receptor I kinase inhibitor – an activator of the Smad pathway). This confirms that asiaticoside induces type I collagen synthesis through the activation of Smad pathway in a TβRI kinase-independent manner.

The influence of asiaticoside on collagen synthesis and keloid-derived fibroblast proliferation was also investigated by Tang *et al.* (2011). Keloid scars occur as results of a pathological wound healing, characterized by hyperproliferation of keloid fibroblasts, overproduction of extracellular matrix, aberrant cytokine and growth factor activities. The TGF-β pathway, especially TGF-β1, is involved in keloid formation. Prolonged healing of the wound can lead to unbalances in TGF-β1 expression and thus can cause fibroproliferative disorders and excessive scar formation. Within R-Smad family, Smad3 mainly mediates collagen production in dermal fibroblasts stimulated by TGF-β. Overexpression or overphosphorylation of Smad in keloid fibroblast in comparison with normal fibroblasts was observed. The asiaticoside inhibits the TGF-β receptors protein and mRNA expression, increases the Smad7 protein and mRNA expression, whereas it did not alter Smad2, Smad3, Smad4, expression and phosphorylated Smad2 and Smad3 (reduction of TGF-βR1 expression leads to the decreased expression of R-Smads) in keloid scars. Smad7, as Smads inhibitor, acts as a negative feedback regulator which is antagonist of R-Smads. Taken together, it seems that asiaticoside has a dual role by promoting wound healing and preventing scar formation.

The ethanolic extract of *C. asiatica* enhanced threefold collagen synthesis of human fibroblast cells compared to the control. The highest collagen synthesis was found at 50 mg/mL of *C. asiatica* extract. This extract demonstrated significant DPPH-radical scavenging activity with 84% inhibition at a concentration 1 mg/mL. The activity was compared to that of grape seed extract and vitamin C (Hashim *et al.*, 2011).

The ursane triterpenoids suppressed the production of NO and secretion of TNF-α in lipopolisaccharide stimulated RAW 264.7 cells; therefore, these compounds are considered to be important anti-inflammatory constituents of *C. asiatica*. Among the analyzed compounds, asiaticoside presented the strongest effect (Nhiem *et al.*, 2011).

The influence of asiaticoside on normal human skin cells was studied by Lee *et al.* (2012). *In vitro* studies proved that asiaticoside affects proliferation of human skin dermal fibroblasts as well as increases migration rates and accelerates attachment of skin cells.

Ruszymah *et al.* (2012) studied the effect of the aqueous extract of *C. asiatica* on re-epithelization of corneal epithelium during wound healing. It has been proven that the extract significantly enhances the migration of rabbit corneal epithelial (RCE) cells in the *in vitro*

wound healing model. At high concentration, it also has an antiproliferative action on the RCE cells.

Asiaticoside enhanced periodontal tissue healing on human periodontal ligament cells (HPDLs). Dose-dependent increases in the levels of mRNA and protein of fibronectin and type I collagen, as well as attenuated metalloproteinase-I mRNA expression, were observed when HPDLs were treated by asiaticoside. Furthermore, asiaticoside promoted osteogenic differentiation of HPDLs (Nowwarote *et al*., 2013).

The various fractions from methanolic extract of C. *asiatica* showed significant antibacterial and antifungal activity against a various microorganisms (Gram-positive, Gram-negative bacteria and fungi) (Ullah *et al*., 2009; Dash *et al*., 2011).

Psoriasis. The anti-psoriatic activity of water extracts of *C. asiatica*, containing asiaticoside and madecasosside on the growth of SVK-14 keratinocytes, was compared with those of water extracts of *Psoralea corylifolia* seeds containing psoralen and synthetic dithranol. The tests were performed on two types of *C. asiatica* and *P. corylifolia* extracts: (i) with addition of polyvinylpolypyrrolidone (PVPP) and (ii) without PVPP responsible for the removal of phenolic compounds. The extracts inhibited keratinocyte replication with IC_{50} values of (i) 209.9 ± 9.8 μg/mL, (ii) 238.0 ± 2.5 μg/mL for *C. asiatica* and (i) 18.4 ± 0.6 μg/mL, (ii) 36.3 ± 3.3 μg/mL for *P. corylifolia*. These results proved that phenolic compounds were not responsible for the inhibitory effect of the extract. The IC_{50} value of dithranol, asiaticoside and madecasosside was 1.2 ± 0.1 μg/mL, 8.0 ± 0.5 μg/mL and 8.4 ± 0.1 μg/mL, respectively. It is worth to note, that although the aqueous extract of the *C. asiatica* herb was not as potent as that of the *P. corylifolia* seed, its constituents, i.e. triterpenoid glycosides, had IC_{50} values similar to those of dithranol (Sampson *et al*., 2001).

In vivo experiments

Wound healing. When applied topically, 1% ointment, cream and gel with aqueous extract of *C. asiatica*, three times a day for 24 days on the open wounds in rats, increased cellular proliferation and collagen synthesis at the wound site, as evidenced by the increase in collagen content and tensile strength. The treated wounds epithelialized faster and the rate of wound contraction was higher as compared to control wounds. The process of healing was the best with gel formulation (Sunilkumar *et al*., 1998).

The activity of *C. asiatica* was studied in relation to normal and delayed-type wound healing in guinea pigs. The animals were treated with 0.2% solution of asiaticoside applied to punch/puncture wounds. After treatment, there was an increase in hydroxyproline content of about 56% and in tensile strength of about 57%. Moreover, an increase in collagen content and better epithelization were reported. A similar effect was obtained in the same animal model by oral administration of asiaticoside (1 mg/kg of body weight), as well as in guinea pigs with experimentally induced diabetes characterized by delayed-type wounds treated with 0.4% asiaticoside solution (Shukla *et al*., 1999a).

The wound healing process depends on antioxidants levels in the wound. After 7 days of twice daily application of asiaticoside (0.2%) on incisional wound in rats, the levels of enzymatic and non-enzymatic antioxidants, e.g. superoxide dismutase (35%), catalase (67%), glutathione peroxidase (49%), vitamin E (77%), and ascorbic acid (36%), in the newly created tissues were elevated (Shukla *et al*., 1999a, 1999b).

Wounds treated with TECA and its separated components: asiatic acid, madecassic acid and asiaticoside were investigated in wound chamber model by Maquart *et al*. (1999). After the stainless steel wound chambers were implanted under the skin of rats, TECA and isolated compounds were injected. Chambers were collected after 7, 14, 21 or 28 days and biochemical and histological analyses were performed. TECA-injected wound chambers were characterized by the increased dry weight, DNA, total protein, collagen, uronic acid and peptidic hydroxyproline content, suggesting the increased remodeling of the extracellular matrix in the wound. Presumably, the tested extract and compounds caused fibroblast proliferation and migration, as well as the production and activation of some growth factors in the wound. The triterpenoid components were also able to stimulate the synthesis of glycosaminoglycans, especially hyaluronic acid synthesis. The stimulating effect on collagen synthesis in human skin fibroblasts was demonstrated for asiaticoside, asiatic, madecassic acid and their combination. However, asiaticoside was active at lower doses than asiatic and madecassic acids.

A mixture of asiatic and madecassic acids was tested on the connective tissue of rats. Following subcutaneous implantation of glass rods, the rats were administered the triterpenic acids orally or subcutaneously. After 3 weeks, irrespective of the administration route, the weight of granuloma of the scar tissue was reduced. The rupture strength and tensile strength of the scar tissue increased. The effect was associated with an increase in the collagen content, as compared to the uninjured tissue (Brinkhaus *et al*., 2000).

The ethanolic extract of the *C. asiatica* facilitated the wound healing in both normal and dexamethasone-suppressed wound. The study was done on Wistar albino rats using incision, excision and dead space wounds models. The extract increased the wound breaking strength in incision wound model, the rate of wound contraction and accelerated the epithelization compared to control wounds. Wet and dry granulation tissue weights, granulation tissue breaking strength and hydroxyproline content in a dead space wound model also increased significantly. The extract had the attenuating effect of dexamethasone healing in all wound models. The results were confirmed by histology observations (Shetty *et al*., 2006).

It was also found that madecassoside was active in burn wound healing, through increasing antioxidative activity and enhancing collagen synthesis, and influencing angiogenesis. After oral administration of this compound at doses 6, 12 and 24 mg/kg to mice facilitatation of wound closure in a time-dependent manner and complete wound closure took place on 20th day in the group receiving 24 mg/kg of madecassoside. A histopathological study showed that madecassoside could alleviate infiltration of inflammatory cells and enhanced epithelization resulting from dermal proliferation of fibroblasts. The tested compound at doses 12

and 24 mg/kg decreased nitric oxide level and malonyl dialdehyde content in the burned tissue. Madecassoside increased the level of reduced glutathione and hydroxyproline, an indicator of collagen synthesis in burned skin. These results confirm a positive effect on fibroblast proliferation and collagen synthesis during burn wound repair. The authors indicate that the effect of madecassoside on wound healing involve a few mechanisms including collagen synthesis, antioxidant activity and accelerated angiogenesis, which play an important role in the formation of new granulation tissue in the proliferation (Liu *et al*., 2008).

Topical application of asiaticoside at a dose of 10 pg, 1 ng or 100 ng/wound area for 20 days on the backs of mice, caused facilitation of burn wound healing through the influence on the level of various cytokines produced in the place of the burn wound. The improvement in burn wound healing might be an outcome of angiogenesis promotion during wound healing in the injured area occurring as a result of the stimulation of vascular endothelial growth factor production. This happens as a result of an expression increase in monocyte chemotactic protein-1 (MCP-1) in keratinocytes and interleukin-1β (IL-β) in macrophages induced by asiaticoside and MCP-1 (Kimura *et al*., 2008).

The effect of different *C. asiatica* extracts on the incision and burn wound was studied in an experimental animal study. All types of extracts used in the study: hexane, methanolic, ethyl acetate and aqueous affect the wound healing process, but the ethyl acetate extract rich in asiatic acid was the most active (Somboonwong *et al*., 2012).

Asiaticoside administered orally, exhibited the potent antipyretic and anti-inflammatory effects in lipopolisaccharide-treated rats. These effects could be associated with the inhibition of pro-inflammatory mediators, including TNF-α and IL-6 levels, COX-2 protein expression and PGE2 production, as well as liver myeloperoxidase activity. Furthermore, asiaticoside increases the level of antiinflammatory IL-10 in serum and up-regulates heme oxygenase-1 (HO-1) expression, an enzyme which protects the liver (Wan *et al*., 2013).

Clinical study

Wound healing. *C. asiatica* extract can shorten the healing process of wound in diabetic patients. The randomized control study included 200 diabetic patients, treated with two capsules of *C. asiatica* extract (50 mg asiaticoside/capsule) three times a day. Results showed that wound contraction was better than in the placebo group. Moreover, the extract suppresses the formation of scar tissue (Paocharoen, 2010).

Scleroderma. Guseva *et al*. (1998) studied the efficacy of orally/topically administered madecassol in patients with systemic sclerosis (SSc) and localized scleroderma (LS). They found that 6 month oral course (30 mg/day) caused softening of the skin lesions, lightening of hyperpigmentation and improvement of general condition of 12 SSc patients. The drug was not effective in patients with progressive disease and in those with diffuse skin lesions. The best response was observed in the area of digital ulcers in SSc patients.

TOXICITY

C. asiatica applied in the recommended doses is not toxic and the possible side effects are rare. It may cause allergic reactions and burning, when used externally. Oral administration of the recommended doses of *C. asiatica* may cause dyspepsia, nausea and headache, and overdose may result in dizziness and drowsiness. *Gotu kola* can cause an increase of glucose level in the blood of diabetic patients, as well as lipids level in the case of coexisting hyperlipidemia (Gruenwald *et al*., 2004).

There are data suggesting the risk of hepatotoxicity of *C. asiatica* in humans treated for 20–60 days (Jorge and Jorge, 2005).

Treatment with *C. asiatica* extracts for more than 6 weeks is not recommended and a 2-week break before the next application must be maintained. No information is available about interactions of preparations containing *C. asiatica* with other drugs, teratogenic effect on the fetus and safety of use by lactating women; hence, preparations containing extracts of this herb are not recommended at this time (Gohil *et al*., 2010).

DISCUSSION

It has been scientifically proven that *C. asiatica* herb can be useful in the treatment of skin diseases, especially in wound healing. Different extracts (TECA, TTFCA, ethanolic and methanolic), as well as individual pentacyclic triterpenes, mainly asiaticoside, madecassoside, asiatic and madecassic acid were investigated. Due to the fact that the studies were carried out on defined extracts, undefined extracts and individual compounds, the results are difficult to compare. However, the evaluation of main compounds activity allows to conclude that the active constituents are pentacyclic triterpenes.

Most *in vitro* studies were carried out using human dermal fibroblasts. It was proven that *C. asiatica* has a great impact on extracellular matrix proteins deposition. It stimulates fibroblasts proliferation, activates Smads pathway, increases the collagen synthesis, decreases the activity of metalloproteinases and thus increases the collagen deposition (Tenni *et al*., 1988; Maquart *et al*., 1990; Bonté *et al*., 1994; Bonté *et al*., 1995; Lu *et al*., 2004a, 2004b; Hashim *et al*., 2011; Tang *et al*., 2011; Nowwarote *et al*., 2013). It also inhibits the inflammatory phase of wound healing (Nhiem *et al*., 2011). Furthermore, the anecdotic studies provide information on proangiogenic (Coldren *et al*., 2003), antioxidative (Hashim *et al*., 2011; Nhiem *et al*., 2011) and antimicrobial (Ullah *et al*., 2009; Dash *et al*., 2011) activity of *C. asiatica* extracts. Taken together, all the abovementioned activities may improve the healing process of wounds and therefore they give a mandate for further *in vivo* studies.

The studies which elucidate the mechanism of wound healing such as changes of gene expression involved in angiogenesis and the activation of Smad pathway provided important information on the effectiveness of asiaticoside as a major active constituent of *C. asiatica* (Maquart *et al*., 1999; Nhiem *et al*., 2011). It can be assumed that the detected mechanism may be representative of *Gotu kola*.

There is also one *in vitro* study focusing on antipsoriatic effect of *C. asiatica* by Sampson *et al.* (2001). The results are promising but unfortunately there are no other studies supporting them. Therefore, there is a need of more studies, preferably in the form of clinical trials to prove the efficacy of *C. asiatica* as an antipsoriatic agent.

Most of the studies on animal models were focused on wound healing. They indicated that *C. asiatica* increases collagen synthesis, as well as proliferation and migration of fibroblasts and thus accelerates the reepithelization and contraction of the wound (Sunilkumar *et al*., 1998; Shukla *et al*., 1999a; Maquart *et al*., 1999; Brinkhaus *et al*., 2000; Liu *et al*., 2008). The efficacy was supported by histology findings (Sunilkumar *et al*., 1998; Shetty *et al*., 2006; Liu *et al*., 2008). Moreover, *C. asiatica* was responsible for antioxidative, anti-inflammatory and proangiogenic activity according to a few studies (Shukla *et al*., 1999a, 1999b; Kimura *et al*., 2008; Liu *et al*., 2008; Wan *et al*., 2013). Together with the *in vitro* studies, it makes *C. asiatica* a good candidate to clinical trials with chronic wounds. Unfortunately, there is just one clinical trial on diabetic patients with wounds (Paocharoen, 2010). However, good clinical response was observed, thus confirming that *C. asiatica* is a potent agent promoting wound healing. The other clinical trial was focused on the assessment of efficacy of *C. asiatica* in SSc and LS patients. It seems that the prominent benefit obtained by oral, as well as topical administration of madecassol was healing of the digital ulcers (Guseva *et al*., 1998). This may rather confirm the efficacy of madecassol in wound healing than in improving of sclerodermic lesions.

In conclusion, although previous studies suggest a positive effect of *Gotu kola* on wound healing, more studies are needed. Current knowledge is insufficient to clearly determine the effectiveness of *C. asiatica* and its preparation in facilitating the wound healing. Moreover, available literature does not clarify the best route and dosage of administration of the *C. asiatica* extract. In order to evaluate the usefulness of the plant in this area, clinical trials should be carried out. However, considering the safety of *C. asiatica*, it should be mentioned that proangiogenic activity of topically applied agents could be connected to the higher risk of neoplasm formation (Griffioen and Molema, 2000). As the proangiogenic activity of *C. asiatica* was proved, caution should be maintained in clinical trials.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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