CHAPTER II

LITERATURE REVIEW

Centella asiatica (Linn.) Urban is known as Bua-bok, Gotu Kola, Asiatic Pennywort, Indian Pennywort, Indian Water Navelwort, Mandukparni, etc. It is a cultivated plant in the family Apiaceae or Umbelliferae that creeping subtropical and topical climates of Asia, Africa, North and South America such as Thailand, Sri Lanka, India etc (1-2).

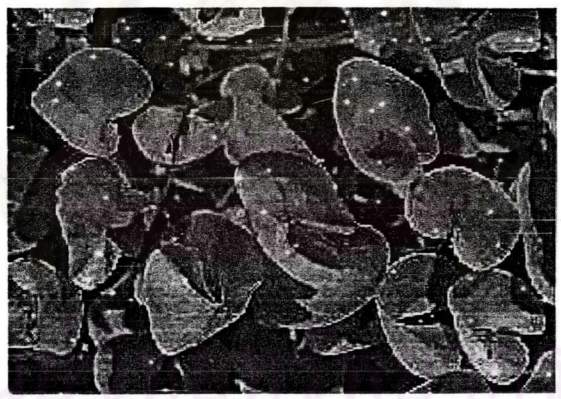

Figure 2.1 Picture of *Centella asiatica* (Linn.) Urban

2.1.Botanical description

A perennial, slender, herbaceous, creeper plant has a smell reminiscent of tobacco and a mildly bitter taste. The leaves are glabrous, kidney shaped 2-5 cm in diameter, with long petioles, arising from the stem nodes in rosettes. The stems

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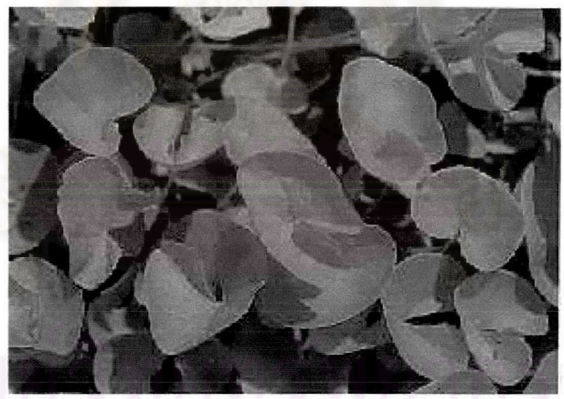

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2.1.Botanical description

A perennial, slender, herbaceous, creeper plant has a smell reminiscent of tobacco and a mildly bitter taste. The leaves are glabrous, kidney shaped 2-5 cm in diameter, with long petioles, arising from the stem nodes in rosettes. The stems

2.2.2 Essential oil (13)

The aerial parts of CA contains 0.1% of essential oil which compose of 80% sesquiterpenoids such as β-caryophyllene, α-humulene and germacrene-D, elemene and bicycloelemene, trans-farnesene.

2.2.3 Flavone derivatives (11)

Quercetin and kaempferol glycosides and astragalin have been found.

2.2.4 Phytosterols (14)

Stigmasterol, sitosterol have been found.

2.2.5 Amino acids (10-11)

The leaf contains alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine and tryptophan.

2.3 Medicinal and pharmacological activities

2.3.1 Wound-healing activity

A wound is a disruption of tissue integrity that is typically associated with a loss of substance. The wound healing process is generally independent of the form of injury. It is convenient to divide the overall process into three overlapping phases (inflammatory phase, proliferative phase, maturational phase).

The inflammatory phase is characterized by homeostasis and inflammation. Collagen exposed during wound formation activates the clotting cascade (both the intrinsic and extrinsic pathway), initiating the inflammatory phase. After injury to tissue occurs, the cell membranes, damaged form the wound formation, release thromboxane A_2, and prostaglandin 2- alpha, potent vasoconstriction. This initial response helps to limit hemorrhage. After a short period, capillary vasodilation occurs secondary to local histamine release, and the cells of inflammation are able to migrate to the wound bed. Platelet, the first response cell, release multiple chemokines, including epidermal growth factor (EGF), fibrinogen, fibronectin, histamine, platelet-derived growth factor (PDGF), serotonin, and von Willebrand factor. These factors help stabilize the wound through clot formation. These mediators act to control bleeding and limit the extent of injury. platelet degranulation also activates the complement

cascade, specifically C5a, which is a potent chemoattractant for neutrophils. The second response cell to migrate to the wound, the neutrophil, is responsible for debris scavenging, complement-mediated opsonization of bacteria, and bacteria destruction *via* oxidative burst mechanism (ie , super oxide and hydrogen peroxide formation). The neutrophils kill bacteria and decontaminate the wound from foreign debris. Leukocytes and macrophages are the next cells present in the wound. Numerous enzymes and cytokines are secreted by the macrophage. These include collagenase, which debride the wound; inteleukins and tumor necrosis factor (TNF), which stimulate fibroblasts (produce collagen) and promote angiogenesis, and transforming growth factor (TGF), which stimulates keratinocytes. This step marks the transition into the process of tissue recostruction (the proliferative phase) (15).

The proliferative phase is the second stage of wound healing. Epithelialization, angiogenesis, granulation tissue formation and collagen depostion are the principal steps in this anabolic portion of wound healing. Epithelialization occurs early in wound repair. If the basement membrane remain intact, the epithelial cells migrate upwards in the normal pattern (equivalent to a first-degree skin burn). The epithelial progenitor cells remain intact below the wound, and the normal destroyed, similar to a second- of third- degree burn, then the wound is re-epithelialized from the normal cells in the periphery and from the skin appendages, if intact. Angiogenesis, stimulated by TNF-alpha, is marked by endothelial cell migration and capillary formation. The new capillaries deliver nutrients to the wound and

help maintain the granulation tissue bed. The migration of capillaries into the wound bed is critical for proper wound healing. The granulation phase and tissue deposition require nutrients supplied by the capillaries, and failure for this to occur results in a chronically unhealed wound. Mechanisms for modifying angiogenesis are under study and gave significant potential to improve the healing process. Granulation tissue formation is the final part of the prolierative phase. Fibroblats differentiate and produce ground substance and then collagen. The ground substance is deposited into the wound bed; collagen is then deposited as the wound undergoes the final phase of repair. Many different cytokines are involved in the proliferative phase of wound repair. The steps and the exact mechanism of control have not been elucidated. Some of the cytokines include PDGF, insulin-like growth factor (IGF), and EGF. All are necessary for collagen formation (15).

Collagen synthesis occurs in several steps. The first stage takes place in the fibroblast and involves the synthesis of polypeptide chains from glycine, proline or hydroxyproline, hydroxylysine and an additional third of other amino acid. Three polypeptide chains are packed tightly together to form the triple helix molecule procollagen. Procollagen is then excreted into the extracellular microtubules. Here, processing and assembly of the still soluble collagen leads to the formation of collagen fibrils and filaments of considerable tensile strength to adapt to the needs of the wound area (15).

The final phase of wound healing is the maturational phase. The wound undergoes contraction, ultimately resulting in a smaller amount of

apparent scar tissue. The entire process is a dynamic continuum with an overlap of each phase and continued remodeling. The wound reaches maximal strength at one year, with a tensile strength that is 30% of normal skin. Collagen deposition continues for a prolonged period, but the net increase in collagen deposition plateaus after 21 days (15).

A titrated extract of *Centella asiatica* (TECA), containing asiatic acid, madecassic acid and asiaticoside, and its separated components were evaluated for their effects in the wound chamber model. TECA-injected wound chambers were characterised by increased dry weight, DNA, total protein, collagen and uronic acid contents. Peptidic hydroxyproline was also increased, showing an increased remodelling of the collagen matrix in the wound. The three purified components of TECA were all able to reproduce the effects of the complete drug (16-17). The effects of TECA and its individual components were checked on human foreskin fibroblast monolayer cultures. TECA increased the collagen synthesis in a dose-dependent fashion whereas a simultaneous decrease in the specific activity of neosynthesized collagen was observed. Asiatic acid was found to be the only component responsible for collagen synthesis stimulation. TECA and all three terpenes increased the intracellular free proline pool. This effect was independent of the stimulation of collagen synthesis (18-20).

The activity of asiaticoside was studied in normal and delayed-type wound healing. In guinea pig punch wounds, topical application of a 0.2%

solution of asiaticoside produced a 56% increase in hydroxyproline, 57% increase in tensile strength, increased collagen content and improved epithelialisation. In streptozotocindiabetic rats, where healing is delayed, topical application of a 0.4% solution of asiaticoside over punch wounds increased hydroxyproline content, tensile strength, collagen content and epithelialisation, thereby facilitating healing. Asiaticoside was also orally active at 1 mg/kg dosing and is thought to be the main active constituent of CA. Asaiticoside enhanced antioxidant levels at an initial stage of wound healing which may be an important contributory factor in the healing properties of this constituent (21). The extract also protected skin against radiation injury (19). Formulations (ointment, cream and gel) of aqueous extract of CA, when applied topically, thrice daily for 24 days on the open wounds in rats increased cellular proliferation and collagen synthesis at the wound site, as evidenced by increase in collagen content and tensile strength (23). Alpha CA cream® contains two main components. The first is an extract from the plant *Bulbine frutescens*. This extract proposed antibacterial and remained increases hydration under the tape by leaving a layer of fatty vesicles of glycoprotein on the skin surface. The second component is the principal terpenoids extracted from the *Centella asiatica* plant. These include asiatic acid, madecassic acid, and asiaticoside. The most beneficial effect appears to be the stimulation of maturation of the scar by the production of type I collagen and the resulting decrease in the inflammatory reaction and myofibroblast production (24).

CA extract is used effectively in the treatment of keloids, leg ulcers, phlebitis, slow-healing wounds, leprosy, surgical lesions, striae distensae and cellulitis. Although applied frequently to damaged skin, the risk of acquiring contact sensitivity to this plant or its constituents is low. AS, AA and MA have been studied in guinea pigs and found to be very weak sensitizers (25-29).

2.3.2 Central Nervous system

The alcoholic extract of CA, when given orally to rats and mice treated with phenobarbitone, significantly prolonged sleeping time of the animals. In the maximum electroshock-induced convulsion test in rats, it significantly reduced the duration of individual convulsions. In a behavioral test, it reduced the duration of the immobility phase, indicating sedative, antidepressive and analgesic actions. Intraperitoneal injections of brahmoside and brahminoside were found to have a CNS-depressant effect in mice and rats (14).

AA has been patented as a treatment for dementia and an enhancer of cognition by the Hoechst Aktiengesellschaft (EP 0 383 171 A2) (30).

An extract of *Centella asiatica* was found to increase brain GABA levels (6). In a study on the effects of *Centella asiatica* (as 500 mg tablets of dried *Centella asiatica*)on mentally-challenged children compared with placebo, children who took the Centella tablet showed significantly improvement in co-operation, memory, c oncentration, attention , vocabulary

and social adjustment (31). Aqueous extract of whole plant (200 mg/kg for 14 days) showed an improvement in learning and memory in both shuttle box and step through paradigms in rats. All doses of aqueous extract (100, 200 and 300 mg/kg) increased the number of avoidances in shuttle box and prolonged the step through latency in step through apparatus in a dose dependent manner, while only two doses 200 and 300 mg/kg of aqueous extract showed significant increase in the step down latency in the step down apparatus and transfer latency (TL) in elevated plus maze (8-9). A two-compartment passive avoidance task test (with rats) showed an improvement in 24-h retention. Assessment of the turnover of biogenic amines (norepinephrine, dopamine and serotonin) showed significant reductions of these amines and their metabolites in the brain following oral administration of a fresh juice (1 ml = 0.38 g fresh leaves), at a dose of 0.18 g/kg for 15 days. The decrease of amine levels was correlated to improved learning and memory in rats (32).

Triterpenoids (active compounds in gotu kola) have been shown to soothe anxiety and boost mental function in mice. A recent study found that people who took gotu kola were less likely to be started by a novel noise (a potential indicator) than those who took placebo. Although the results of this study are somewhat promising, the dose used in this study was extremely high, making it difficult to draw any conclusions about how gotu kola might be used by people with anxiety. Gotu Kola significantly attenuated the peak acoustic startle response (ASR) amplitude 30 and 60 minutes after treatment. Gotu Kola had no significant effect on self-rated mood, heart rate, or blood

pressure. These preliminary findings suggest that Gotu Kola has anxiolytic activity in humans as revealed by the ASR (33).

2.3.3 Antiulcerogenic activity

The antiulcerogenic activity of the fresh juice of CA was studied against ethanol-, aspirin-, cold restraint stress- and pyloric ligation-induced gastric ulcers in rats. When given orally at doses of 200 and 600 mg/kg twice daily for 5 days, the drug showed significant protection against all the above experimental ulcer models. This effect was thought to be due to the strengthening of mucosal defensive factors. Oral administrations of CA extract (0.05, 0.25 and 0.50 g/kg) before ethanol administration significantly inhibited gastric lesion formation (by 58-82%) and decreased mucosal myeloperoxidase (MPO) activity in a dose-dependent manner. It prevented gastric mucosal lesions by strengthening the mucosal barrier and reducing the damaging effects of free radicals (34-35). Extract of CA inhibited significantly gastric ulceration induced by cold and restraint stress (CRS) in Charles-Foster rats, Antiulcer activity of plant extract was compared with famotidine (H2-antagonist) and sodium valproate (anti-epileptic). Plant extract, farmotidine and sodium valproate showed a dose dependent reduction of gastric ulceration. Plant extract increased brain GABA level which was also dose dependent. Pretreatment with bicuculline methiodide (specific GABA-antagonist) at the dose level of 0.5 mg/kg intramuscularly (im), reversed the antiulcerogenic

activity of both plant extract and sodium valproate. Bicuculline as such did not induce gastric ulceration in normal rat (36).

2.3.4 Spasmolytic activity

The alcoholic extract of CA at a concentration of 10 mg/ml showed spasmolytic activity on isolated guinea pig ileum (37).

2.3.5 Antitubercular activity

An injection of 0.5 ml of a 4% solution of hydroxyasiaticoside, derivative of asiaticoside from CA, was given in guinea pigs, inoculated 15 days previously with tubercle bacillus. It reduced the number of tubercular lesions in the liver, lungs, nerve ganglions and spleen decreased the volume of the spleen over that of untreated control animals, thereby displaying antitubercular activity. AS have been reported to be active against *Mycobacterium tuberculosis, Bacillus leprae* and *Entamoeba histolytica* (38).

2.3.6 Antimicrobial activity

AS at a concentration of 10 mg/ml showed antibacterial activity against *Pseudomonas pyocyaneus* and *Trichoderma mentagrophytes* (39).

2.3.7 Antiviral activity

The alcoholic extract and water extracts of CA showed antiviral activity against Herpes simplex type II virus (40-41).

2.3.8 Antilarval activity

A new triterpenoid glycoside 3-O-[α-L-arabinopyranosyl] 2α,3β,6β,23α-tetrahydroxyurs-12-ene-28 oic acid exhibited activity against larvae of *Spilarctia oblique* (42).

2.3.9 Immunomodulatory activity

An alcoholic extract of CA showed stimulated effect on the reticulo-endothelial system (RES) in mice and an in vitro study of the aqueous extract demonstrated a positive effect on both the classic and alternative pathways of complement activation (43). One study involving 13 females animal with scleroderma found that gotu kola decreased joint pain, skin hardening, and improved finger movement. The usage of madecassol (asiaticoside) in tablet, ointment and powered form was found to be efficacious in the treatment of chronic or subchronic systemic scleroderma with limited skin involvement and inprogressive and/or advanced focal scleroderma (44).

2.3.10 Venous Insufficiency and Varicose veins

Guto kola is effective in the treatment of venous insufficiency and has been shown to reduce ankle edema, foot swelling and capillary filtration in rate and to improve microcirculatory parameters (45-52).

When blood vessels lose their elasticity, blood pools in the legs and fluid leaks out of the blood vessels, causing the legs swelling (venous insufficiency). In a study of 94 people with venous insufficiency, those who took gotu kola reported a significant improvement in symptoms compared to those who took placebo. In another study of people with varicose veins, ultrasound examination revealed improvements in the vascular tone of those who took guto kola (53). Another double-blind study with 40 patients suffering chronic venous insufficiency found that Guto kola extract (60 mg/kg for 30 days) significantly improved ankle circumference, vascular tone and leg volume compared with baseline (54).

2.3.11 High Blood Pressure

The variation of capillary filtration rate (CFR), ankle circumference (AC), and ankle edema (AE) was evaluated in three groups of patients with venous hypertension (ambulatory venous pressure greater than 42 mmHg) and

in a group of normal subjects before and after treatment for four weeks with Total Triterpenic Fraction of Centella Asiatica (TTFCA), a venoactive drug acting on the microcirculation and on capillary permeability. Group A (20 patients) was treated with TTFCA 60 mg tid; Group B (20 patients) was treated with 30 mg tid; Group C (12 patients) was treated with placebo; and Group D (10 normal subjects) was treated with TTFCA 60 mg tid in an open study. Capillary filtration rate was assessed by venous occlusion plethysmography, ankle edema by a new system called AECT (Ankle edema coin tester). Subjective symptoms of venous hypertension were assessed by an analogue scale line considering four symptoms: swelling sensation, restless lower extremity, pain and cramps, and tiredness. CFR, AC, and AE were significantly higher in patients in comparison with normal subjects. After four weeks of TTFCA treatment there was a significant decrease of the abnormally increased CFR, AC, and AECT time in patients. This was also greater in the higher dose group. No significant change was observed in the placebo group and in normal subjects treated with TTFCA. Symptoms were also significantly improved in the two groups treated with the active drug according to the dose. No significant changes were observed in the placebo group (55-58). In another double-blind clinical trial involving 87 patients with chronic venous hypertensive microangiopathy, two dosage forms of CATF (30 mg/day and 60 mg/day) were applied for 60 days and no unwanted effects were observed (59-61). The results also confirmed the efficacy of CATF in a dose-dependent manner. The effects of the CATFs on enzymes involved in

mucopolysaccharide metabolism supported the hypothesis that the extract acts on basic metabolism in the connective tissue of the vascular wall (62). The levels of basal serum uronic acid and enzymes involved in mucopolysaccharide metabolism (beta-glycuronidase, beta-N-acetylglucosaminidase, and arylsulfatase) were elevated in patients with varicose veins, indicating an increased mucopolysaccharide turnover. After treatment (60 mg/day for three months) the above enzyme levels fell progressively. In a study of people with heart disease and high blood pressure, those who took abana (an Ayurveic herbal mixture containing guto kola) experienced a significant reduction in diastolic blood pressure (pressure on blood vessels when heart in at rest) compared to those who took placebo. Further studies are needed to determine whether gotu kola alone, some other herb in the Ayuvedic mintre, or the particular combination of all the herbs in the remedy is responsible for the beneficial effect.

2.3.12 Insomnia

Because of sedative effects demonstrated in animals, gotu kola has been used to help people suffer from insomnia (63).

2.3.13 Antitumor Properties

A crude extract and purified fraction of CA were tested on different tumor cell line models. Both the crude extract and purified fraction showed cytotoxicity against *Erlich ascites* and Dalton's lymphoma ascites tumor cells in a concentration-dependent manner. However, no cytotoxicity effects were detected against normal cell lines. The oral administration of the extracts (crude or purified) retarded the development of solid and ascites tumors in mice (64).

2.3.14 Hepatitis

Researchers found improvement in 5 of 12 clients with chronic hepatic disorders, treated with a titrated extract of CA (64-65).

2.4 Applications

2.4.1 Parts used

Aerial parts were used as folk medicine. Guto kola is available as teas, dried herb, tinctures, capsules, tablets and ointments.

2.4.2 Traditional and modern use

The leaves and stems of the gotu kola plant are widely used to treat a variety of illness, particularly in traditional eastern medicine. Historically, gotu kola has been used to treat syphilis, hepatitis, stomach ulcers, mental fatigue, epilepsy, diarrhea, fever and asthma. Today, American and European herbalists use gotu kola for disorders that cause connective tissue swelling, such as scleroderma, psoriatic arthritis(arthritis occurring in conjunction with psoriasis), and rheumatoid arthritis. Recent studies confirm some of the traditional uses and also suggest possible new application for gotu kola, such as lowering high blood pressure, treating venous insufficiency (pooling of blood in the veins, usually in the legs, boosting memory and intelligence), easing anxiety and speeding wound healing (2,5).

2.5 Overview of the analytical method

There are several methods for qualitative and quantitative determination of active constituents of CA. Titrimetric method was conventionally used to determine the triterpene acids (asiatic acid, madecassic acid) and glycosides (asiaticoside and madecassoside). This method can determine only the terpene acids content. For determination of glycosides, sample must be hydrolyzed to acids before titration. This method is non-selective, non-specific and lacks of the precision and accuracy (69).

A colorimetric method is used to determine the glycosides by reaction with anthrone reagent. Anthrone reagent tests for the presence of glycosides by hydrolyzing polysaccharides to monosaccharide then forming a color compound in the presence of the monosaccharide. The complex will vary in color from green to black depending upon the amount of glycoside present. The triterpene acids do not interfere. This method is non-selective, non-specific (66).

Thin-layer chromatography (TLC) method was used for qualitative and quantitative (in combination with HPTLC scanner) determination of active constituents of CA. TLC plate silica gel GF254 and hexane:ethylacetate: diethylamine (8:2:0.2) was used for qualitative determination in CA (Standard of ASEAN Herbal Medicine) (67). Arunya Sribusarakum used chloroform:methanol:water (15:7:1) used to resolve AA, MA, terminolic acid, AS and MS and then detected with 0.2%anthrone reagent for qualitative determination in crude extract (65). For quantitative determination of active constiuents of CA, Manit Naklampa reported chloroform:methanol:water (40:30:4) as developing solvent used for isolation of AS and MS. The developed plate was sprayed with 10% sulfuric acid in ethanol and heated at 110 °C for 10 min and finally scanned with HPTLC scanner (68).

Presently, HPLC method has become a method of choice for analysis of Herbal medicine. P.K. Inamdar (69) determined AA, MA, AS and in the crude plant extracts and preparations using C18 column and acetonitrile-water gradient system as mobile phase. Arunya Sribusarakum (66) reported a reversed-phase gradient HPLC for separation of AS, MA and AA in preparations. R.K. Verma

used 1%Trifluoroacetic acid:methanol(30:70) as mobile phase for determination of asiaticoside in crude extract (70). For preparation, P. Morganti *et., al.* were reported acetonitrile-water gradient reversed-phase HPLC that was used to determinate asiatic acid, madecassic acid and asiaticoside in Transdermal patch (71).