Gotu Kola (*Centella asiatica* L.): An Under-utilized Herb

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ABSTRACT

The short growing season and harsh climate found in many parts of Canada necessitates development of both field and greenhouse-based plant production. Gotu kola (*Centella asiatica* L. Urban) is a member of the Apiaceae family, which is characterized by its constantly growing roots, and long copper-coloured stolons (runners) with long internodes and roots at the base of each node. Also known as Indian pennywort, it is a perennial creeping plant native to India, China, Japan, North Africa and Sri Lanka. Gotu kola has been used as a therapeutic herb in India, China and Indonesia for thousands of years. Its ability to heal wounds, to improve mental complications and to treat skin lesions are the main reasons for its wide spread use in those countries. In the western world, the crop is becoming popular due its ability to boost mental acuity and improve circulation. Growth of gotu kola on the Canadian Prairies has met with limited success and potential for field production of the crop is slim due to unfavourable growing conditions. It appears that annual *in vitro* production of the plant through tissue culture and clonal propagation in the greenhouse is necessary prior to transfer of the plant to the field. Alternatively, continuous propagation of gotu kola as an herb grown under greenhouse conditions appears to show considerable promise. The plant can successfully be grown in combined culture with fish in a closed recirculation aquaponic system that appears competitive with most highly productive commercial greenhouse ventures.

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INTRODUCTION

Gotu kola (*Centella asiatica* L.), also known as *Centella* and Indian pennywort, is a perennial creeping plant native to India, China, Japan, North Africa and Sri Lanka. It flourishes in water-logged, damp and swampy areas, but growth also can be observed along stone walls, in rocky and sunny areas at elevations up to 2500 m above sea level, although generally in climates with high humidity (Ohwi 1965; Kartnig and Hoffman-Bohm 1992; Awang 1998).

Gotu kola has small fan shaped green leaves and light violet coloured flowers (Brinkhaus *et al*. 1998a). The word 'gotu' in the Sinhalese language stands for 'conical shape' and 'kola' stands for leaves, which collectively describes this herb as a plant with 'conical shaped leaves' (Bandara, pers. obs., 2010). The generic name used for gotu kola is Hydrocotyle which was introduced about 1700 A.D. (Madaus 1938) and is derived from the Greek words ηυδορ = water, and κοτυλε = cup which allude to the aquatic habitat and cup-shaped leaves of the plant. During the 17th century

Invited Review

Table 1 Taxonomy of the gotu kola plant.

Classification	Name
Kingdom	Plantae
Division	Magonoliaphyta
Class	Magnolipsida
Order	Apiales
Family	Apiaceae
Genus	*Centella*
Species	*Centella asiatica* (L.) Urban

Source: http://www. ayushveda.com

colonists from the Dutch colonies of Indonesia also referred to gotu kola as Codagam or Kutakan (Brinkhaus *et al.* 1998a).

Besides traditional use of the plant as a leafy vegetable, Gotu kola also is well known as a traditional medicinal plant that has been in use since pre-historic times and that has been used as a therapeutic herb in India, China and Indonesia for thousands of years (Kartnig 1988). Castiglioni (1958) suggested that gotu kola is similar to the plant 'manduka parni' which is listed in the *Susruta Samhita*, a Sanskrit compendium of all of the major concepts of Ayurvedic medicine which was written ~1200 B.C. (Brinkhaus *et al.* 1998a); in fact, by 500 A.D. gotu kola is seen formally listed among the medicinal plants included within east Indian textbooks of ayurvedic medicine. Ability of gotu kola to promote wound healing, to improve mental acuity and to treat skin lesions were the main reasons for its use in those countries. In the western world, the crop is becoming popular due its ability to boost mental acuity and improve circulation.

Recent studies have confirmed some of the traditional as well as new applications of the herb; e.g., gotu kola is able to reduce high blood pressure and venous insufficiency, improve memory and intelligence, help with varicose veins, act as an anti-depressant, and reduce stress as well as accelerate wound healing (Poizot and Dumoz 1987; Awang 1998; Brinkhaus *et al.* 2000; Belcaro *et al.* 2001; De Sanctis *et al.* 2001; Kumar and Gupta 2002; Dong *et al.* 2004; Rao *et al.* 2005b; Gupta and Flora 2006). Among the twenty-some species from the genus *Centella*, the *asiatica* species is the only source of additives now used in some commercial drug and cosmetic products. *Centella asiatica* is the most ubiquitous species of *Centella*. It is found in Southeast Asia, Sri Lanka, in parts of China, in the western South Sea Islands, Madagascar, South Africa, in the southeast of the U.S.A, Mexico, Venezuela and Colombia, as well as in eastern regions of South America. All remaining species of *Centella* grow mainly in South Africa (Karting and Hoffmann-Bohm 1992). The herbal material, however, is primarily collected in India, Sri Lanka, Madagascar and South Africa. Aerial parts of the plant are predominantly used for medicinal purposes (Bhavan 1992; Awang 1998). In tropical climates the plant can be grown and harvested throughout the year and cost-effectively dried in the sun.

BOTANICAL DESCRIPTION OF GOTU KOLA

Gotu kola is a slender, creeping plant that belongs to the Apiaceae (Umbelliferae) family, genus *Centella* (**Table 1**), which contains 20 different species. In addition to the name gotu kola, which is usually used in scientific work, the synonym *Hydrocotyle asiatica* L. also is commonly used (Brinkhaus *et al.* 2000). The stems of the plant are green to reddish green in color and have long-stalked, green leaves with rounded apices. The leaves are borne on pericladial petioles, are smooth-surfaced, thin and soft, with palmate nerves and can measure from 1 to 5 cm in width. Nodes found on the stem give rise to long petioles (5-15 cm) which hold the leaves.

The gotu kola plant is characterized by constantly growing roots and long copper-coloured stolons (runners), with wide-spaced internodes that have roots at the base of each node (Kartnig 1988). The root stocks consist of rhizomes which grow vertically down and are covered with root hairs. Wahundeniya (2007) used plant morphological characteristics, such as the number of runners per plant, number of leaves per plant, length of the stalk and leaf attributes (type, shape, margin, breathe and colour) to categorize gotu kola accessions collected throughout Sri Lanka into two main groups, bush and runner types. The bush type gotu kola group does not produce runners and has medium sized leaves with long leaf stalks, whereas the runner type group has the ability to produce a runner, and can be further categorized into four subgroups by considering leaf size and appearance of the leaf margins.

The flowers (typically 3-6 units) from gotu kola are pinkish to red in color and grow on short pedicels situated
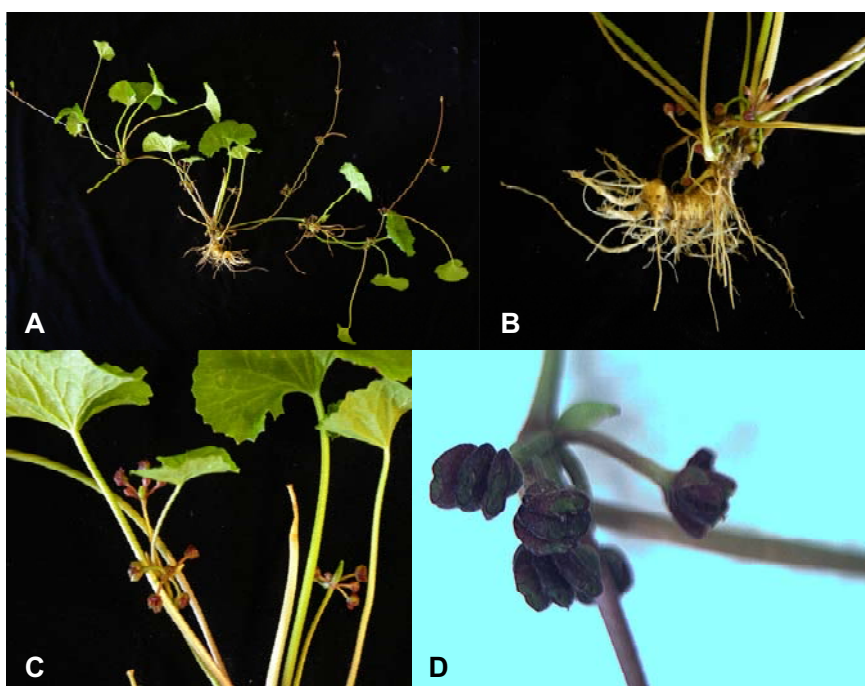

Fig. 1 Vegetative and reproductive parts of the gotu kola plant. (**A**) Whole plant with runners and reproductive parts. (**B**) Rhizome with roots. (**C**) Umbel type flower head. (**D**) An umbel with immature fruits.

Table 2 Different names used by selected countries for gotu kola.

Country	Name	Reference
Chinese	Luei Gong Gen, Tungchian	Dr. Yueshu Li, pers. comm.
English	Gotu kola, Indian Pennywort	Brinkhaus *et al.* 2000;
French	Hydrocotyle asiatique	http://www.ayushveda.com
German	Asiatischer Wassernabel	Brinkhaus *et al.* 2000
Indonesian names		Brinkhaus *et al.* 2000
Sumatra	Kaki kuda	
Java	Kaki kuda, Pegagan, Antanan geda, Gagan-gagan, Gang-gagan, Kerok batok, Panegowan, Rendeng, Calingan rambat, Kos tekosan.	
Sulawesi	Pagaga, Tungake-tungke.	
Bali	Papaiduh, Pepiduh, Piduh	
Flores	Puhe beta, Kaki kuta, Tete karo, Tete kahdo.	
Gujrati	Khand bhrami	http://www.ayushveda.com
Hindi	Bengsaag	
Italian	Idrocotile	Brinkhaus *et al.* 2000
Japanese	Tsubo-kusa	
Malay	Pegaga	Ee Lynn Lee, pers. comm.
Mauritius	Bavilacqua	Brinkhaus *et al.* 2000
Sanskrit	Manduk parni	http://www.ayushveda.com
Sinhalese	Gotu kola	Dr. Manjula Bandara, pers. comm.
Tamil	Vallarai, Kodangal	Dr. Perumal Vijayan, pers. comm.

within auxiliary umbels, in which the fruits of each umbel are enclosed within a pericarp made up of large elliptical bracts (Brinkhaus *et al.* 1998a). Main vegetative (leaves, rhizomes, roots, runners, etc.) and reproductive parts (flower heads, immature fruits, etc.) of the gotu kola plant are shown in **Fig. 1**. Each flower bears five stamens and two styles. The fruits are densely reticulate, distinguishing *H. asiatica* from other species of *Hydrocotyl* which have smooth, ribbed or warty fruit. Names used in different countries of the world for gotu kola are given in **Table 2**.

TRADITIONAL USES FOR GOTU KOLA

The gotu kola plant has been used for medicinal purposes since prehistoric times. Locals as well as traditional healers from the South Sea Islands, Indonesia, Madagascar, and from near the Malay Peninsula have used fresh extracts of gotu kola both topically as a treatment for wounds and internally for therapeutic applications (Kartnig 1988). People from India and Africa have also used preparations of the plant for the treatment of leprosy (Madaus 1938; Kartnig 1988) and successful application of gotu kola in treating for the treatment of leprosy was reported for the first time in 1852 (Madaus 1938). Since 1887 more wide-spread clinical use of gotu kola as a therapeutic agent for the treatment of leprous lesions has been documented.

Gotu kola was officially included in the Indian Pharmacopoeia in the 19th century (Kartnig 1988). Some reported uses of the plant are for the treatment of bladder inactivity, physical and mental exhaustion, diabetes, eye disease, stomach problems and icterus (jaundice) in traditional Asian medicine (Matsuda *et al.* 2001a). East Africans also used gotu kola leaves to treat fever and stomach upset as well as for scrophula, a form of tuberculosis affecting the lymph nodes, and syphilis (Watt and Breyer-Brandwijk 1962). Aerial parts of the plant have been used by the Chinese to treat fever, dysentery, urinary tract infections, hepatitis and jaundice (Kan 1986). Some also have claimed that gotu kola is an effective antidote for mushroom (*Gelsemium elegans* L.) and arsenic-poisoning. In addition, the plant has been used externally as a poultice to treat snakebites (Leung and Foster 1996) as well as for treatment of fractures and sprains (Kartnig 1988). Recently, gotu kola was introduced as a health food supplement in North America (Matsuda *et al.* 2001a).

In Sri Lankan cuisine, gotu kola is used as a green salad; in Sinhalese the herb is known as 'Gotu kola Sambola'. The salad consists of finely chopped gotu kola leaves mixed with grated coconut and finely chopped green chillies, chilli powder, finely chopped dried fish pieces, turmeric power and lime or lemon juice. In addition, Sri Lankan people prepare a gotu kola slurry known in Sinhalese as 'Gotu kola kenda' which is prepared by adding a boiled gotu kola leaf extract to well boiled rice; coconut kernel milk is added to this and re-boiled for about 5 min to complete the dish (M. Bandara, pers. comm., 2010).

People in Malaysia believe that gotu kola improves vision, eliminates toxins from the blood stream, and that it can cool down the body during hot and dry weather. However, even in areas where the plant is common, it is often overlooked. Demand is highest during the hot season when people feel they need to cool down or when herbalists recommend it for treatment. It is not as popular as other wild greens used as vegetables. It is not commonly sold in city markets; rather, one is more likely to find it sold with the vegetables in rural or farmers' markets. People in rural areas of the northern States of Peninsular Malaysia also gather and eat it (Thean Pheh, pers. comm., 2010). In these areas a salad known as *ulam* is prepared from a mixture of gotu kola, shrimp, onion and coconut and then eaten (Thean Pheh, pers. comm., 2010). In addition, gotu kola drinks are now available in pop cans (imported) or are available as a freshly squeezed juice which the locals prefer, believing that it is more authentic and potent. Freshly squeezed drinks are sold by street hawkers on demand; these consist of fresh leaves with the petioles ground in a grinder to extract the juice. Most customers prefer to drink the concentrated dark green juice straight, while others like it diluted a little with water and sweetened with sugar.

CHEMICAL COMPOSITION

Phyto-chemical analysis of gotu kola has revealed that it contains more than 70 constituents including triterpenic acid, triterpenoid saponins, polyacetylenes, flavones, sterols, and lipids (Yu *et al.* 2006). A summary of major constituents reported to occur in gotu kola is presented in **Table 3**. Trterpene is a major important component of gotu kola, regarded as a marker constituent in terms of quality control (Zheng and Qin 2007). James and Dubery (2009) provides a comprehensive review on biosynthesis, chemical structure and biological activities of pentacyclic triterpenoids occur in gotu kola. The flavonoids and terpenes found in gotu kola are secondary plant metabolites derived from phenylpropanoids and acetyl-CoA respectively (Brinkhaus *et al.* 1998b). According to Castellani *et al.* (1981) the main bioactive compounds from gotu kola responsible for its biological effects are asiaticoside, asiatic acid and madecassic acid. Asiaticoside is a triterpene glycoside that occurs in three forms, asiaticoside, asiaticoside A and asiaticoside B,

Table 3 Major bioactive constituent groups and constitutes reported to occur in gotu kola.

Constituent group	Constituent	Reference	Constituent group	Constituent	Reference
Triterpenic acids	Asiatic acid	Brinkhaus *et al*. 1998a; Zheng and Qin 2007	Essential oil	β-Caryophyllene	Brinkhaus *et al*. 1998a; Oyedeji and Afolayan 2005
	Madasiatic acid			Terpene acetate	
	Madecassic acid	Brinkhaus *et al*. 1998a; Yu *et al*. 2006		Pinene	
	Thankunic acid	Brinkhaus *et al*. 1998a		α-Humulene	Oyedeji and Afolayan 2005
	Indocentoic acid	Yu *et al*. 2006		Biclyclogermacrene	
	Euscaphic acid			Germacrene B	
	Terminolic acid			Elemene	Qin *et al*. 1998
	Isothankunic acid	Brinkhaus *et al*. 1998a		Farnesol	
	2α,3β, 20,23-tetrahydroxyurs-28-oic acid (common name has not given)	Yu *et al*. 2006	Flavone derivatives	Quercetin glycoside	Brinkhaus *et al*. 1998a; Yu *et al*. 2006
	3β-6β-23-trihydroxyolean-12-en-28-oic acid (common name has not given)			Kaempferol	
	3β-6β-23-trihydroxyurs-12-en-28-oic acid (common name has not given)			Astragalin	Brinkhaus *et al*. 1998a
	Bayogenin			Catechin	Zainol *et al*. 2003
	Centic acid	Zheng and Qin 2007		Rutin	
	Betulinic acid			Naringin	
	Cenellic acid		Sesquiterpenes	Elemene	Brinkhaus *et al*. 1998a
	Brahmic acid			Bicycloelemene	
	Indocentic acid			*Trans*-farnesene	
	Centic acid			Ermacrene D	
Triterpenic acid sugar esters	Asiaticoside	Brinkhaus *et al*. 1998a; Matsuda *et al*. 2001b; Kim *et al*. 2007; James and Dubery 2009		Elemene	
	Asiaticoside A	Brinkhaus *et al*. 1998a			
	Asiaticoside B	Brinkhaus *et al*. 1998a; Matsuda *et al*. 2001b			
	Asiaticoside C	Jiang *et al*. 2005			
	Asiaticoside D				
	Asiaticoside E				
	Asiaticoside F				
	Braminoside	Brinkhaus *et al*. 1998a			
	Brahmoside				
	Brahminoside				
	Thankuniside				
	Isothankniside				
	Centellasaponins A	Matsuda *et al*. 2001a			
	Centellasapogenol				
	Sceffoleoside A	Matsuda *et al*. 2001b			
	Centellasaponins B				
	Centellasaponins C				
	Centellasaponins D				
	Centelloside	Zheng and Qin 2007			
	Madecassoside				
Triterpenic steroids	Stigmasterol	Brinkhaus *et al*. 1998a			
	Sitosterol				

all of which differ in the composition of their R-groups.

Previously, confusion over the types of triterpenoid compounds found in gotu kola has arisen because the source of raw materials used in studies of the plant originated from different geographical locations, sometimes resulting in contradictions within the chemical literature (Kartnig 1988); e.g., asiaticoside has been isolated from *Hydrocotyle asiatica* (Bontems 1941) while madecassic acid has been isolated from plants found in Madagascar (Boiteau and Chanez 1967). Dutta and Basu (1968) found the triterpene, isothakunic acid in gotu kola extracts, along with thankunic acid, brahmic acid, isobrahmic acid and their glycoside derivatives, while Pinhas (1969) was able to identify the triterpene, madasiatic acid in his extracts. Brahmic acid later was shown to be identical to madeccasic acid, while isobrahmic acid was shown to be a mixture of both asiatic and madecassic acids (Rao and Seshadri 1969).

An oleanane-type triterpene called centellasapogenol A and its oligoglycoside variant, centellasaponin A can be extracted from gotu kola using methanol (Matsuda *et al*. 2001a). These compounds inhibit the activity of aldose reductase, a key enzyme in the polyol pathway which catalyzes the reduction of glucose to sorbitol. In cells, diffusion of sorbitol across membranes does not occur readily. In diabetic patients sorbitol can accumulate within intercellular spaces resulting in chronic complications such as cataracts (Matsuda *et al*. 2001a). When a methanolic extract of gotu kola was partitioned in an ethyl-acetate/water mixture to create an ethyl-acetate-soluble fraction and a water-soluble fraction, significant inhibition of aldose reductase activity by the ethyl-acetate fraction (IC_{50} 0.25 µg mL^{-1}) was identified. Chromatography of the ethyl-acetate fraction revealed the presence of centellasapogenol A, madecassic acid, kaempferol 3-*O*-β-D-glucuronide, petuletin, and 2, 5-dihydroxybenzoic acid, while the water-soluble fraction was found to contain the glycosides centellasaponin A, madecassoside, scheffikeisude A, and asiatic acid. In a related study, Matsuda *et al*. (2001b) also identified the

Table 4 Major triterpenoid compound groups presence in gotu kola based on their geographical origin.

Triterpenoids	Origin
Braminoside	India
Brahmoside	India
Brahminoside	India
Thankunic acid	Northeast India
Isothakunic acid	Northeast India
Thakuniside	Northeast India
Isothakuniside	Northeast India
Madasiatic acid	Madagascar

Source: Brinkhaus *et al.* 2000.

Table 5 Phytochemicals extracted from gotu kola.

Compound	Plant part
Asiatic acid	Plant
Asiaticoside	Plant
Betulic acid	Plant
Betulinic acid	Plant
Brahmic acid	Plant
Brahminoside	Plant
Brahmoside	Plant
Campesterol	Plant
Camphor	Essential oil
Centellic acid	Plant
Centellinic acid	Plant
Centellose	Plant
Centoic acid	Plant
Cineole	Essential oil
Dodecane	Essential oil
Isobrahmic acid	Plant
Isothankunic acid	Plant
Isothankuniside	Plant
Madasiatic acid	Plant
Madecassic acid	Plant
Madecassoside	Plant
Medicassic acid	Plant
Oxyasiaticoside	Plant
Thankunic acid	Plant
Thankuniside	Plant

Source: http://www.chromadex.com/Literature/Lists/GotuKola.pdf

ursane-type triterpene oligoglycosides, centellasaponins B and C, as well as another oleanane-type triterpene oligo-glycoside, centellasaponin D.

Additional studies of ethanolic extracts from gotu kola using Fast Atom Bombardment Mass Spectroscopy (FAS-MS), Infra red (IR) spectroscopy and Nuclear Magnetic Resonance (NMR) technologies, have lead to identification of the triterpenoid glycosides asiaticoside C, D, E and F (Jiang *et al.* 2005). Yu *et al.* (2007) were able to isolate docosyl ferulates, bayogenin, 3β-6β-23-trihydroxyolean-12-en-28-oic acid, 3β-6β-23-trihydryurs-12-en-28-oic acid and D-gulonic acid from gotu kola. The triterpenic steroids reported to occur in *C. asiatica* are stigmasterol and sitosterol. Triterpenic acids and their sugar esters identified by Kartnig and Hoffmann-Bohm (1992) also appear to contain slight differences in their chemical structures according to geographical origin of the plants studied. A summary of triterpenoid compounds presence in gotu kola with different origin is given in **Table 4**. Additional variation in the chemical content of gotu kola has been identified by looking at composition of the plant both above and below ground level; e.g., compared to above ground parts of the plant, the below ground parts contain low concentrations of at least 14 different types of polyacetylenes (Schulte *et al.* 1973).

Biting on gotu kola leaves will bring a sense of pungency, most likely due to the presence of essential oils. It has been reported that gotu kola contains approximately 0.1% essential oils, which include ρ-cymol, α-pinene, allyl mustard oil (Salgues 1969), camphor, cineole and n-dode-

cane (Castellani *et al.* 1981). *Trans*-β farnesene, germacrene D, and β-caryophyllene also have been identified using gas chromatography (Asakawa *et al.* 1982).

Major constituents of gotu kola leaves indicate that they contain 89.3% water, 6.9% carbohydrate, 2% fibre, 1.6% protein, and 0.6% fat. Samples consisting of 100 g of leaves contained 1.6% ash, 414 mg K, 170 mg Ca, 30 mg P, and 3.1 mg Fe, as well as 6.58 mg β-carotene, 0.15 mg thiamine, 0.14 mg riboflavin, 1.2 mg niacin, and 4 mg ascorbic acid (Duke 1992). ChromaDex Inc., a commercial biochemical company provides a list of extractable chemical compounds found in gotu kola, some of which are given in **Table 5**.

Table 6 Summery of studies conducted to assess various effects of gotu kola extracts *in vivo* and *in vitro*.

Animal studies, clinical trials or laboratorial research conducted	References
First report of a double-blind, placebo-controlled study on the anxiolytic effects of gotu kola in healthy humans. Study reported that the attenuated acoustic startle response in healthy human subjects significantly increased following a single 12g oral dose of gotu kola.	Bradwejn *et al.* 2000
Centella asiatica extracts contained anti-HSV1 and anti-HSV2 activities, determined by plaque inhibition assay. Exerted effects synergistically with other medicinal plant extracts.	Yoosok *et al.* 2000
Centella extract exerted protective effects against ethanol-induced gastric mucosal lesions, by strengthening the mucosal barrier and reducing the damaging effects of free radicals.	Cheng and Koo 2000
Oral intake of combined extracts of vitamin E, rutin, sweet clover and *Centella* lead to significant improvement in clinical symptoms associated with chronic venous insufficiency.	Cataldi *et al.* 2001
Total triterpenic fraction of *C. asiatica* exerted protective effects against chronic venous insufficiency, diabetic microangiopathy, venous hypertension, oedema, microcirculation alterations and enhanced modulation of collagen synthesis.	Cesarone *et al.* 2001a
Titrated extract of *C. asiatica* treatment effected changes in expression of genes involved in angiogenesis, responded strongly to asiatic and madecassic acids.	Coldren *et al.* 2003
Additive anti-convulsion activity of ethyl acetate fraction of *C. asiatica* extract and other anti-epileptic drugs in rats. Reduced the effective dose for three other anti-epileptic drugs by ~60-75%. Suggestive potential of *C. asiatica* as an adjunctive medication for epileptic patients.	Vattanajun *et al.* 2005
Leaf extracts of *C. asiatica* significantly promote wound healing through faster epithelization, wound contraction, increased tissue granulation and hydroxyproline content, even in dexamethasone-suppressed wound healing rat models	Shetty *et al.* 2006
Asiatic acid (AA) collectively demonstrated mitochondrial protectivity in mice via prevention of lipopolysaccharide and D-galactosamine-induced acute liver injury, following pre-treatment with 20, 50 or 100 ng kg^{-1} AA. Up-regulation of the important mitochondrial protein as well as the inhibition of the mitochondrial permeability transition (MPT) was suggested as the anti-hepatotoxicity mechanism of AA.	Gao *et al.* 2006
Chloroform/methanol (C/M) extracts of *C. asiatica* conferred protective effects against neurodegeneration in monosodium glutamate-treated rats. The 80:20 C/M extracts administered at 100 and 200 mg kg^{-1} to rats demonstrated significant improvement in catalase, superoxide dismutase and lipid peroxides levels in the hippocampus and striatum regions which are associated with the antioxidative activities of *C. asiatica*. A significant neuroprotective effect was apparent as observed in the locomotor activity as well as in the CAI hippocampus region.	Ramanathan *et al.* 2007

MEDICINAL PROPERTIES

Gotu kola possesses anti-inflammatory, anti-ulcerogenic, anxlytic, nervine, and diuretic acting compounds in addition to acting as a circulatory stimulant; the plant also can possess both anti-viral and bactericidal properties (Bradwejn *et al.* 2000; Winston and Maimes 2007). Thus, gotu kola is one of the few traditional medicinal plants that has been subjected to extensive chemical studies and clinical investigations (Brinkhaus *et al.* 2000). Some of the studies are of superior quality according to standardized scientific criteria, and have shown positive effects of gotu kola in the treatment of skin diseases, especially in improving venous insufficiency and expediting wound healing. Selected studies are briefly elaborated on below while other works are summarized in **Table 6**.

Wound healing

Use of gotu kola extracts in Madagascar, Sri Lanka, and India for the treatment of leprosy, lupus, varicose ulcers and eczema is widely recognized (for review articles see Kartnig 1988; Awang 1998; Brinkhaus *et al.* 2000). Introduction of the gotu kola plant to the western world occurred when it was imported into France in the mid-1800s, and included in the French Pharmacopoeia in 1884 due its healing abilities (Widgerow *et al.* 2000). In general, extracts of the plant containing the glycoside madecassoside possesses anti-inflammatory properties, while those containing asiaticoside appear to stimulate would healing. Comprehensive animal studies have been conducted to examine the wound healing ability of gotu kola extracts using rats and guinea pigs as model systems. Rats that were repeatedly wounded were fed 100 mg kg^{-1} body weight of gotu kola extract containing asiaticoside and glycoside madecassoside, exhibited accelerated healing (Poizot and Dumez 1978). In another study, rats and mice treated with topically applied gotu kola-based triterpenoids recovered faster from wounds than animals that were orally fed extracts from the plant; topically applied extract rapidly penetrated to subcutaneous tissues and abdominal muscles (Viala *et al.* 1977). In these studies Asiatic acid was absorbed later than madecassic acid and topical applications of the compounds were observed to penetrate to the blood plasma and deeper tissues. Shukla *et al.* (1999b) further investigated the wound healing ability of asiaticoside both *in vivo* and *in vitro* using guinea pig and diabetic rats (delayed healing) models. Wounds were artificially inflicted in these animals, and concentrations of asiaticoside ranging from 0.05-0.4% were applied topically, twice daily for 7 days in order to treat the injuries. For the *in vitro* study, chick chorioallantoic membranes (CAM) were damaged and then treated with asiticoside in order to assess its effect on angiogenic activity. Increased collagen synthesis and tensile strength was observed in wound tissues in both normal and diabetic animals, indicating an enhanced rate of wound healing in tissues treated with asiaticoside. Asiaticoside also promoted angiogenesis by stimulating formation of new blood vessels the wounded tissues.

In a more recent study, Shetty *et al.* (2006) looked at both normal and dexamethasone-treated (suppressed healing) rats in order to assess the wound healing potential of ethanol extracts of gotu kola. They reported a significant increase in wound healing in steroid-treated rats given the extract, as well as an increase in levels of hydroxyproline, indicating enhanced collagen synthesis and fiber stabilization within the wounded tissues. Levels of epithelialization and contraction of extract-treated wounds also were higher in treated tissues compared to controls.

Antioxidants can play a significant role in the wound-healing process. Newly formed tissues from excision-type cutaneous wounds in rats given a topical application of 0.2% asiaticoside twice daily for 7 days had increased levels of both enzymatic and non-enzymatic antioxidants (Shukla *et al.* 1999a); *i.e.*, levels of superoxide dismutase, catalase, glutathione peroxidase, vitamin E and ascorbic acid all increased as a result of the asiaticoside treatment. However, continued application of asiaticoside for 14 days resulted in no significant difference in antioxidant levels compared to those of the control suggesting that asiaticosides are able to enhance production of antioxidants early in the healing process which may be an important contributing factor to the healing properties of this substance.

Gotu kola extracts are able to promote wound healing in humans (Poizot and Dumez 1987). During this process wounded tissues initially pass through a hemostasis phase which involves solidification of the wounded tissues involving constriction of the blood vessels, formation of a platelet plug and coagulation of the blood to form a clot. This process is followed by a second inflammatory phase and a proliferative phase leading to healing of a wound. Gotu kola appears to be effective in the proliferative phase of wound healing by stimulating type-1 collagen synthesis within the connective tissue (Bonte *et al.* 1995; Mackay and Miller 2003). This stimulates scar maturity and is especially helpful in scar management following the decrease in inflamematory response and during myofibroblast production within the proliferative phase (Widgerow *et al.* 2000). It appears that Asiatic acid and asiaticoside stimulate collagen synthesis (Suguna *et al.* 1996; Sunilkumar *et al.* 1998; Mackay and Miller 2003) and increase levels of antioxidants in cells, respectively, resulting in more rapid wound healing (Tenni *et al.* 1988; Maquart *et al.* 1990; Shukla *et al.* 1999b).

The ability of extracts from gotu kola to stimulate production of connective tissue components may be useful in rejuvenation of other aged or damaged tissues. S*triae gravidarum* or stretch marks often form in women following pregnancy. An anti-striae skin cream, Trofolastin® (marketed in Europe only), containing gotu kola extract along with tocopherol and collagen-elastin hydrolysate, was used to treat 80 pregnant women (Mallol *et al.* 1991); each received either a topical application of the cream or a placebo once a day for 12 weeks. Significantly fewer striae were observed in women using the cream compared to the placebo group. While women in the placebo group with a history of striae during puberty developed stretch marks, women with a similar history in the treatment group appeared to gain a significant protective effect, strongly suggesting that gotu kola containing creams may be useful in addressing this as well as other similar cosmetic problems.

Varicose veins

Varicose veins develop from the malfunction of valves within the blood vessels which under normal conditions maintain the unidirectional flow of blood towards the heart. They are seen as swollen veins that become raised above the skin surface and appear twisted and bulging, and dark purple to blue in colour. These anomalies in the valves result in backing up of blood flow and pooling of blood within the veins, leading towards formation of varicose veins (U.S. National Institute of Health 2007). Pointel *et al.* (1987) evaluated the efficacy of an extract of gotu kola in treating venous insufficiency of the lower limbs in 94 patients. The study was conducted over two months, with the patients divided in 3 groups, each treated with an extract consisting of 120 mg day^{-1}, 60 mg day^{-1} or a placebo, respectively. Although influence of the different doses was not significant, they reported considerable improvement overall in the treated groups based on both a subjective symptoms-based evaluation by patients as well as an objective plethysmographic assessment of venous volume in each of the groups.

Belcaro *et al.* (1990) investigated variations in venous permeability and microcirculation in patients with moderate to serious hypertension. Using a vacuum suction chamber (VSC), which produces a wheal on the skin that generally disappears in less than 60 minutes in normal subjects, they measured the disappearance time (DT) of the wheal in subjects treated with a total triterpenic fraction of gotu kola (TTFG) for 2 weeks. A significant decrease in DT was ob-

served in both subjects with moderate and serious symptoms, indicating improvements in microcirculation and capillary permeability after treatment with TTFG. In another study by Belcaro *et al.* (2001), local capillary filtration in patients with ankle oedema caused by venous hypertension was evaluated using the VSC device and by assessing rate of ankle swelling (RAS). Fifty test subjects were treated with 60 mg TTFG twice daily for 4 weeks. A significant reduction in RAS (34% of initial value) and DT values (48% of initial value) was observed as a result of the treatment, suggesting that treatment with TTFG can lead to improvement in microcirculation, skin and subcutaneous tissue perfusion and function (Belcaro *et al.* 2001).

De Sanctis *et al.* (2001) also conducted a randomized placebo-controlled clinical trial to evaluate the efficacy of TTFG in treating oedema and venous hypertension. The study involved 62 patients, who were divided into 4 groups; *i.e.*, groups A, B, and C exhibited venous hypertension while group D represented a normal control group. Groups A, B and C were treated 3 x daily for 4 weeks with either 60 mg TTFG, 30 mg TTFG or a placebo, respectively. Group D received a similar treatment as group A. Test subjects from each of the groups were evaluated for capillary filtration rate (CFR), ankle circumference (AC) and ankle oedema (AE). Results from the study revealed a significant decrease in CRF, AC and AE in the groups treated with TTFG; this effect was greater in the higher dose group, suggesting that TTFG was efficient in alleviating the symptoms of venous hypertension. Both the placebo and normal subject groups reported insignificant changes in treatment effect.

Another study by Cesarone *et al.* (2001a) evaluated the efficacy of TTFG in improving microcirculation in conditions of venous hypertension and microangiopathy. Forty subjects with severe venous hypertension symptoms were randomized into treatment (60 mg twice daily) and placebo groups, for 8 weeks. Clinically important differences in venous flux at rest, rate of ankle swelling, as well as signs and symptoms of venous hypertension were observed over an eight week period in the TTFG treated subjects, suggesting that TTFG treatment can improve venous microangiopathy. In a related study by, ability of TTFG to alleviate oedema and microcirculation problems during long distance flights were assessed (Cesarone *et al.* 2001b). Symptomatic patients traveling for more than three hours in economy class were randomized into a treatment group and a control group. 60 mg TTFG was given to the treatment group twice a day, for 2 days prior to the flight, on the day of the flight, and the day after the flight. Both the rate of ankle swelling (RAS) and oedema level were reported to be significantly lower in the treatment group than in controls, suggesting that TTFG might be used as a possible oedema treatment option for patients taking long haul flights.

Memory and brain function

Whole plant preparations of gotu kola appear to improve the memory of treated subjects (Mukharji 1953); e.g., addition of the plant to the diet of rats improved on their maze learning ability (Rao *et al.* 1999). Mentally retarded children fed gotu kola also have been reported to exhibit improvement in general mental abilities (Rao *et al.* 1973; Dash *et al*. 1996). In pharmacological studies, plant extracts of gotu kola appeared to act by depressing activity within the central nervous system (Chatterjee *et al.* 1992). In rats suffering from lithium pilocarpine induced epilepsy, treatment with gotu kola resulted in anti-lipid peroxidative and anti-epileptic activities (Katare and Ganachari 2001) and was beneficial in improving memory in the normal controls (Mukharji 1953). A selection of the research studies conducted on the effect of gotu kola extracts on memory and brain function are summarized below.

Rao *et al.* (2005a) assessed the effect of gotu kola fresh leaf juice on leaning and memory during the neonatal period of 7-day-old Wistar rats. Three separate groups of rats were given three doses of gotu kola juice at 2, 4 and 6 mL kg^{-1} body weight, and another two groups were maintained as saline-treated and untreated controls. These rat groups were further subdivided based on the duration of the treatment; *i.e.*, for 2, 4 and 6 weeks. Rats receiving high doses of the juice for a short duration showed an improvement in learning behavior, as well as rats that received any dose of gotu kola during either the four or six week treatment (Rao *et al.* 2005a).

In another animal study, Rao *et al.* (2005b) assessed the effects of gotu kola on brain function and neuronal maturation following the birth of mice. Three month old male Swiss albino mice were injected orally with graded doses (200, 500, 700 and 1000 mg kg^{-1} body weight) of gotu kola aqueous extract for 15 days in order to identify an effective dose for use as a cognitive enhancer / nootropic agent. Behavioral (open field, dark/bright arena, hole board and radial arm maze tests) and biochemical (acetylcholine esterase activity) tests, as well as a histological assessment of dendritic arborization were carried out. Performance of juvenile and young adult mice was significantly improved in the radial arm maze test for spacial memory and in the hole-board test for exploratory behavior; however, locomotor activity in the mice was not altered relative to the control. Treatments also resulted in increased acetylcholine esterase activity in the hippocampus. Results suggest that treatment of juvenile and young adult mice with gotu kola during postnatal development can influence neuronal morphology and promote higher brain function. Mice receiving low doses of gotu kola completed the radial arm maze test for spatial memory faster than those receiving higher doses of the plant; this may be explained by the depressant effects observed with high dose treatments (Rao *et al.* 2005b). These results suggest that administration of gotu kola to young individuals might improve learning and memory.

Cognitive behaviour and oxidative stress

Kumar and Gupta (2002) conducted an animal study to evaluate behaviour and oxidative stress within the brains of cognitively impaired streptozocin (STZ)-treated rats following administration of aqueous extracts of gotu kola. Rats treated for 21 days with levels of plant extract ranging from 100 to 300 mg kg^{-1} body weight demonstrated a dose-dependent increase in cognitive behaviour; however, improvements in oxidative stress were only observed at higher doses of the plant. They also reported that treatment with gotu kola appeared to prevent cognitive deficits and oxidative stress in the brain, two characteristics important to development of Alzheimer's disease in the elderly.

Gupta and Flora (2006) conducted an animal trial in order to evaluate the effects of an aqueous extract of gotu kola on arsenic-induced oxidative stress in rats. Extracts administered for 4 weeks at 100 to 300 mg kg^{-1} body weight day^{-1} were given to the rats along with arsenic dissolved in the drinking water. In the arsenic-exposed rats δ-aminolevulinic acid dehydratase (ALAD), glutathione (GSH) and superoxide dismutase (SOD) levels were depleted in the red blood cells while activity of thiobarbituric acid reactive substances (TBARS) increased. A similar pattern was observed in liver tissues. Increases in TBAR activity within the kidney and the brain were accompanied with increased concentrations of arsenic in the blood and soft tissues. Aqueous extracts of gotu kola demonstrated significant protection against depressed ALAD activity and restored GSH levels in the blood. Results of the study suggest that concomitant supplementation with gotu kola during arsenic exposure may confer protection against oxidative stress.

A Canadian study by Wijeweera *et al.* (2006) evaluated anxiolytic effects of gotu kola for the treatment of anxiety using plant materials of different genotypic origins, different organic extracts of the plant, as well as the efficacy of specific asiaticoside extracts. Rats fed the plant at 200 mg kg^{-1} and 500 mg kg^{-1} body weight were evaluated using the Elevated Plus Maze (EPM), open field test, social interaction test and the Vogel test (punished-drinking test) in

order to evaluate levels of anxiety in the test animals. Plant materials from Madagascar had a higher content of asiaticoside than those tested from the Indian subcontinent. At the higher dosage, a pronounced anxiolytic effect on the rats was observed in five out of the seven anxiety tests given. Three different organic extracts (made up in hexane at 212 mg kg^{-1}; ethyl-acetate at 111 mg kg^{-1}; and methanol at 3047 mg kg^{-1} body weight) of gotu kola were administered p.o. (pharmacology of orally) in condensed milk to the rats. Concentrations of asiaticoside were most abundant in the methanol extract and lowest in the hexane extract (due to its relatively more polar chemical structure). Only methanol and ethyl-acetate extracts showed anxiolytic effects in the behavioural tests, strongly indicating the presence of a pharmacologically active component in these extracts. Significant improvements for the parameters measured in the rats were observed in a dose-dependent manner. In another trial, a saturation in anxiolytic activity was observed at higher dosages.

Other medicinal properties

Asiatic acid, a major triterpene compound in gotu kola, was found to cause dose- and time-dependent cell death in the cell line U-87MG, via apoptosis, Ca^{2+}-mediated necrotic cell death, and activation of caspase-9 and caspase-3 containing pathways (Cho *et al*. 2006). It was also found that Asiatic acid is cytotoxic in a wide variety of cancer cell lines ranging from breast cancer to melanomas and myelomas (Hsu 2005), and has been reported to exhibit cytotoxic effects against cells situated in G2 of the cell cycle via Ca^{+2} release and p53 upregulation (Lee *et al*. 2002). Anti-inflammatory and healing effects resulting from asiatic acid treatment appear to occur through inhibition of nitric oxide (NO) synthesis (Guo *et al*. 2004); treatment with Asiatic acid also appears to prevent UVA-mediated photoaging, amyloid-induced neurotoxicity, and to possess anti-ulcer and anti-hepatofibric activities (Jew *et al*. 2000; Lee *et al*. 2000; Soo *et al*. 2003; Dong *et al*. 2004). Mook-jung *et al*. (1999) investigated the protective effects of asiaticoside derivatives on apoptosis and beta-amyloid-induced neurotoxicity. Twenty-eight derivatives were tested, including asiatic acid and asiaticoside, six of which demonstrated strong inhibition of amyloid-beta-induced cell death in B103 cells at micromolar concentrations. Asiatic acid was found to be the most effective compound in protecting against free radical-induced cell death, as well as capable of lowering free radical concentrations within the cell, suggesting that asiaticoside derivatives have significant potential as therapeutic agents for the treatment of Alzheimer's disease.

PRODUCTION POTENTIAL OF GOTU KOLA IN THE TROPICS AND ON THE CANADIAN PRAIRIES

Field production

Despite the fact that gotu kola has been used as a medicinal plant since ancient times (Aziz *et al*. 2007), limited progress have been made in characterization of different accessions of the plant, development of its agronomic aspects or crop improvement (Perera 1990, 1991). Thus, research on improvement of gotu kola cultivars with production potential for herb and bioactive compound production is needed.

Gotu kola grows in the wild in the southern USA, as well as in tropical and subtropical parts of Australia, the southern parts of Africa, and the tropics of South America (Kartnig and Hoffmann-Bohm 1992). The herb prefers to grow in marshy areas as well as along riverbanks in the tropics. Gotu kola is characterized by its ability to multiply sexually by seeds, and vegetatively by stolons that produce roots at nodes which are separated by long internodes (Mathur *et al*. 2000). Generally, gotu kola is propagated from pieces of stolen together with stems and roots. Harvesting of aerial parts of the herb is usually carried out

throughout the year (Herbs2000.com 2010). Gotu kola is a shade loving plant and grows in a moist habitat at altitudes up to 2500 m above see level. Under favourable moist and shady environments a gotu kola crop typically can be harvested six months after planting in the field, in particular in the tropics (Kartnig and Hoffmann-Bohm 1992). However, under optimum growing conditions such as those found in central Sri Lanka, the first cut of a gotu kola crop grown there can occur 2.5 months after being planted in the field with herb yield ranging from 11.7 to 22.8 MT ha^{-1} (Wahundeniya 2007). Mathur *et al*. (2000) evaluated 16 gotu kola accessions with a wide range of morphological traits, for herb and asiaticoside yields under different levels of shading and sub-topical field conditions found on the Indo-Gangetic plains during the winter season. Herb and asiaticoside yields of the accessions looked at ranged from 470 to 2730 kg ha^{-1} and 1.0 to 9.8 kg ha^{-1}, respectively. Among those tested, thirteen accessions produced their highest herb and asiaticoside yields under 50% shading, while two accessions produced high yields under full light conditions; this suggests that high yields of triterpenoid, saponin rich gotu kola can be produced in the field under both shade and full light conditions by selecting for genotypes adapted to the respective growth conditions.

Variation in asiaticoside content in gotu kola also has been shown to be associated with plant origin. For example, a gotu kola population growing at high altitude contained a significantly higher content of asiaticoside than plants grown at a lower altitude (Das and Mallick 1991). Gupta *et al*. (1999) also reported that asiacoside contents in plants varied from 0.42 to 1.17 % on a dry weight basis in four lines of field-grown gotu kola from India. Similarly, gotu kola from Madagascar contained higher contents of bioactive compounds than those from India (Roullard-Guellec *et al*. 1997), while the asiaticoside content of gotu kola from Malaysia was comparable to that from India (Gupta *et al*. 1999). Wahundeniya (2007) reported that in general, in gotu kola, the non-runner producing trait (bush type) is considered to be an undesirable agronomic trait from the point of view of herb productivity and weed control, compared to the runner type which has an ability to cover the ground in a short time and suppress weed emergence. Bush type gotu kola accessions tend to have a reduced herb yield because of restricted nutrient availability, a trait that is associated with its restricted root system. In addition, higher herb yields can be attributed to large leaf size and a long leaf stalk.

Consumers prefer gotu kola accessions with tender leaves as a vegetable, but high yielding gotu kola accessions with rough leaf traits could be used in the food processing industry in dehydrated products (Wahundeniya 2007). In studies by Wahundeniya (2007) herb yields from the 1st cut of gotu kola harvested in the season ranged from 11.7 to 22.8 MT ha^{-1}, while yields from the 2nd cut harvested ranged from 7.8 to 55.8 MT ha^{-1} and the 3rd from 3.6 to 18.5 MT ha^{-1} for crops harvested 2.5, 4.5 and 7.5 months after field planting. Overall, based on agronomic traits, a moderate herb yielding runner type accession of gotu kola with medium size, dark green tender leaves, medium stalk length and light purple colouration at the base, was selected as the best accession for use as a vegetable (Wahundeniya 2007). Bioactive compound content of these accessions however, was not considered as a selection criterion.

A two-year preliminary study was conducted under field conditions on the southern Canadian prairies using two gotu kola genotypes (small-leaf and large-leaf types) and two planting dates (early and mid spring planting); the study showed that gotu kola has limited potential as a commercial field-grown herb on the southern Canadian prairies, due to low productivity (620 kg ha^{-1}), which was closely associated with a combination of cool soil temperatures in the spring, high light intensity, and a short growing season (Bandara, unpublished data). Under the field conditions experienced, the gotu kola leaves appeared purple in color and exhibited stunted growth, indicating that the plants

were grown under stress, even though an adequate supply of plant nutrients was provided. Further studies are being done to evaluate the crop in fields situated in the shade and under low tunnel plastic cover conditions.

Greenhouse production

A three-year greenhouse study conducted at the Crop Diversification Centre South in Brooks, Alberta, Canada, confirmed that gotu kola can be successfully grown on a soilless (Premier Pro-Mix High Porosity) growing medium with an average herb productivity of 1.1 kg m^{-2} when harvested six weeks after transplanting (M. Bandara, unpublished data). Regrowth of gotu kola grown as an herb under greenhouse conditions two weeks after harvesting produced dense new growth with a rapid turn-around.

Preliminary greenhouse studies also are in progress at the Crop Diversification Centre South (CDCS) in Brooks, Alberta, Canada to evaluate the performance of various gotu kola crop species grown in an aquaponics production system. Aquaponics involves the combined culture of fish and plants in closed recirculation systems. Waste nutrients in the aquaculture effluent are used to produce crop plants. The effluent is treated (nutrient uptake, nitrification, etc.) in the plant component and returned to the fish rearing tanks. Aquaculture effluent typically supplies 10 of the 13 required plant nutrients in adequate amounts, with only Ca, K and Fe needing supplementation. Continuous generation of nutrients from fish waste prevents nutrient depletion while uptake of nutrients by the plants prevents nutrient accumulation, extends water use, and reduces discharge to the environment, highlighting this approach as a highly desirable "Green" technology. With this approach, water used in the system can be re-circulated continuously for periods of one year. The balance between nutrient generation and removal in the system also reduces the need for water quality monitoring, making it less labour intensive. In the system ammonia excreted by fish through their gills is used by the plants as a nitrogen source, resulting in a balanced pH within the water. Nutrient balance is achieved by maintaining an optimum ratio between daily fish feed input and the plant growing area used (Rakocy 1988).

The aquaponics research facility at CDCS was established in 2002 with provincial government funding; it uses tilapia (*Oreochromis nitotious*) as the fish species and has been developed using three major plant species, cucumbers (*Cucumis sativus*), tomatoes (*Solanum lycopersicum*) and sweet basil (*Ocimum basilicam*). In addition, over 60 other crop species, including medicinal herbs and vegetables, such as bitter melon (*Momordica charantia*), rosemary (*Rosmarinus officinalis*), echinacea (*Echinacea angustifolia*), lettuce (*Lectuca sativa*) and gotu kola have been evaluated for their productivity. The aquaponics system at CDCS consists of four fish rearing tanks (5 m^3 each) and four raft hydroponic troughs (20 m^2 each). In addition to fish produced within the system average fruit yields of tomatoes and cucumbers were 20.7 and 33.4 kg plant^{-1} year^{-1}, respectively, which exceeded average production of commercial greenhouses in Alberta that employ conventional hydroponic technology. Furthermore, average herb yield of sweet basil varied from 13 to 42 kg m^{-2} year^{-1} (Savidov 2007).

Gotu kola plants grown in the aquaponics facility exhibited interveinal chlorosis at the early growth stage; this condition appeared to be the result of a magnesium deficiency, although the plants did recover about three weeks after transplanting. This aquaponic, fish-plant integrated system appears to be an appropriate method for gotu kola production in prairie regions of Canada. Under these growth conditions crops produced an average of 1.8 kg of fresh weight gotu kola m^{-2} six weeks after transplanting. In the same growing system, sweet basil produced an average aboveground biomass of 2.0 kg of fresh weight m^{-2} six weeks after transplanting (Bandara and Savidov, unpublished data). Leaf and runner production of a gotu kola crop

Fig. 2 Gotu kola and basil crops growing in an aquaponics system at the Crop Diversification Centre South, Brooks, Alberta in 2010.

Fig. 3 Gotu kola runners producing in an aquaponics system at the Crop Diversification Centre South, Brooks, Alberta in 2010.

in the aquaponics system at CDCS is shown in **Figs. 2** and **3**. In the future, identification of a plant genotype or landrace of gotu kola with high and stable herb productivity and content of bioactive compounds will be important for commercial cultivation of plants with medicinal relevance.

In vitro production

In general, tissue culture technology (*in vitro*) has been successfully used for the commercial production of pathogen-free plants (Debergh and Maene 1981; Khan *et al.* 2009) to conserve the germplasm of rare and endangered species (Fay 1992; Naidu *et al.* 2010), and as a component of gene introduction protocols (Kim *et al.* 2007). Moreover, plant cell and tissue culture has been used in attempt to increase the production if bioactive secondary metabolites of pharmaceutical interest (Giri and Naraseu 2000; Mangas *et al.* 2006; Mangas *et al.* 2008; Mangas *et al.* 2009; Bonfill *et al.* 2010; Yendo *et al.* 2010). Thus, development of effective growth medium and also identification of superior explant source are critical for successful *in vitro* production of specific bioactive compounds in gotu kola (Omar 2004; Mangas *et al.* 2008; Yendo *et al.* 2010). Several protocols have thus been developed for gotu kola plant regeneration systems (Khan *et al.* 2009; Bonfill *et al.* 2010; Hernández-Vázquez *et al.* 2010). In a study by Omar *et al.* (2004) revealed that sucrose as a single factor which was positively

improved growth of callus obtained from gotu kola leaf in suspension culture; increasing sucrose concentration from 3.32 to 6.68% (w/v) resulted in an increase in dry cell weight from 10 to 27 g L^{-1}. Furthermore, the optimum concentrations of growing medium for yielding 27.4 g L^{-1} dry cell weight were 6.68% (w/v) sucrose, indole-3-acetic acid (IAA) 0.84 mg L^{-1}, and 6-benzylaminopurine (BAP) 1.7 mg L^{-1}. Naidu *et al.* (2010) developed a protocol for callus induction and plantlet regeneration form nodal explants of gotu kola and reported that Murashige and Skoog's (MS) medium supplemented with α- naphthaleneacetic acid (NAA) 4 mg L^{-1} in combination with 2,4-dichlotohpenoxy-acetic acid (2,4-D) 2 mg L^{-1} proved effective for callus induction with a frequency of 92%, and BAP 1.5 mg L^{-1} and 6-furfurylaminopurine (kinetin) 1.5 mg L^{-1} was effective for well-organized regeneration of gotu kola. In a study comparing explants sources of gotu kola, Roa *et al.* (1999) reported that stem explants and stolon proved to be the best for callus induction followed by leaf, and the best combination of growth regulators for maximum callus induction was NAA 2 mg L^{-1}+ kinetin 0.5 mg L^{-1}.

Successful plant regeneration via culturing of embryonic and organogenic calli (Patra *et al.* 1998; Martin 2004; Paramageethan *et al.* 2004), and clonal propagation of axilliary buds (Tiwari *et al.* 2000) of gotu kola has spawned interest in the *in vitro* production of secondary metabolites such as asiacoside in intact plants and also in tissue culture has been achieved using chemical elicitors (Ning *et al.* 1994; Kim *et al.* 2004, 2007). Kim *et al.* (2004) studied the effects of different elicitors on whole plant cultures using yeast extract, copper (II) chloride, cadmium chloride and methyl jasmonate. They reported a 1.44- to 1.64-fold increase in asiaticoside production by adding 0.1 g L^{-1} yeast extract and 0.1 mM methyl jasmonate to the growth medium, respectively (Kim *et al.* 2004). Also, determination of asiaticoside content in various tissues of the plant was found to be highest in the leaves and lowest in the root, suggesting that production of asiaticoside in gotu kola is tissue-specific, hence justifying use of whole plant cultures to produce this secondary metabolite (Kim *et al.* 2004, 2005, 2007).

Transformed root (hairy root) cultures have shown to be a good model for the study of many secondary metabolites. *Agrobacterium tumefaciens* or *A. rhizogenes*-mediated transformation has been widely utilized for the introduction of functionally important genes into plants. Aziz *et al.* (2007) experimented with *A. rhizogens*-transformed roots from gotu kola and determined the content of asiaticoside and madecassoside found in different organs of greenhouse-grown plants and cultured cells of two morphological distinct lines (F and S phenotypes) of Malaysian origin. They reported that differences in asiaticoside and madecassoside content exist not only between the F- and S-line, but also among different tissues of the plant, as well as between greenhouse-grown and *in vitro*-grown plants. They further found that triterpenoid accumulation was highest in the leaves, which is in agreement with the findings of Kim *et al.* (2004). However, they were unable to detect any triterpenoids in the transformed roots, and attributed this to a gap in the biosynthetic pathway of triterpenoids which may be caused by a lack of interaction between the roots and other metabolite-producing tissues.

In another attempt to enhance production of asiaticoside in transformed root cultures, Kim *et al.* (2007) utilized *A. rhizogens* strain R1000, which harbours hygromycin phosphotransferase and green fluorescence protein genes for root transformation; methyl jasmonate is used as a the chemical elicitor to induce expression of the inserted genes. No asiaticoside was detected in hairy root cultures despite the abundant growth observed. However, upon application of methyl jasmonate at 0.1 mM for three weeks, significant accumulation of asiaticoside (7.12 mg g^{-1} dry weight) was observed. Thus, up-regulation of pertinent gene expression and application of chemical elicitors can be manipulated to optimize the *in vitro* production of secondary metabolites in these plants.

SUMMARY

Gotu kola is a traditional medicinal plant that has been used since prehistoric times, mainly for the treatment of wounds in humans and animals. It has been used in folk medicine by people from India, Madagascar, Sri Lanka and the Indonesian islands, among many others, as well as in the treatment of leprosy. Modern use of the plant is for the treatment of blood clots and cosmetic problems such as varicose veins, hypertrophic scars and keloids as well as to prevent formation of post-birth stretch marks in women. Wound healing properties of gotu kola are attributed to presence of triterpenoid compounds such as Asiatic acid, asiaticoside, madecassic acid and madecassoside in the plant. These compounds are hypothesized to stimulate production of type I collagen, an essential connective tissue used in the process of wound healing. Gotu kola extracts were also shown to demonstrate sedative and anti-depressive effects. Recent animal studies and clinical trials also have shown anti-oxidative and anxiolytic effects, supporting its anti-cancer and anti-anxiety properties, respectively. It has also been implicated as a potential therapeutic agent for Alzheimer's disease. Toxicity studies conducted on gotu kola report no significant safety issues except for a few isolated negative incidents in which symptoms were alleviated upon discontinuation of treatment with the herb. Gotu kola is currently consumed as a raw salad in Asian regions, in countries such as Sri Lanka, Thailand and Malaysia or its juice processed as a cooling beverage in these areas, for both local and the export market. In North America and Europe, gotu kola is used externally in the form of poultice to be applied to sore areas to encourage wound healing. However, it is mostly used as a plant source to extract economically important compounds such as asiatic acid, asiaticoside, madecassic acid and madecassoside, and is sold in capsulated forms as supplements. Despite the fact that gotu kola genotypes show a considerable variation in productivity and bioactive compound content, limited attention has been paid to crop improvement activities for the crop. Growth of the plant in prairie regions of Canada will require use of greenhouse facilities. In particular, an aquaponic system has been shown to be favourable as a growing environment for gotu kola herb production. However, *in vitro* production of the plant metabolites is actively being researched as an alternative to using field-grown plants as a source of bioactive compounds, where less controllable environmental factors may give rise to greater variation in the levels of its medicinal compounds.

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