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Centella asiatica: from folk remedy to the medicinal biotechnology – a state revision

Helmi Yousif Alfarra, Mohammad Nor Omar*

Department of Biotechnology, Kulliyyah (Faculty) of Science, International Islamic University Malaysia, Jalan Istana, Bandar Indera Mahkota, 25200, Kuantan, Pahang, Malaysia

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Abstract

Centella asiatica or "gotu kola" has been used since long time ago as an ethno-pharmacological plant and supposed to be a potent medicinal plant for its various pharmacological effects favorable for human health. Many studies described the noteworthy protective effect of *C. asiatica* against numerous diseases. Biological activities of *C. asiatica* have been linked to the most major compounds in it. This state paper is a part of our ongoing research on *C. asiatica*, its activity, isolation of novel metabolites, and applying the biotechnological methods to improve this plant and its phytochemical activities.

* **Corresponding Author:** Mohammad Nor Omar ✉ mnoromar@iiu.edu.my

Introduction

The revision of 'Ethno-medicine' or 'Ethno-pharmacology' or what is known by traditional medicine turn out to be very essential and precious in the current era in the perspective of discovering plant sp. to develop a phytomedicine.

Centella asiatica is one of the pioneers' herbs that is working powerfully in the traditional remedy such as skin problems treating, wounds healing, nerves and brain cells stimulating, that why in India *C. asiatica* has been called the "Brain food or the "Brain tonic (Singh *et al.,* 2010; Zheng C. J. and Qin 2007).

The present revision states an overview of our up to date knowledge and understanding on the botany, ethno-pharmacological uses, historical development of therapeutic applications, pharmacology and clinical-therapeutic use of *C. asiatica*.

Botany of C. asiatica

Centella consists of about 50 species, occupies tropical and subtropical areas. This genus is a member of the plant family *Apiaceae (Umbelliferae*) and comprises the most ever-present species *Centella asiatica* (L.) Urb. (Hydrocotyle asiatica L.). This permanent trail plant accompaniments plentifully in humid marshy regions 700 meters above sea level and is a small, herbaceous(non-woody) annual plant of the subfamily Mackinlaya(James and Dubery 2009; Zheng C. J. and Qin 2007). *C. asiatica* leaves' sizes are quite unstable; the petioles are commonly five to ten, longer than the lamina, which is 10-40 mm long and 20-40 mm, sometimes up to 70 mm, wide, the leaves are swaps over, sometimes clustered simultaneously at the nodes, kidney-shaped or circular or most likely egg-shaped and have palmate (cordate) nervation, regularly with seven veins, and a crenate margin. Young leaves show a few trichomes on the lower surface while adult leaves are glabrous. The inflorescence (flowers), if present, is a single umbel which generally contains three flowers, sometimes two or four but this is not common; the flowers are very small about two mm pentagonal (have five sides) and have an inferior ovary; the fruit, a brownish-grey, orbicular cremocarp, up five mm long, is very flattened laterally and has seven to nine prominent curved edges and this plant has been known to have a soft aroma(Jamil *et al.,* 2007; Pharmacopoeia 2010; Zheng C. J. and Qin 2007).

Phyto-distribution and synonyms

C. Asiatica is domestic in South East Asia countries such as Malaysia and Indonesia, India, Pakistan, Sri Lanka, part of China, southern and the middle Africa, Nepal, Australia, the South Pacific, the Western South Sea Islands, Western Himalayas', Madagascar, South-East U.S., Venezuela, Mexico, Colombia and Eastern South America(Brinkhaus *et al.,* 2000; ICS-UNIDO, 2006; Jamil *et al.,* 2007). This plant is commonly known as Pegaga in Malaysia, Gotu Kola in Chinese and Indian Pennywort in English and it has different names and synonyms around its geographic distribution, (Table 1) shows different names around the different ethnic (Brinkhaus *et al.,* 2000; Hashim *et al.,* 2011; Zainol N.A. *et al.,* 2008; Zhang F. L. *et al.,* 2008).

Ethno-pharmacological uses of C. asiatica

C. asiatica has been used and utilized as a traditional herbal medicine in Malaysia and all other regions of Asia and its aboriginal areas since ages, It has been used in different traditions with different recipes and diverse prescriptions by various ancient cultures and tribal groups as demonstrated as in (Table 2). In India, *C. asiatica* it has been described under the name of *Mandukaparni* and used in *Ayurveda* medicine, in China, it has been listed as one of the Traditional Chinese Medicine (TCM) too. The areal parts as well as the whole plant, fresh or dried have been used for medicinal purposes(Hashim *et al.,* 2011; Jamil *et al.,* 2007; Singh *et al.,* 2010; Zainol N.A. *et al.,* 2008).

C. asiatica has been reported to have different biological activities by a number of researchers here we put together the entire outcomes that have been reported up to date and share it with the researchers and the readers to be updated with the foundlings about *C. asiatica*.

Table 1. The different Names of *Centella asiatica*.

Country / Region	Language	Common name	Reference
Malaysia	Bahasa Malay	Pegaga	(Hashim *et al.,* 2011, Zainol N.A. *et al.,* 2008)
China	Chinese	Luei Gong Gen or Tung chian	(Brinkhaus *et al.,* 2000, Hashim *et al.,* 2011)
USA	English	Indian Pennywort	(Brinkhaus *et al.,* 2000)
Indonesian names	Bahasa Indonesia	Pegagan and Kaki Kuda	(Brinkhaus *et al.,* 2000, Hashim *et al.,* 2011)
Sumatra	-	Kaki Kuda	(Brinkhaus *et al.,* 2000)
Jawa	Javanese	Kaki Kuda, Pegagan, Antanan gede, Gagan-gagan, Gang-gagan, Kerok barambat, Kos tekosan	(Brinkhaus *et al.,* 2000)
Sulawesi	-	Pagaga, Tangke-tungke	(Brinkhaus *et al.,* 2000)
Bali	-	Papaiduh, Pepiduh, Piduh	(Brinkhaus *et al.,* 2000)
Elores	-	Puhe beta, Kaki kuta, Tete karo, Teto kadho	(Brinkhaus *et al.,* 2000)
Italy	Italian	Idrocotile	(Brinkhaus *et al.,* 2000)
Japan	Japanese	Tsubo-kusa	(Brinkhaus *et al.,* 2000)
Mauritius	Mauritian Creole	Bavilacqua	(Brinkhaus *et al.,* 2000)
Spain	Spanish	Blasteostimulina (asiaticoside)	(Brinkhaus *et al.,* 2000)
France	French	Hydrocotyle Asiatique	(Brinkhaus *et al.,* 2000)
Fiji	Fijian/ Fiji Hindi	Totodro	(Singh *et al.,* 2010)
Cook Islands	Cook Islands Maori	Kapukapu	(Singh *et al.,* 2010)
Hawaii	Hawaiian	Pohe Kula	(Singh *et al.,* 2010)
Indian names :		mandookaparni	(Jamil *et al.,* 2007; Singh *et al.,* 2010)
Assam	Assamese	Manimuni	(Jamil *et al.,* 2007; Singh *et al.,* 2010)
Bengali	Bengali	Brahammanduki, Thankuni, Tholkuri	(Jamil *et al.,* 2007)
Bihar		Chokiora	(Jamil *et al.,* 2007)
Bombay		Karinga, Karivana	(Jamil *et al.,* 2007)
Deccan		Vallari	(Jamil *et al.,* 2007)
Gujrati		Barmi, Moti brahami	(Jamil *et al.,* 2007)
	Hindi	Bemgsag, Brahama-manduki, Khulakhudi, Mandookaparni	(Jamil *et al.,* 2007)
Kanarases		Brahmisoppu, Urage,	(Jamil *et al.,* 2007)
	Urdo	Kodagam, Kodangal, Kutakam; Brahamii	(Jamil *et al.,* 2007)

Phytochemicals of C. asiatica

C. asiatica has been reported to have plentiful phytochemicals, e.g., (terpenes, saponins, glycosides, alkaloids, flavonoids and others) as secondary metabolites(Jamil *et al.,* 2007). Looking for bio-active compounds of *C. asiatica* is a vital target that catches the attention of the many researchers around the world. Various phytochemicals of *C. asiatica* since long time ago, have been linked to the plant environment and origin. The most major components of *C. asiatica* have been divided to major collections, these groups involved triterpenoids saponins and their aglycones correspondents (*asiaticoside*, *madecassoside* and *asiatic* and *madecassic acids*), and pectin, volatile oil, traces of alkaloids, and others(Hashim 2011; Vohra *et al.,* 2011) different types of compounds have been isolated by different researchers all over the world since more than three decades ago, the number of these isolated constituents ranges up to 70. Here we review and report the most major bio-chemicals of *C. asiatica*. *Terpenes, C. asiatica* terpenes group involves many forms of terpenes these forms include the *Monoterpenes* and sesquiterpenes which contains *α*-copaene, *α*-pinene, *β*-elemene, *β*-caryophyllene, *β*-pinene, *trans-β*-farnesene, *γ*-terpinene, myrcene, bornyl acetate,germacrene *D* and bicycloelemene. The most available and most bio-active type of these terpenes are the pentacyclic *Triterpenes*, which include the most vital and interested glycosides and

glycons, which may belong to either ursane or oleanane. *Asiaticoside*, *asiatic acid*, *madecassoside* and *madecassic acid*, are the triterpenes that have been suggested by many studies to be the soul source of the medicinal activity of *C. asiatica*. Orhan, (2012) reviewed and reported triterpenes that have been isolated before 2012 from *C. asiatica*, and others like thankunic acid, isothankunic acid, betulinic acid, asiaticin, centellin, centellicin, bayogenin, brahmic acid, centellasapogenol A, centellasaponins A-D, terminolic acid, 3*β*,6*β*,23- trihydroxyolean-12-en-28-oic acid, 3*β*,6*β*,23-trihydroxyurs- 12-en-28-oic acid, 3-O-[*α*-L-arabinofuranosyl] 2*α*,3*β*,6*β*,23- *α* tetrahydroxyurs-12-en-28-oic acid, pomolic acid, ursolic acid, 3-epimaslinic acid, 23-O-acetylasiaticoside B and 23-O-acetylmadecassoside(Brinkhaus *et al.,* 2000; Orhan 2012; Williamson 2002).

Table 2. The Ethno-pharmacological uses of *Centella asiatica*.

Country/Ethnic group	Prescribed for	Used part	Utilization mode	References
Malaysia	hypertension, diarrhoea and urinary tract infections, a detoxicant, diuretic and to lower blood pressure and decrease heart rate. As cardiodepressant, hypotensive, weakly sedative, tonic, treatment for skin diseases	Leaves and Whole plant	Tea of the plants, dried herb	(ICS-UNIDO, 2006; Jamil *et al.,* 2007)
India	asthma, skin disorders, ulcers and body aches, improving memory, as a nervine tonic and in treatment of dropsy, elephantiasis, gastric catarrh, kidney troubles, leprosy, leucorrhoea and urethritis, in maternal health care, treatment of stomach disorders and also as a vegetable, cure dysentery and improve memory power			(Jamil *et al.,* 2007)
China	dysentery and summer diarrhoea, vomiting, jaundice and scabies, Hansen's disease (leprosy), nosebleeds, tonsillitis, fractures, measles, tuberculosis, urinary difficulties, as a endocrine tonic and as an 'adaptogen', have diuretic properties, Snow plant" for the reason of its cooling properties, longevity and virility		drunk an infusion,	(Jamil *et al.,* 2007)
Nepal	rheumatism, indigestion, leprosy, poor memory, cooling property to body and stomach, kill germs from wounds, cure leprotic wound		leaf juice, Crushed leaf and root extract, Decoction of leaves	(Jamil *et al.,* 2007)
Bangladesh/ Kavirajes/ Chalna area	treat multiple ailments like dog bite, asthma, carminative, itching, leucorrhoea, malaria, tumour and wounds		whole plant is utilized	(ICS-UNIDO, 2006; Jamil *et al.,* 2007)
Fiji	treating Childhood tidal fevers, eye problems, fractures, swollen joints, rib pain and unwanted pregnancy			(Jamil *et al.,* 2007)
Madagascar	leprosy, tuberculosis			(Jamil *et al.,* 2007)
Brazil	elephantiasis and leprosy, the whole plant especially the leaves are used for preparation of hair oil, to prepare chutney, hasuvale, tambali and toddy			(Jamil *et al.,* 2007)
Brunei Darussalam	In urinary tract infection, stones	Leaves		(ICS-UNIDO, 2006)
Philippines	As diuretic, in wounds	Leaves		(ICS-UNIDO, 2006)
Kenya	wound healing			(Indena, 2011)
Central africa and Cape	Wounds and sores are treated topically. Used as a remedy for leprosy, tuberculosis and syphilis	Whole plant		(Indena, 2011)

Asiaticoside was isolated from *C. asiatica* leaves for the first time early in 1950s(Polonoski 1951). It supposed to be the parental resource of *asiatic acid* as it is produced during the metabolic path way of the metabolites production by a hydrolysis reaction of the sugar moiety. *Asiaticoside*, is a marked bio-active constituent in *C. asiatica* as it has been reported to have antimicrobial effects and it has some promising

uses as wound a healing agent together with madecassic and asiatic acids(Grimaldi *et al.,* 1990; Hausen 1993). *Asiatic acid* is another triterpen from *C. asiatica* which is the aglycone of the *asiaticoside*, which also have its special bio-activity, it is reported to have power over cell division in human hepatoma, melanoma cells as well as cytotoxic activity on fibroblast cells and other various pharmaceutical actions(Coldren *et al.,* 2003; Hashim *et al.,* 2011). *Madecassoside*, is the second major glycoside that is reported also to have therapeutic activities, and *madecassic acid* is its glycon structure that has been reported to have capacity to stimulate collagen synthesis(Reihani and Azhar 2012).

Flavonoids, such as apigenin, kaempferol (3-g1ucosylkaempferol and 7-glucosylkaempferol), quercetin (3-glucosylquercetin), rutin, patuletin, castilliferol, castillicetin, stigmasterol, β- sitosterol and myricetin have been found in *C. siatica,* and apigenin was reported as the most abundant among the others(Vohra *et al.,* 2011).

Other phyto-constituents

Amino acids such as glutamate, serine and alanine in addition to another 17 amino acids have been reported to be found in the different parts of *C. asiatica*. Serine, glutamate and alanine have been found to be the majors among the others. *Carotenoids, Alkaloids* (e.g., Hydrocotylin), Ascorbic acid (vitamin C or L-dehydroascorbic acid), Some polyacetylenes such as (3-hydroxy-8-acetoxy-pentadeca-1,9-diene- 4,6-diyne; pentadeca-2-9-diene-4, 6-diyne-1-ol acetate; 3,8-diacetoxypentadeca-1, 9-diene-4,6-diyne; Pentadeca-1, 8- diene-4, 6-diyne-3,10-diol and 3-hydroxy-10-acetoxy-pentadeca-1,8-diene-4,6-diyne), vitamin B and C, tannins, pectic acid, sugars, oligosaccharide and more have been reported to be found in *C. asiatica*. Phenolic compounds are commonly known as antioxidants, are found in *C. asiatica* leaves with different range's start from 3.23g up to 11.7g/100g, In addition, it contains minerals such as potassium, calcium, phosphorus, sodium and ferrous(Reihani and Azhar 2012; Vohra *et al.,* 2011; Zainol M. K. *et al.,* 2003; Zheng C. J. and Qin 2007).

Wound healing and anti-inflammatory effect of C. asiatica

The process of wound healing from first to last passes through a versatile operation involving various types of cells. Keratinocytes and fibroblasts of epidermal and dermal layers of the skin play important roles in this pathway(Lee *et al.,* 2012). In 1950's was the first research to examine the *asiaticoside* role in wound healing, and this study gave an idea that *asiaticoside* has the ability to heal wound and ulcer. It has been reported that a mixture of C. *asiatica* selected terpenes (CAST) can help the formation of wound tissue and improved the tensile strength of the newly made skin after local application on rat wounds(Rosen *et al.,* 1967; Tsurumi *et al.,* 1974), it was also reported that a customized *C. asiatica* extract increased speed of rats chronic wounds healing after oral administration of 100 mg/kg. Subsequently, an *in-vitro* work evaluated the effect of the CAST mixture on cultured human vein and skin fibroblasts, and it was concluded that proline incorporation and stimulate collagen biosynthesis was increased, which has been confirmed the *in-vivo* studies of the using of alcoholic extract of *C. asiatica* leaves. Another study concluded that 25 µg/mL of CAST induced the cell layer fibronectin on cultures of human skin fibroblast more over it was reported that 15-70 µg/mL of CAST enhanced the rate of endothelial cells adherence in the culture, and showed a interesting activity on fibronectin and PGI2 production. Also, it was verified that a mixture of *C. asiatica* selected terpens can slow down the platelet aggregation stimulated by collagen, ADP, and arachidonic acid. Some clinical researches showed that daily addmision of 60-120 mg from CAST, for 30-90 days have been improved the subjective and the objective symptoms of the primary or secondary chronic venous insufficiency of the lower limbs(Indena, 2011). Other clinical studies(Belcaro *et al.,* 1990a; Belcaro *et al.,* 1990b; Cesarone *et al.,* 1992) have been showed that CAST can interfere a range of stages of venous disease for instance venous

wall changes, transform of connective metabolism, endothelial distress, impairment of microcirculation. It has been reported that CAST can encourage collagen synthesis *in-vitro* and reduces serum lysosomal enzymes in patients with varicose veins(Arpaia *et al.,* 1990). *C. asiatica* triterpenes have reduced capillary permeability and enhanced microcirculatory parameters and ankle edema in the patients who are suffering from chronic venous deficiency linked with venous hypertension in addition to the diabetic microangiopathy patients(Belcaro *et al.,* 1990a; Belcaro *et al.,* 1990b). C. asiatica triterpenes also, have shown to influence the lymphatic task in the lymphatic and postphlebitic edema patients(Cesarone *et al.,* 1991). all the experiments have been reported that CAST, strongly demonstrated to be useful in reducing the symptomatology related to chronic venous insufficiency such as tiredness, phlebodynia, itching in the legs, and night cramps, which went out in a large number of patients(Lee *et al.,* 2012; Shim *et al.,* 1996).

C:\Users\User\Downloads\Maquart, 1999Maquart *et al.,* (1999) and his team studied the effect of the Titrated Extract from *C. asiatica* (TECA) on wound healing, TECA included *asiatic acid*, *madecassic acid* and *asiaticoside* as well as the effect of each components separately in the wound chamber model. they inserted the Stainless steel wound chambers surgically under the skin of rats and injected them by either TECA or the purified components. The team's results showed that TECA-injected wound chambers were characterized by increased dry weight, DNA, total protein, collagen and uronic acid contents, likewise, Peptidic hydroproline was also increased, showing an increased remodeling of the collagen matrix in the wound. Then again, The three separated compounds of TECA were all able to mimic the effects of the mixture, with a few variations depending on the compund, thus, *Asiatic acid* and *asiaticoside* appeared to be the most active of the three triterpenes. *Asiaticoside* showed activity on collagen synthesis at low doses only, moreover, Maquart *et al.,* (1999) study has reported that TECA was also able to stimulate glycosaminoglycan synthesis as well as the collagen(Maquart *et al.,* 1999). It was reported that extract of *C. asiatica* containing madecassic acid, asiatic acid and asiaticoside known as Madecassol, speed up cicatrisation and fix of injure. Moreover, asiaticoside found to endorse fibroblasts proliferation and extracellular matrix synthesis in wound healing (Srivastava *et al.,* 1997). *C. siatica* extract ointment, α-tocopherol and collagen-elastin hydrolysates was associated with less women developing stretch marks(Young and Jewell 2000). Triterpene composition of the leave extract of *C. asiatica* has been showed that the highest collagen synthesis was found at 50 mg/mL of the extract(Hashim *et al.,* 2011

A study has been investigated the effect as anti-pruritic and anti-inflammatory of *C. asiatica* extract in rats and anti-allergic *in-vitro* using sheep (Capra hircus) serum method and compound 48/80 induced mast cell degranulation method. *C. asiatica* extract was administered in rats orally, the results has been showed that the extracts of *C. asiatica* demonstrated anti-allergic, anti-pruritic and anti-inflammatory behavior(George *et al.,* 2009).

Another study results confirmed the useful effects of the *C. asiatica* extract for treating the diabetic wounds in people with diabetes mellitus(Nganlasom *et al.,* 2008).

C:\Users\User\Downloads\Somboonwong, 2012Somboonwong *et al.,* (2012) investigated the wound curing activities of chronological hexane, ethyl acetate, methanol, and water extracts of *C. asiatica* in scar and partial-thickness burn wound in rats models. they also reported that the used *C. asiatica* extracts has relieved the wound remedial development in incision and burn wounds equally. In addition it was found that the analysis of the different extracts on thin layer chromatography have verified that the major phyto-constituents were β-sitosterol, asiatic acid, and asiaticoside and madecassocide in the hexane, ethyl acetate and methanol extracts, correspondingly and then concluded that it might be the *asiatic acid* in the ethyl acetate extract the most active constituent for wound healing process(Somboonwong *et al.,* 2012). Ruszymah *et al.,*

(2012)C:\Users\User\Downloads\Ruszymah, 2012 have been examined *C. asiatica* effects of on the proliferation and migration of rabbit corneal epithelial (RCE) cells as an in-vitro model, they found that RCE cells show significant enhancement of migration rate compared to the control group at concentrations up to 62.5 ppm, they concluded, that supplementation of *C. asiatica* aqueous extract at low concentrations could be useful to help in the healing of corneal epithelium wound(Ruszymah *et al.,* 2012). In an *In-vitro* and *In-vivo,* Wu *et al.,* (2012) have investigated the effect level of the four main triterpenes of *C. asiatica*. Primary human skin fibroblasts from healthy human foreskin samples, have treated with concentration range of the four constituents, and collagen synthesis, cell proliferation, MMP-1/TIMP-1 balance, and TGF-*β*/Smad signaling pathway have examined *In-vitro*. In addition, *In-vivo*, the four compounds have orally administered to mice for two weeks after burn injury, then, the speed and quality of healing the wound, in addition to TGF-*β*1 levels in the skin tissues, have studied. The results have concluded that *asiaticoside* and *madecassoside* are the most active constituents of *C. asiatica* which is responsible for healing the burn wound, while, *asiatic acid* and *madecassic acid* were not. Furthermore, *in-vitro*, *madecassoside* for pro-collagen type III synthesis showed to be more effective than *asiaticoside* with *P* value 0.0446, for wound healing speed with *P* value 0.0057, and *P* value 0.0491 for wound healing pattern *in vivo*, correspondingly)(Wu *et al.,* 2012).

Lee *et al.,* (2012) studied *asiaticoside* effects on an *in-vitro* system, they found that in a wound closure seeding model, *asiaticoside* has increased the migration rates of skin cells, they also concluded that *asiaticoside* has improved the primary skin cell adhesion, in addition to that they have reported that *asiaticoside* induced the increase of the number of normal human dermal fibroblasts(Lee *et al.,* 2012).

Paolino *et al.,* (2012) studied the possibility of ultra-deformable vesicles as a feasible relevant delivery system for *asiaticoside*. they used Ultra-deformable vesicles with sodium cholate molar fractions, their results showed that sodium cholate molar fraction of 0.2 was the most suitable topical carriers for *asiaticoside* and *asiaticoside*-loaded ultra-deformable vesicles with this molar fraction obtained the best degree of collagen bio-synthesis in human fibroblasts. they have concluded that the greatest *in-vitro* skin permeation of *asiaticoside* with 10 times increased due to the using of Ultra-deformable vesicles(Paolino *et al.,* 2012). In another study mice were pretreated with 45 mg/kg *Asiaticoside* in addition to GW9662 an hour before the cecal ligation and puncture (CLP), the survival, inflammatory mediators and signaling molecules, lung injury, and Peroxisome proliferator-activated receptor-γ (PPAR-γ) have evaluated 24 hours after CLP. *Asiaticoside* drastically has reduced CLP-induced the mortality, lung pathological damage, the infiltration of mononuclear, polymorphonuclear (PMN) leucocytes and total proteins. Also, *Asiaticoside* has repressed CLP-induced the activation of mitogen-activated protein kinases (MAPKs) and nuclear factor-κB (NF-κB), the expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) protein in lung tissues, and the production of serum tumor necrosis factor (TNF-α) and interleukin-6 (IL-6). The study concluded that the expression of PPAR-γ protein in lung tissue has up-regulated. Additionally, GW9662 - the PPAR-γ inhibitor- has notably reversed the effects of *asiaticoside* in the diseased mice. as a brief, the results have proposed that *asiaticoside* might be efficiently defend against septic lung injury stimulated by CLP and the underlying mechanisms might be correlated with up-regulation of PPAR-γ expression to some level, which slow down passageway of the MAPKs and NF-κB(Zhang Li-na *et al.,* 2011). *Asiaticoside,* 5, 10 and 20 mg/kg per day has orally admitted for three days to a mice, after that Lipopolysaccharide(LPS)/D-galactosamine(D-GalN) (LPS/D-GalN) has injected. The results showed major protection. This protection confirmed by the reduction of the important amino-transferases, hepatocytes apoptosis and caspase-3, improvement of mortality and development of liver pathological damage. The experiments has shown that *asiaticoside*

has reduced the increase of phospho-p38 MAPK, phospho-JNK, phospho-ERK protein and TNF-alpha mRNA expression in liver tissues and plasma TNF-alpha, which advocated that *asiaticoside* has a significant hepato-protective behavior on LPS/D-GalN-induced liver damage and the probable moode of action might be associated with the inhibition of TNF-alpha and MAPKs(Zhang L. *et al.,* 2010), the same group has worked on the kidney injuries and found that *asiaticoside* has a protective effects against sepsis-induced acute kidney injury, which possibly related to the inhibition of IL-6 in serum and iNOS protein in kidney tissues(Zheng J. *et al.,* 2010). Another study on *asiaticoside* showed it reduces the fibroblast proliferation, it also, inhibited mRNA expressions and type I and type III collagen protein. Additionally, the expression of TGF-βRI and TGF-βRII at the transcriptional and translational level has reduced. Besides, *asiaticoside* has increased the expression of Smad7 protein and mRNA, along with, it can be said that *asiaticoside* might be an alternative suplement for treatment and prevention of wounds and keloids(Tang B. *et al.,* 2011).

In a most recent study, different techniques such as MTT assay for cell number count, reverse transcription-polymerase chain reaction for mRNA expression analysis, Western blot analysis and immuno-cyto-chemistry for protein synthesis confirmation and alkaline phosphatase activity to determine the Osteogenic differentiation has been used to confirm the proficiency of *asiaticoside* on wound healing. Human periodontal ligament cells (HPDLs) have treated with 25, 50, and 100 μg/mL *asiaticoside*, then, its effects on protein synthesis, proliferation, and osteogenic differentiation in the HPDLs have examined. The results have recommended the possible application of *asiaticoside* for encouraging the curing of periodontal tissue(Nowwarote *et al.,* 2013).

Primary keloid-derived fibroblasts KFs, originating from human earlobe keloids, has purified and cultured, after that, they were treated with 10, 30, and 100μM of *madecassoside*. In transwell migration assays and scratch-wound-closure assays, KF migration has significantly suppressed by *madecassoside* treatment. Also, KFs which treated with *madecassoside* has demonstrated a decrease in F-actin filaments, as exposed by fluorescein iso-thiocyanate (FITC)-phalloidin staining and confocal microscopy. *Madecassoside,* by Western blot analysis has showed extraordinarily attenuate the phospho-rylation of cofilin, p38 MAPK and phosphatidylinositol-3-kinase (PI3K)/AKT signaling, as a result it was suggested that *madecassoside* might be of immense use in the curing and/or avoidance of hypertrophic keloids and wounds(Song *et al.,* 2012).

Acetic acid as anti-inflammatory has reduced the paw edema at the fourth and fifth hours after λ-carrageenan (Carr) administration and improved the actions of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in the liver tissue. *acetic acid* also, has reduced the nitric oxide (NO), tumor necrosis factor-α (TNF-α), and interleukin-1β (IL-1β) levels. In addition, it decreased cyclooxygenase (COX-2), Carr-induced inducible nitric oxide synthase (iNOS), and nuclear factor-κB (NF-κB) expressions in the edema paw. Huang *et al.,* (2011) has linked the mechanisms of anti-inflammatory effects of *asiatic acid* to the reduction of MDA, iNOS, COX-2, and NF-κB levels of the edema paw by increasing the activities of SOD, CAT, and GPx in the liver.

A new reported urasane, *asiaticoside* G [2α,3β,23,30-tetrahydroxyurs-12-en-28-oic acid 28-O-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester, has been isolated by Nhiem *et al.,* (2011) and its anti-inflammatory activities were studied on lipopolysaccharide (LPS)-stimulated RAW 264.7 cells. The results showed that production of nitric oxide and tumor necrosis factor-α on the concentration of 100 μM have been inhibited by 77.3% and 69.0%, respectivelyC:\Users\User\Downloads\Nhiem, 2011. Wan *et al.,* (2012) experiments, have confirmed that *Asiaticoside* can inhibit lipopolysaccharide (LPS)-induced fever and inflammatory response, including serum tumor necrosis factor (TNF)-α and interleukin

(IL)-6 production, liver myeloperoxidase (MPO) activity, brain cyclooxygenase-2 (COX-2) protein expression and prostaglandin E(2) (PGE(2)) production. *Asiaticoside* also, has increased serum IL-10 level, liver heme oxygenase-1 (HO-1) protein expression and activity. Moreover, it was observed that the suppressive effects of *asiaticoside* on LPS-induced fever and inflammation have reversed by pretreatment with ZnPPIX, a HO-1 activity inhibitor. In conclusion, it can be said that *asiaticoside* has the antipyretic and anti-inflammatory effects in LPS-treated rat and these effects may perhaps linked with the inhibition of pro-inflammatory mediators, as well as TNF-α and IL-6 levels, COX-2 expression and PGE(2) manufacture, in addition to MPO action, which could be mediated by the up-regulation of HO-1C:\Users\User\Downloads\Wan, 2012.

At the end the total conclusion of the results suggested that *C. asiatica* and its components could adapt the connective tissue metabolism, supports the healing of wounds and also develops microcirculation of tissue. *C. asiatica* leaves' extract and *asiaticoside* have been reported to be well tolerated in experimental animals especially by oral route.

Neurological effects of C. asiatica

In South east Asian countries, *C. asiatica* has been used for different neurological instabilities.

C. asiatica tablets administered orally to mentally retarded children proved major increase in general capacity and behavior patterns(Rao-Appa *et al.,* 1973; Young and Jewell 2000).

Asiaticoside effect has been tested on a putative anxiolytic male mice by using a number of experimental paradigms of anxiety. Researchers found that ten mg/kg of *asiaticoside* have "significantly increased head-dipping counts and duration as well as diazepam (0.3 mg/kg)" (Chen Si Wei *et al.,* 2006). Antidepressant effects of *asiaticoside* has been examined on mice, it was suggested that *asiaticoside* could have antidepressant activity(Liang *et al.,* 2008). In addition Haleagrahara and Ponnusamy Haleagrahara and Ponnusamy (2010) reported that administration of *C. asiatica* was effective in protecting the brain against Parkinson disease and the neuro-degerative disordersC:\Users\User\Downloads\Haleagrahara, 2010. In another study, male mice has exposed to 5 mg/kg of *C. asiatica* prophylaxis for ten days, and after two days followed by 3-nitropropionic-acid 3-NPA administration 75 mg/kg and the level of the oxidative stress in the cytoplasm of the brain area has evaluated. The results showed that *C. asiatica* prophylaxis has ameliorated the 3-nitropropionic-acid (3-NPA) - induced oxidative stress, furthermore, it was assumed that the prophylactic protection presented by *C. asiatica* extract against neuro-toxicant exposure might be mainly because of its capacity to recover thiols, GSH and antioxidant defenses in the brain of mice(Shinomol and Ravikumar 2010).

A study has reported that extracts of *C. asiatica* (n-hexane extract, chloroform extract, ethyl acetate extract, n-butanol extract) have anti-convulsant and neuro-protective action, therefore, *C. asiatica* extracts are proficient to be used for efficient managing in epileptic seizures healing(G *et al.,* 2010).

It was observed that administration 30 mg/kg of *asiatic acid* have been facilitated passive avoidance and active avoidance on memory and learning. thus, it might be helpful for memory and learning with less consequence in blood pressure in supporting memory and learning increases(Nasir *et al.,* 2011).

Wanasuntronwong *et al.,* (2012) studied the effect of a standardized extract of *C. asiatica* containing triterpenoids not less than 80% on anxiolytic. The study showed a promising effect on both acutely and chronically stressed animals, the team concluded that this effect possibly will mainly be because of *madecassoside* and *asiaticoside* which may suggest a possible use of *C. asiatica* extracts for the treatment of both acute and chronic anxiety in the pathological state.

Neuro-protective effects of *asiaticoside* has been investigated by Xu C. L. *et al.,* (2012a), in the rats model of Parkinsonism induced by 1-methyl-4-phenyl- 1,2,3,6-tetrahydropyridine(MPTP). The study found that using the *asiaticoside* has protected dopaminergic neuron by antagonizing MPTP induced neurotoxicity and has improved locomotor dysfunction, moreover, it has significantly attenuated the MPTP-induced decrease of dopamine in the striatum. Experiments showed that the content of MDA has been considerably declined whereas the GSH level has notably increased in *asiaticoside*-treated groups. Besides, *asiaticoside* has increased the Bcl-2/Bax percentage. At the end the research group have sum up their results with the statement that *asiaticoside* has showed an effective behaviour in reversing MPTP induced Parkinsonism via its neuroprotective properties as well as the antioxidant activity, keeping the metabolic balance of DA, and increasing ratio of Bcl-2/Bax(Xu C. L. *et al.,* 2012a). Major increase has been shown in the dendritic length - intersections - and dendritic branching points in amygdaloid neurons (the area responsible with memory and learning) of the rats treated daily with 6 mL/kg of *C. asiatica* leave extract for 6 weeks or long time. The neuronal dendritic growth indicates that *C. asiatica* has the properties that enable it to enhance neuronal dendrites in stress and memory disorders and other neurodegenerative(Mohandas Rao *et al.,* 2012). 150 and 300 mg/kg per day for 6 weeks of *C. asiatica* has introduced to male Wistar rats. The extract appreciably enhanced memory performance, oxidative defense decreased aluminum concentration, acetylcholinestrease activity, caspase-3 and reversal of mitochondrial enzyme activity. Results showed the neuro-protective latent of *C. asiatica* against aluminum-induced cognitive dysfunction and mito- oxidative damage(Prakash and Kumar 2012). One more study has been examined *C. asiatica* water extract effects of on activity of subtypes of phospho-lipase A2 (PLA2) in primary cultures of rat cortical neurons, it found and concluded that cPLA2 and sPLA2 behaviors were repressed *in-vitro* by *asiaticoside* present as majority component in *C. asiatica* water extract, which point toward that the extract could be a nominee for the treatment of neuro-degenerative process(Defillipo *et al.,* 2012). Various concentrations of *C. asiatica* ethanolic extract 100, 200 and 300 mg/kg body weight once daily for three weeks have presented orally to male Wistar rats, then subjected to right middle cerebral artery occlusion for two hours followed by 22 hours reperfusion. It was shown that *C. asiatica* administration has been reduced infarction volume and enhanced neurobehavioral activity as well as the repair of histological morphology of brain in middle cerebral artery occlusion rats. Besides, it has showed that *C. asiatica* extract supplementation to middle cerebral artery occlusion group has decreased the level of thiobarbituric acid reactive species, reinstated the glutathione content and increased the activities of antioxidant enzymes-catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase and superoxide dismutase in a dose-dependent manner in ischemic rats(Tabassum *et al.,* 2012). *C. asiatica* water extract has presented a good therapeutic mechanism and novel phytochemical of potential significance to the treatment of Alzheimer's disease(Soumyanath *et al.,* 2012).

Zhang X. *et al.,* (2012) have found that *asiatic acid* (0.01 to 1.0 μmol/l) in the primary neurons has reduced the C(2)-ceramide-induced cell death and mitochondria membrane potential. Also, it declined cellular manufacturing of reactive oxygen species following C(2)-ceramide treatment. 1.0 μmol/l of *asiatic acid*, has partially counteracted the pro-apoptotic effects of the C(2)-ceramide through decreasing the cytosolic release of HtrA2/Omi, the up-regulation of Bax and caspase 3, as well as the dephosphorlyation of ERK1/2. The results reported that *asiatic acid* can defend neurons from C(2)-ceramide-induced cell death by antagonizing mitochondria-dependent apoptosisC:\Users\User\Downloads\Zhang, 2012.

Other studies showed that *C. asiatica* can be antidepressant and memory enhancing. It has been stated that the whole triterpenes had antidepressant action and caused significant decrease of the

corticosterone level in serum(Chen Y. *et al.,* 2003). In addition, it was reported that *C. asiatica* aqueous extract has been showed noteworthy effects on learning and memory in addition to the reduction of the levels of norepinephrine, 5-HT and dopamine and their metabolites in the brain(Nalini *et al.,* 1992).

Anti-Oxidant, Cytotoxic and Anti-tumor effects of C. asiatica

Malignant tumors became as the top reason to death in our present life. A number of studies have been conducted to check the effects of *C. asiatica* and its components on the tumor cells on cytotoxicity and anti-oxidant. These studies have been reported that oral supplementation of *C. asiatica* extract as well as its partially purified fractions have encouraged apoptosis in solid and Ehrlich Ascites tumor, also it made the life span of the mices with tumors increased and other researchers reported that *Asiatic acid* have an anticancer effect on skin cancer (Babu and Paddikkala 1994; Park *et al.,* 2005).

Babykutty *et al.,* (2008) have published the results of their experiment on the ability of the methanolic extract of *C. asiatica* as apoptosis inducer, using different cancer cell lines. The results have found that *C. asiatica* extracts induced apoptosis in MCF-7 cells, then they cocluded the possibility to use extract of the *C. asiatica* as a part of herbal medicines treatments of tumor cellsC:\Users\User\Downloads\Babykutty, 2008. *C. asiatica* aqueous extracts have showed important DPPH scavenging action, with an IC(50) value of 31.25 microg/mL. The extracts showed to have capability against rat glioma cell lines, mouse melanoma and human breast cancer, with IC(50) values of 1000.0, 698.0 and 648.0 microg/mL correspondingly(Pittella *et al.,* 2009).

Asiatic acid also has been studied by Tang X. L. *et al.,* (2009), the study examined the ability of *asiatic acid* to inhibit the growth of the cancer cells, it has reported that the *asiatic acid* obviously inhibited the cancer cells propagation and apoptosis of SW480 human colon cancerC:\Users\User\Downloads\Tang, 2009. The *n*-hexane, carbon tetrachloride, chloroform and aqueous soluble fractions of *C. asiatica* methanolic extracts have been examined in the brine shrimp lethality bioassay and showed a significant cytotoxic potentials having LC50 1.254, 0.826, 3.866 and 5.366µg/ml respectively(Ullah *et al.,* 2009), in another study, *C. asiatica* methanolic extracts fractions have showed a sensible to strong antioxidant activity, the chloroform and aqueous soluble fraction demonstrated the best antioxidant action with the IC50 value of 4.0 µg/ml and 7.0 µg/ml, respectively(Ullah *et al.,* 2009). Furthermore, studies showed that *C. asiatica* extracts and its active components have repressed CYP2C9, CYP2D6 and CYP3A4 actions with varying strength with CYP2C9 being the most liable isoform to inhibition. The major inhibition has observed for *asiatic acid* plus ethanol and dichloromethane extracts, implying involvement of semi-polar components of *C. asiatica* in the effect. The study has proposed that *C. asiatica* could cause drug-herb exchanges through CYP2C9 inhibition(Pan *et al.,* 2010). It was also observed that dichloromethane extracts strongly inhibited the CYP2C19 activity(Pan *et al.,* 2011). Another study has showed that after two months of treatment using daily doses 500 and 750 mg of *C. asiatica* has improved lower extremity strength accessed using the 30-s chair stand test. Mato *et al.,* (2011) also reported that high doses of *C. asiatica* might increase the life satisfaction subscale in the physical function subscale, especially in the lower extremities of the elderlyC:\Users\User\Downloads\Mato, 2011. Hashim *et al.,* (2011) have studied the triterpene composition of *C. asiatica* leaves and they reported that the bioactivity as an antioxidant was (84%) in comparison with other natural antioxidants.

A study on *madecassoside* has reported that concentrations of 10, 30, 100 µmol./L *madecassoside* might reverse morphological changes, elevate cell viability, increase glutathione levels, along with the reduce of lactate dehydrogenase and malondialdehyde levels. *Madecassoside,* also, attenuated apoptosis, stopped the start of caspase-3 and the loss the mitochondria membrane potential, and the phosphorylation of p38 mitogen-activated protein kinase (MAPK) in HUVECs, then it was

concluded that *madecassoside* can keep HUVECs from oxidative injury, which possibly accomplished by slowing down cell apoptosis via protection of mitochondria membranes and down regulation of the activation of caspase-3 and p38 MAPK(Bian *et al.,* 2012). A dose of 10 mol./L of *asiatic acid* has been considerably reduced apoptotic cell death and reduced reactive oxygen species, stabilized the mitochondrial membrane potential, and endorsed the expression of PGC-1α and Sirt1. In the mice's models, oral administration of 100 mg/kg *asiatic acid* has been notably attenuated cognitive insufficiency in the Morris water maze test, and restored lipid peroxidation and glutathione and the activity of SOD in the hippocampus and cortex to the control levels. Doses of 50 and 100 mg/kg *asiatic acid* also attenuated neuronal damage of the pyramidal layer in the CA1 and CA3 regions. In conclusion it can be said that *asiatic acid* can attenuate glutamate-induced cognitive deficits of mice and keeps SH-SY5Y cells against glutamate-induced apoptosis *in-vitro*(Xu M. F. et al. 2012b). And most recently Thoo *et al.,* (2013) have been studied, The synergistic antioxidant properties of *C. asiatica* ethanolic extracts mutually with α-tocopherol. They have concluded that the interactions between *C. asiatica* and α-tocopherol to reveal diverse degrees of interactions that can increase the antioxidant activityC:\Users\User\Downloads\Thoo, 2013.

Anti-Microbial effect of C. asiatica

Fractions of the methanolic extract which dissolved in *n*-hexane and chloroform has been tested as antimicrobial, the crude extracts demonstrated remarkable antibacterial and antifungal activity against sixteen microorganisms. The *n*-hexane, carbon-tetrachloride, chloroform and aqueous soluble partitionates of the methanolic soluble fractions showed average zones of inhibition ranged from 7-15 mm, 8-12 mm, 8-16 mm and 8-13 mm, respectively, at a concentration of 400 µg/disc. (Ullah *et al.,* 2009). It was reported also that *asiaticoside* has been shown to be helpful in the healing of leprosy and certain forms of tuberculosis(Singh *et al.,* 2010; Zheng C. J. and Qin 2007).

The methanolic extract of *C. asiatica* showed MIC of >500_g/ml against MA and MS *mycobacterium* species of where, streptomycin had IC50 value of 1.14 and 0.17_g/ml against MA and MS, respectively, in micro broth dilution assay(Gautam *et al.,* 2007). Alcoholic extract of *C. asiatica* also illustrated activity against *Entamoeba histolytica* as anti protozoal effect(Dhar *et al.,* 1968).

Cardio-protective effect of C. asiatica

Alcoholic extract of *C. asiatica* gave an idea that it has a strong cardio-protective activity in limiting ischemia-reperfusion stimulated myocardial infraction in rats(Pragada *et al.,* 2004).

The therapeutic effect of one of the major Triterpenes of *C. asiatica*, *madecassoside*, has been evaluated on rat cardiac dysfunctions during sepsis induced by lipopolysaccharide, in addition to the potential mechanism. This study has found that pretreatment of the rats with 20 mg/kg of *madecassoside* significantly repressed the increase of plasma TNF-alpha, delayed the fall of mean arterial blood pressure, and attenuated the tachycardia induced by LPS. More over it was observed that *madecassoside* has prevented the LPS-induced nuclear factor-kappa B (NF-kappaB) translocation from the cytoplasm into the nucleus, and inhibited the LPS-induced phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) and p38. The research team concluded that their results suggested *madecassoside* to reduce LPS-stimulated TNF-alpha creation through the blocking of ERK1/2, p38 and NF-kappaB pathways in cardiomyocytes and as consequences it might have cardioprotective property in LPS-mediated sepsis(Cao *et al.,* 2010

Radio-protective effects of C. asiatica

C. asiatica could be good in preventing radiation encouraged behavioral changes during clinical radiotherapy. *C. asiatica* extracts reported to exhibit a similar UV safety result to OMC at 10% concentration(Hashim *et al.,* 2011). An *et al.,* (2012) in their work on evaluation of the defensive effects of *C. asiatica* Titrated extract against ultraviolet B

(UVB) damage in human keratinocytes using microRNA (miRNA) expression profiling analysis found that the extract has demonstrated low cytotoxicity in normal human HaCaT keratinocytes at <5 μg/ml doses, furthermore, UVB 50 mJ/cm2 irradiation has considerably reduced the cell viability, and the extract treatment has decreased the UVB toxicityC:\Users\User\Downloads\An, 2012.

Immuno-modulating effects of C. asiatica

C. asiatica methanolic extracts as well as the isolated pectin and some saponins have been reported to be immuno-stimulating actively and showed a preliminary immunomodulatory outcome(Jayathirtha and Mishra 2004; Wang *et al.,* 2003). The effects of *C. asiatica* extracts injection on chronic renal failure rats has been studied by Pang *et al.,* (2010) have found that High-doses of compound *C. asiatica* enema has considerable healing results on the chronic renal failure ratsC:\Users\User\Downloads\Pang, 2010.

Anti-diapetic extract of C. asiatica

Babish *et al.,* (2010) group has reported that among a 203 screened botanical products, *C. asiatica* showed to have a greatest activity as anti-diabetic herbC:\Users\User\Downloads\Babish, 2010.

And Chauhan *et al.,* (2010) in their study noted that *C. asiatica* ethanolic and methanolic extracts have shown significant protection and reduced the levels of the blood glucose to the normal in glucose tolerance tests. The highest decrease in the blood glucose in alloxan induced diabetic rats has been observed after three hours with dose 250 mg/kg of the body weight. As a conclusion Chauhan *et al.,* (2010) found that both extracts have been shown a noteworthy anti-diabetic action equivalent with that of glibenclamideC:\Users\User\Downloads\Chauhan, 2010. Another study has been reported that at a dose of 200 mg/kg of *C. asiatica* ethanolic extract anti-diabetes activity in streptozotocin diabetic male wistar rats was significant(Gayathri *et al.,* 2011). Recently, a study on *Asiatic acid* derived from *C. asiatica*, found that it might be a potential bio-active compound to adjust the metabolism of the carbohydrate by adapting the key, regulatory enzymes in streptozotocin (STZ)-induced diabetic rats(Ramachandran and Saravanan 2013).

Sliming effect

Extracts of *C. asiatica* demonstrated a remarkable increase in the cyclic adenosine mono phosphate content with a subsequent rise in the non-esterified fatty acids content in human adipocytes(Tholon *et al.,* 2002).

Conclusion and Remarks

Hundreds of years ago *centella asiatica* has been reported to be used widely in the traditional remedy due to the believe of its medicinal properties, however, there were no experimental evidences and clinical results. Therefore, attention on *C. asiatica* and studies on this plant, its medicinal efficacy and how to improve its bio-active compounds specially the triterpenoids production has been greater than before and it is clearly shown that the effects of *C. asiatica* studies on wound healing were the most and the common published articles. Although, there are many studies and experiments have been reported in this literature, it has been noted that most of the old as well as the new studies have been concluded by statements such as " Further development of ECa 233 as an anxiolytic agent should be carried out (Wanasuntronwong *et al.,* 2012), ~. However, further research is essential (Mato *et al.,* 2011), "Further studies will be required to assess the generality of present findings to other species and behaviourial paradigms (Chen Si Wei *et al.,* 2006), "efforts continue further to define the activity of this class of compounds and to discover new wound healing agents (Shim *et al.,* 1996) and others. That why it is very important to review and continue the research on this plant and share our findings with the pool of these findings related to the *C. asiatica*. This state review article is a part of an ongoing research to isolate the major bio-active compounds from *C. asiatica* and applying the biotransformation techniques and biotechnological methods on the plant

and its compounds in order to produce novel secondary metabolites with more potent activity. It can be concluded that this "status" paper might be a small gate on this still unclear plant that might have a significant medicinal.

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