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

Manjula S. Bandara1* • Ee L. Lee1,2 • James E. Thomas2

1 Crop Diversification Centre South, Alberta Agriculture and Rural Development, 301 Horticultural Station Road East, Brooks, AB, T1R 1E6, Canada
2 Department of Biological Sciences, University of Lethbridge, Lethbridge AB, T1K 2M4, Canada

Corresponding author: * manjula.bandara@gov.ab.ca

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 China, India 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.

Keywords: aquaponics, asiatic acid, asiaticoside, cognitive behaviour, gotu kola, madecassic acid, memory, triterpenoid saponins, varicose veins, wound healing

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; AA, asiatic acid; AC, ankle circumference; AE, Ankle oedema; ALAD, aminolevulinic acid dehydratase; BAP, 6-benzylaminopurine; CDFS, Crop Diversification Centre South; CFR, capillary filtration rate; C/M, chloroform/methanol; DT, disappearing time; EPM, elevated plus maze; FAS-MS, fast atom bombardment mass spectrometry; GSH, glutathione; IAA, indole-3-acetic acid; IR, infra red; MPT, mitochondrial permeability transition; MS, Murashige and Skoog; NAA, α-naphthaleneacetic acid; NMR, nuclear magnetic resonance; RAS, rate of ankle swelling; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substance; TTGF, total triterpenic fraction of gotu kola; VSC, vacuum suction chamber

CONTENTS

INTRODUCTION................................................................................................................... 20
BOTANICAL DESCRIPTION OF GOTU KOLA................................................................................... 21
TRADITIONAL USES FOR GOTU KOLA................................................................................... 22
CHEMICAL COMPOSITION........................................................................................................... 23
MEDICINAL PROPERTIES ........................................................................................................... 25
Wound healing.................................................................................................................. 25
Varicose veins............................................................................................................... 26
Memory and brain function...................................................................................... 26
Cognitive behaviour and oxidative stress........................................................... 27
Other medicinal properties .................................................................................... 27
PRODUCTION POTENTIAL OF GOTU KOLA IN THE TROPICS AND ON THE CANADIAN PRAIRIES...................... 28
Field production........................................................................................................ 28
Greenhouse production.......................................................................................... 29
In vitro production.................................................................................................... 29
SUMMARY....................................................................................................................... 29
REFERENCES................................................................................................................... 29

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 ὑδρό = water, κοτύλα = cup which allude to the aquatic habitat and cup-shaped leaves of the plant. During the 17th century
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 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-eight 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 (Kartnig and Hoffmann-Böhm 1992). The herbal material, however, is primarily collected in India, Sri Lanka, Madagascar and South Africa. Aerial parts of the plant are predominately 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 pericladiad 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...
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 *Hydrocotyle* 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 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 widespread 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 scrofula, 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 et al. 2000b) 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 widespread 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 scrofula, 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). East Africans also used gotu kola leaves to treat fever and stomach upset as well as for scrofula, 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 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, polysaccharides, flavones, sterols, and lipids (Yu et al. 2006). A summary of major constituents reported to occur in gotu kola is presented in Table 3. Triterpene 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, asaitic acid and madecassic acid. Asiaticoside is a triterpene glycoside that occurs in three forms, asiaticoside A and asiaticoside B,
Hydrocotyle asiatica (Kartnig 1988); e.g., asiaticoside has been isolated from
resulting in contradictions within the chemical literature
generated from different geographical locations, sometimes
source of raw materials used in studies of the plant ori-
compounds found in gotu kola has arisen because the
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 orig-
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
by the ethyl-acetate fraction (IC 50 0.25 μM mL−1) was iden-
create an ethyl-acetate-soluble fraction and a water-soluble
kola was partitioned in an ethyl-acetate/water mixture to
diabetes patients sorbitol can accumulate within intercellular
spaces resulting in chronic complications such as cataracts
of sorbitol across membranes does not occur readily. In dia-
ductase, a key enzyme in the polyol pathway which cata-
trapped from gotu kola using methanol (Matsuda et al.
2001a). These compounds inhibit the activity of aldose re-
ductase, a key enzyme in the polyol pathway which cata-
lyzes the reduction of glucose to sorbitol. In cells, diffusion
of sorbitol across membranes does not occur readily. In dia-
betic 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 (IC50 0.25 μM mL−1) was iden-
tified. Chromatography of the ethyl-acetate fraction re-
trated from gotu kola using methanol (Matsuda et al.
2001a). These compounds inhibit the activity of aldose re-
ductase, a key enzyme in the polyol pathway which cata-
lizes the reduction of glucose to sorbitol. In cells, diffusion
of sorbitol across membranes does not occur readily. In dia-
betic 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 (IC50 0.25 μM mL−1) was iden-
tified. Chromatography of the ethyl-acetate fraction re-

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

<table>
<thead>
<tr>
<th>Constituent group</th>
<th>Constituent</th>
<th>Reference</th>
<th>Constituent group</th>
<th>Constituent</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triterpenic acids</td>
<td>Asiatic acid</td>
<td>Brinkhaus et al. 1998a; Zheng and Qin 2007</td>
<td>Essential oil</td>
<td>β-Caryophyllene</td>
<td>Brinkhaus et al. 1998a; Oyedeji and Afolayan 2005</td>
</tr>
<tr>
<td></td>
<td>Madasassic acid</td>
<td>Brinkhaus et al. 1998a; Yu et al. 2006</td>
<td>Terpene acetate</td>
<td>Pinene</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Madecassic acid</td>
<td>Brinkhaus et al. 1998a; Yu et al. 2006</td>
<td>o-Humulene</td>
<td>Bicyclolgermacrene B</td>
<td>Oyedeji and Afolayan 2005</td>
</tr>
<tr>
<td></td>
<td>Thankunic acid</td>
<td>Brinkhaus et al. 1998a</td>
<td>Germacrene B</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Indocentoic acid</td>
<td>Yu et al. 2006</td>
<td>Elemene</td>
<td></td>
<td>Qin et al. 1998</td>
</tr>
<tr>
<td></td>
<td>Eucapthic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Terminolic acid</td>
<td>Brinkhaus et al. 1998a</td>
<td>Flavone derivatives</td>
<td>Quercetin glycoside</td>
<td>Brinkhaus et al. 1998a; Yu et al. 2006</td>
</tr>
<tr>
<td></td>
<td>Isothakunic acid</td>
<td></td>
<td>Kaempferol</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2α,3β, 20,23-tetrahydroxyurs-28-oic acid (common name has not given)</td>
<td>Brinkhaus et al. 1998a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3β-6β,23-trihydroxylean-12-en-28-oic acid (common name has not given)</td>
<td>Yu et al. 2006</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3β-6β,23-trihydroxyurs-12-en-28-oic acid (common name has not given)</td>
<td>Brinkhaus et al. 1998a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bayogenin</td>
<td>Zheng and Qin 2007</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Centic acid</td>
<td>Zheng and Qin 2007</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Betulinic acid</td>
<td></td>
<td>Catechin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Centic acid</td>
<td></td>
<td>Ruin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brahmic acid</td>
<td></td>
<td>Naringin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Indocentoic acid</td>
<td></td>
<td>Sesquiterpenes</td>
<td>Bicycloelemene</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Centic acid</td>
<td>Eleme</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triterpenic acid sugar esters</td>
<td>Asiaticoside</td>
<td>Brinkhaus et al. 1998a; Matsuda et al. 2001b; Kim et al. 2007; James and Dubery 2009</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Asiaticoside A</td>
<td>Brinkhaus et al. 1998a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Asiaticoside B</td>
<td>Brinkhaus et al. 1998a; Matsuda et al. 2001b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Asiaticoside C</td>
<td>Jiang et al. 2005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Asiaticoside D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Asiaticoside E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Asiaticoside F</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brahminoside</td>
<td>Brinkhaus et al. 1998a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brahminoside</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thankaniside</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isothankaniside</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Centellasapins A</td>
<td>Matsuda et al. 2001a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Centellasapogenol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Scetfalecoside A</td>
<td>Matsuda et al. 2001b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Centellasapins B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Centellasapins C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Centellasapins D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Centelleside</td>
<td>Zheng and Qin 2007</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Madecassoside</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triterpenic steroids</td>
<td>Stigmasterol</td>
<td>Brinkhaus et al. 1998a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sitosterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

©2011 Global Science Books
ursane-type triterpene oligoglycosides, centellasaponins B and C, as well as another oleane-type triterpene oligoglycoside, centellasaponin D.

Additional studies of ethanolic extracts from gotu kola using Fast Atom Bombardment Mass Spectroscopy (FAMS), Infrared (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-trihydroxyurs-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 are stigmasterol and sitosterol.

Triterpenic acids and their sugar esters identified by Kartnig 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 4 Major triterpenoid compound groups presence in gotu kola based on their geographical origin.

<table>
<thead>
<tr>
<th>Triterpenoids</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brahmoside</td>
<td>India</td>
</tr>
<tr>
<td>Brahminoside</td>
<td>India</td>
</tr>
<tr>
<td>Thankunic acid</td>
<td>Northeast India</td>
</tr>
<tr>
<td>Isothakunic acid</td>
<td>Northeast India</td>
</tr>
<tr>
<td>Isothakuniside</td>
<td>Northeast India</td>
</tr>
<tr>
<td>Madasatic acid</td>
<td>Madagascar</td>
</tr>
</tbody>
</table>


### Table 5 Phytochemicals extracted from gotu kola.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Plant part</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asiatic acid</td>
<td>Plant</td>
</tr>
<tr>
<td>Asiaticoside</td>
<td>Plant</td>
</tr>
<tr>
<td>Betulic acid</td>
<td>Plant</td>
</tr>
<tr>
<td>Betulinic acid</td>
<td>Plant</td>
</tr>
<tr>
<td>Brahmic acid</td>
<td>Plant</td>
</tr>
<tr>
<td>Brahminoside</td>
<td>Plant</td>
</tr>
<tr>
<td>Brahmoside</td>
<td>Plant</td>
</tr>
<tr>
<td>Campesterol</td>
<td>Plant</td>
</tr>
<tr>
<td>Camphor</td>
<td>Essential oil</td>
</tr>
<tr>
<td>Centelic acid</td>
<td>Plant</td>
</tr>
<tr>
<td>Centellinic acid</td>
<td>Plant</td>
</tr>
<tr>
<td>Centellose</td>
<td>Plant</td>
</tr>
<tr>
<td>Cenotic acid</td>
<td>Plant</td>
</tr>
<tr>
<td>Cineole</td>
<td>Essential oil</td>
</tr>
<tr>
<td>Dodecane</td>
<td>Essential oil</td>
</tr>
<tr>
<td>Isobrahmic acid</td>
<td>Plant</td>
</tr>
<tr>
<td>Isothankunisic acid</td>
<td>Plant</td>
</tr>
<tr>
<td>Isothakuniside</td>
<td>Plant</td>
</tr>
<tr>
<td>Madasatic acid</td>
<td>Plant</td>
</tr>
<tr>
<td>Madecasatic acid</td>
<td>Plant</td>
</tr>
<tr>
<td>Madecassoside</td>
<td>Plant</td>
</tr>
<tr>
<td>Medicasatic acid</td>
<td>Plant</td>
</tr>
<tr>
<td>Medecasoside</td>
<td>Plant</td>
</tr>
<tr>
<td>Oxyasiticoside</td>
<td>Plant</td>
</tr>
<tr>
<td>Thakunic acid</td>
<td>Plant</td>
</tr>
<tr>
<td>Thanikunic acid</td>
<td>Plant</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Animal studies, clinical trials or laboratorial research conducted</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>First report of a double-blind, placebo-controlled study on the antiulcer effects of gotu kola in healthy humans. Study reported that the attenuated acute startle response in healthy human subjects significantly increased following a single 12g oral dose of gotu kola. Centella asiatica extracts contained anti-HSV1 and anti-HSV2 activities, determined by plaque inhibition assay. Exerted effects synergistically with other medicinal plant extracts. Centella extract exerted protective effects against ethanol-induced gastric mucosal lesions, by strengthening the mucosal barrier and reducing the damaging effects of free radicals. 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. 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. Tritrated extract of C. asiatica treatment effected changes in expression of genes involved in angiogenesis, responded strongly to asiatic and madecassic acids. 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. Leaf extract 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. 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. Chloroform/methanol (CM) 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 CA1 hippocampus region.</td>
<td>Bradwejn et al. 2000</td>
</tr>
</tbody>
</table>
MEDICINAL PROPERTIES

Gotu kola possesses anti-inflammatory, anti-ulcerogenic, anxiolytic, nerve, 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 leprous, lupus, varicose ulcers and eczema is widely recognized (for review articles see Karting 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-nineteenth century to 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 wound 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 in order to treat the wounds. In an in vitro study, chick chorioallantoic membranes (CAM) were damaged and then treated with asiaticoside 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-I collagen synthesis and the reorganization of 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 inflammatory 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; Maqurt 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. Striae graviarum or stretch marks often form in women following pregnancy. An anti-striae skin cream, Trofolastin\(^\text{a}\) (marketed in Europe only), containing gotu kola extract along with tocopherol and collagen-elastin hydrolysate, was used to treat 80 pregnant women (Mallool 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 into 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 wheel on the skin that generally disappears in less than 60 minutes in normal subjects, they measured the disappearance time (DT) of the wheel 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 (46% 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 increase in CFR, 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 Cesaroni 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 (Cesaroni et al. 2001b). Symptomatic patients travelling 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 brain preparations of gotu kola appear to improve the memory of treated subjects (Mukharji 1953; e.g., administered in the diet or at the rate of 200 mg kg\(^{-1}\) body weight). 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 a lipid oxidative and anti-epileptic activities (Katara 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 learning 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 mg 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 (acetylcholinesterase 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 acetycholine 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 a clinical 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 per day 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. (pellets) and orally (solution) in contrasted 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 (glioblastoma multiforme) in combination with doxorubicin. Concentrations of asiatic acid were optimal in the methanol extract of gotu kola (Pimentel et al. 2000). Asiatic acid treatment was seen to cause Ca²⁺ 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-hepatofibrin 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 can be harvested three months after field planting (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 has also 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 asiaticoside 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 (Roulard-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 texture could be transformed to a 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. 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 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 larger 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 soil-less (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.

Further 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 niloticus*) as the fish species and has been developed using three major plant species, cucumbers (*Cucumis sativus*), tomatoes (*Solanum lycopersicum*) and sweet basil (*Ocimum basilicum*). 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 (*Lactuca sativa*) and gotu kola have been evaluated for their productivity. The aquaponics system at CDCS consists of four fish rearing tanks (5 m³ each) and four raft hydroponic troughs (20 m² 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 intercalary chlorosis at the early growth stage; this condition appeared to be the result of a magnesium deficiency, although the plants did recover within 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 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 from gotu kola and reported that Murashige and Skoog's (1962) medium supplemented with α-naphthaleneacetic acid (NAA) 4 mg L⁻¹ in combination with 2,4-dichlorophenoxiacetic 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-furfurylamino purine (kinetin) 1.5 mg L⁻¹ was effective for well-organized regeneration of gotu kola. In a study comparing explants sources of gotu kola, Rao 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 callus (Patra et al. 1998; Martin 2004; Paramageethan et al. 2004), and clonal propagation of axillary bud plating (Aziz et al. 2000) of gotu kola has spawned interest in the in vitro production of secondary metabolites such as asiaticoside 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) experiment with A. rhizogenes-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 tissues (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 triterpenoid content in roots that attributed this to the position of the gene 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. rhizogenes strain R1000, which harbours hygromycin phosphotransferase and green fluorescence protein genes for root transformation; methyl jasmonate is used as a 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 related 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 incidences were attributed 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 capsule 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.

**References**


Boiteau P, Chanez M (1967) Isolation of madecassic acid, a new triterpen
ursane- and oleanane-type triterpene oligoglycosides, centellasaponins B, C, and D, from Centella asiatica cultivated in Sri Lanka. Chemical and Pharmaceutical Bulletin (Tokyo) 49 (10), 1368-1371


Mukharji B (1953) Indian Pharmaceutical Codex: Indigenous Drugs (Vol I), Council of Scientific and Industrial Research, New Delhi, India, 431 pp


Rao PS, Seshadri TR (1969) Variation in the chemical composition of Indian samples of Centella asiatica. Current Science 38, 77-79


Shukla A, Raski AM, Dhawan BN (1999a) Asiatioside-induced elevation of antioxidant levels in healing wounds. Phytotherapy Research 13 (1), 50-54


