CHAPTER FOUR

Gotu Kola (*Centella asiatica*): Nutritional Properties and Plausible Health Benefits

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Abstract

Centella asiatica L. (Gotu Kola) is a nutritionally important plant and a valued traditional medicine in South East Asia. In this review, the chemical composition, nutritional values, and health benefits of C. asiatica have been discussed in detail to emphasize its usage as traditional food and medicine. C. asiatica is one of the most commonly used green leafy vegetables (GLVs) in some countries including Sri Lanka due to its high amounts of medicinally important triterpenoids and beneficial carotenoids. It is consumed in the form of GLVs and in the preparation of juice, drink, and other food products. It is also known to contain vitamins B and C, proteins, important minerals, and some other phytonutrients such as flavonoids, volatile oils, tannins, and polyphenol. In vitro and in vivo studies have shown important health benefits like antidiabetic, wound-healing, antimicrobial, memory-enhancing, antioxidant, and neuroprotecting activities. However, detailed scientific approaches on clinical trials regarding health benefits and nutritional values of C. asiatica are limited, hindering the perception of its benefits, mechanisms, and toxicity in order to develop new drug prototypes. In vitro studies have shown that the method of processing C. asiatica has an impact on its nutritional values and health-related beneficial compounds. The composition of its compounds is influenced by different biotic and abiotic factors which need to be studied in detail to provide information to the public in order to maximize the usage of this valuable plant.

1. INTRODUCTION

Centella asiatica L. (Gotu Kola) Urban (Syn. Gotu Kola coriacea Nannfd., Hydrocotyle asiatica L., Hydrocotyle lunata Lam., and Trisanthus cochinchinensis Lour.) is a tropical medicinal plant from Apiaceae family native to Southeast Asian countries such as India, Sri Lanka, China, Indonesia, and Malaysia as well as South Africa and Madagascar (Jamil, Nizami, & Salam, 2007). It is native to the warmer regions of both hemispheres. This plant grows wild in damp, shady places up to 7000 ft. and can be commonly seen along banks of rivers, streams, ponds, and irrigated fields. It also grows along stone walls or other rocky areas at elevation of approximately 2000 ft. in India and Sri Lanka (Sayasinha, Warnasuriya, & Dissanayake, 1999). The plant is also

indigenous to China, the western South Sea Island, Australia, Madagascar, Southern United States, and insular and continental tropical America. This slender usually creeping herb is especially abundant in the tropical regions. The other common names of the plant are Asiatic Pennywort, Indian Pennywort, Thick-leaved Pennywort, and Gotu Kola. *C. asiatica* has been used as a medicinal herb for thousands of years in India, China, Sri Lanka, Nepal, and Madagascar. It is one of the chief herbs for treating skin problems, to heal wounds (Shukla et al., 1999; Somboonwong, Kankaisre, Tantisira, & Tantisira, 2012), and for revitalizing the nerves and brain cells, hence primarily known as a "Brain food" in India, and many ailments in the body.

2. HISTORY OF GOTU KOLA AND ANCIENT USES

Available literature reveals that Gotu kola has been used as a medicine in India from time immemorial. It had been used in Indian Ayurvedic practice and is well known for promoting longevity. With time, its value was further identified and it started being used in skin treatment topically and internally. Thus, it was used in curing leprosy, lupus, and eczema. The plant "Manduka parni" which is mentioned in the Susuita samhita, an ancient Hindu text, is believed to be C. asiatica. Interestingly, nineteenth-century American eclectics who were well aware of the medicinal properties of plants that were used to treat leprosy have reported use of close relatives of C. asiatica. In France, the herb extract was accepted as a drug during the 1800s. It has also been recorded that it was finally the Gotu kola treatment which was able to cure Dr. Boiteau in 1852, who was suffering from leprosy for several years. Another significant outcome is that Brisbane doctors exhibited the juice of Gotu kola as a medicine, at the Centennial International Exhibition of Melbourne in 1888 (Sayasinha et al., 1999). In China, it was popular as juvenile agent. A Chinese herbalist, Li Ching Yun had been reported to live allegedly for 256 years, surviving 23 wives as a result of his regular use of Gotu kola (Sayasinha et al., 1999).

3. MORPHOLOGY AND DISTRIBUTION

C. asiatica (L.) is a prostrate, faintly aromatic, stoloniferous, perennial, usually creeper herb that attains height up to 15 cm (6 in.). However, there are some giant types which attain even up to 25 cm (10 in.) in height. Stem is glabrous, striated, rooting at the nodes. Leaves are emerging alternately in clusters at stem nodes, long petioles, 2–6 cm long and 1.5–5 cm wide,

orbicular-reniform, sheathing leaf base, crenate margins, glabrous on both sides. Flowers are in fascicled umbels, each umbel consisting of three to four white to purple or pink flowers, flowering occurs in the month of April–June. Fruits are borne throughout the growing season in approximately 2 in. long, oblong, globular in shape, and strongly thickened pericarp. Seeds have pendulous embryo which are laterally compressed (Seevaratnam, Banumathi, Premalatha, Sundaram, & Arumugam, 2012).

However, there are several morphotypes of *C. asiatica* that have been reported from different countries of the world. Figures 1–6 show some of

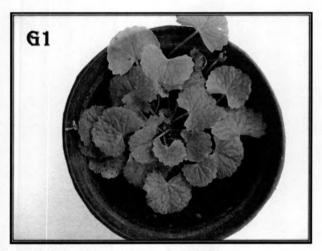


Figure 1 G1 Morphotype of C. acsiatica (wel gotukola).

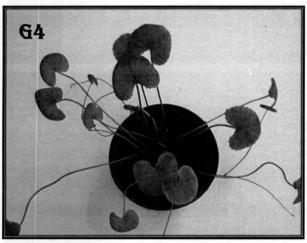


Figure 2 G4 Morphotype of C. acsiatica.

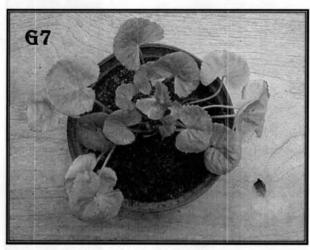


Figure 3 G7 Morphotype of C. acsiatica (bush type).

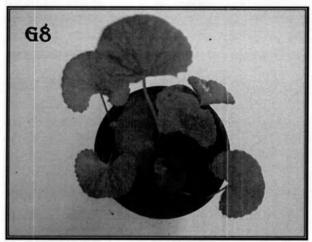


Figure 4 G8 Morphotype of C. acsiatica (giant type 1).

the different morphotypes found in Sri Lanka (Wijekoon, Salim, & Ekanayeka, 2001). Some of them show somewhat different characteristics from those described above. Table 1 shows the morphometric characters of *C. asiatica* types reported from Sri Lanka (Wijekoon et al., 2001).

C. asiatica flourishes extensively in shady, marshy, damp and wet places such as paddy fields river banks forming a dense green carpet and plant can maximize its growth and yield in habitat with sandy loam rather than clayey soil (Devkota & Pramod, 2009).

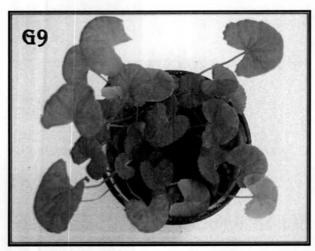


Figure 5 G9 Morphotype of C. acsiatica.



Figure 6 G12 Morphotype of C. acsiatica (giant type 2).

Morphotype	G1	G2	G3	G4	G5	G6	G 7	G8	G9	G10	G11	G12
No. of leaves/ plant	14	11	8	9	16	15	17	10	19	10	15	15
No. of plantlets/ plant	3	7	6	9	6	4	2	4	10	4	5	5
Plant type	Runner	Runner	Runner	Runner	Runner	Runner	Bush	Runner	Runner	Runner	Runner	Runner
Leaf length/cm±SE	0.92±0.08	2.14±0.48	2.37±0.19	2.08±0.12	3.81±0.24	5.4±0.27	2.14±0.18	3.22±0.21	3.21 ± 0.34	1.83±0.16	1.75±0.12	3.58±0.27
Leaf width/cm±SE	1.87±0.15	3.54±0.19	3.69±0.21	3.34 ± 0.27	5.78±0.20	8.02±0.23	3.79±0.22	5.54±0.35	5.18±0.21	2.20±0.15	2.76±0.15	5.93 ± 0.35
Thickness of leaf	Thin	Medium	Thin	Thick	Thick	Medium	Medium	Thick	Thick	Thin	Thin	Thick
Texture of leaf	Smooth	Smooth	Medium	Rough	Smooth	Smooth	Medium	Rough	Medium	Smooth	Medium	Rough
Leaf color	Green	Green	Light green	Green	Dark green	Green	Green	Dark green	Dark green	Green	Green	Dark green
Leaf margin	Slightly dentate	Deeply dentate	Dentate with a pattern	Slightly entire	Slightly dentate with pointed base	Deeply crenate	Slightly dentate	Deeply dentate, pointed	Slightly entire	Slightly dentate	Slightly dentate	Crenate
Petiole length/cm±SE	2.27 ± 0.25	9.88±0.37	3.14±0.31	10.19 ± 0.55	11.06±0.69	19.78 ± 1.64	4.02±0.18	5.26 ± 0.59	6.15 ± 0.23	6.73 ± 0.40	3.32 ± 0.27	12.96 ± 1.64
Petiole diameter/cm±SE	0.09 ± 0.00	0.14±0.03	0.12±0.03	0.21 ± 0.02	0.18 ± 0.03	0.34±0.04	0.15 ± 0.03	0.27 ± 0.04	0.23 ± 0.03	0.09 ± 0.01	0.17 ± 0.04	0.25±0.04
Internodal length/cm±SE	5.37±0.60	10.54±0.60	7.05±0.63	7.34±0.41	12.19±0.83	15.30 ± 0.87		6.53 ± 0.80	10.40±0.67	8.33 ± 0.96	6.25 ± 0.51	13.58 ± 1.09
Presence of pigments	Absent	Absent	Absent	Absent	Present	Absent	Present	Absent	Absent	Absent	Present	Present
Abundance of flowers	Abundant 1-2 flowers	Abundant 1–2 flowers	Clearly abundant 3-5 flowers	Abundant 1–2 flowers	Clearly abundant 3-5 flowers	Clearly abundant 3-5 flowers	Rare	Clearly abundant 1-2 flowers	Clearly abundant 3-5 flowers	Abundant 1-2 flowers	Abundant 1–2 flowers	Clearly abundant 1–2 flowers

Wijekoon et al. (2001).

4. PROCESSING AND USAGE OF GOTU KOLA

Due to its various medicinal and nutritional properties, it is used as a traditional medicine, as a leafy vegetable, and as a beverage in many countries. The nutritional value of *C. asiatica* is mainly due to its richness in carotenoids and vitamins C and B complex.

The herb is commonly used as porridge for feeding preschool children in Sri Lanka in order to combat nutritional deficiency (Cox, Rajasuriya, Soysa, Gladwin, & Ashworth, 1993). This nutritious porridge is known as "Kola kenda," a popular herbal mix of rice and greens enjoyed for generations, usually eaten before breakfast in Sri Lanka. C. asiatica is also a traditional green leafy vegetable (GLV) and locals consume it as "Gotu kola sambola" or salad, where gotu kola is first sliced very finely, then mixed with grated coconut flakes, red onions, and a few extra spices for seasoning.

In Malaysia and Indonesia, *C. asiatica* is commonly eaten fresh as vegetable ("ulam" and salad) especially by the local Malay and Javanese populations (Huda-Faujan, Noriham, Norrakiah, & Babji, 2007). The salads are eaten together with the main meal and can act as an appetizer. Besides eaten raw, it is also cooked as a part of a soup or as a main vegetable. Due to its mild bitterness, it is always cooked and served with the addition of coconut milk and/or shredded coconut and sometimes sweet potatoes and potatoes are added. Due to its therapeutic use, the whole plant including leaves, stem, and root are consumed (Brinkhaus et al., 2000). It is used as a health tonic and processed into cordial drinks and is available at some markets as a ready-made juice (Mohd Ilham, 1998). Fresh, unprocessed plants are also blended to make juice.

In Thailand and India, it is used as vegetable, tonic drink, and juice (Punturee, Wild, & Vinitketkumneun, 2004). *C. asiatica* herbal tea is made by adding a cup of boiled water over either dried or fresh *C. asiatica* plant tissues, letting it brew a few minutes before drinking (Hashim et al., 2011). Either a mixture of many different herbal plants or a single plant may be used when brewing this tea. It is believed that *C. asiatica* herbal tea is a source of antioxidants with many beneficial effects (Huda-Faujan et al., 2007; Naithani, Nair, & Kakkar, 2006).

4.1 Home Remedies

The whole plant mixed with *Drymaria cordata* and *Oxalis comiculata* is boiled and taken to cure dysentery. A syrup of the leaves with ginger and black

pepper is taken for coughs. The leaf juice with palm jaggery is given to women as a tonic after delivery (Sayasinha et al., 1999).

Mixed with bath spa water, it is used in treatment for eczema. Pills prepared from a paste of leaves of the plant and *Ocimum sanctum* Linn, and black pepper are used in the treatment of intermittent fever (Sayasinha et al., 1999).

The powdered leaves of *C. asiatica* make into an ointment to treat elephantiasis, enlarged scrotum, and other affected areas which is prepared with clarified butter or vaseline containing 1 oz. of powdered leaves of *C. asiatica*, while the liquid extract is given internally with a dosage of one to five drops three times a day (Sayasinha et al., 1999).

The powder of the leaves is given in three to five grain doses three times a day for leprosy, scrofula, syphilis, and ulcers. At the same time, some of the powder may be sprinkled on the ulcers or a poultice made of the fresh leaves ground into a paste may be applied (Sayasinha et al., 1999). For ulcers and burns, freshly ground flowers are applied to the affected parts, while around half a gram of powder is taken orally. For syphilitic skin disease, 20 ml of leaf juice is taken orally and some of it is applied externally. About 1.5 g leaves, dried and powdered leaves, are ingested per day as a nervine tonic. To get relief from pain, about 10 ml of fresh plant juice is taken orally twice daily. Powdered *C. asiatica* mixed with lime has been used for the treatment of sores in babies. Leaf juice stops irritation caused by prickly heat. Powdered leaves have been snuffed in cases of ozaena.

4.2 Cosmetic Uses

C. asiatica with astringent tannins and soothing essential oils, which are excellent ingredient for toning and stimulating the skin, is ideal for skin care formulations and also offers protective care. The flavonoids are also used in hair care products where it stimulates the peripheral circulation of the scalp and will promote healthy scalp condition and prevent hair loss. The flavonoids are also to aid stimulates the peripheral circulation of the scalp made them valuable in hair care products where it promotes healthy scalp condition and prevent hair loss (Sayasinha et al., 1999).

4.3 Commercial Products

Due to high value of the extracts of *C. asiatica*, it has been developed into various commercial products and launched in the markets around the world. Some products launched in the market containing *C. asiatica* are reported in

Singh, Gautam, Sharma, and Batra (2010). Oral preparations, capsules, tablets, syrups, tonics, and also have incorporated with some other products such as tea have been launched for various conditions namely, to improves mental abilities, to enhance vascular support, as an antistress formulas, to enhance immunity, as a lipid-lowering agent, and for many other valuable properties; some of them are listed below under the health benefits. Also there are new topical preparations for various indications: nourishing skin creams, eye treatment serums, baby skin care creams, cleansers, and moisturizers (Singh et al., 2010).

5. NUTRIENT COMPOSITION

Generally, GLVs are a rich source of minerals (including iron, calcium, potassium, and magnesium) and vitamins, including vitamins K, C, E, and many of the B vitamins. They also provide a variety of phytonutrients including β -carotene, lutein, neoxanthin, and zeaxanthin, which protect human cells from damages eyes from age-related problems among many other effects.

Macronutrients found in *C. asiatica* are mainly proteins, carbohydrates, and fibers. According to three studies done in the previous decade, the content of nutrients shows relatively close values but in some instances, big variations are also seen (Das, 2011; Hashim, 2011; Joshi & Chaturvedi, 2013). Macronutrient contents of *C. asiatica* are summarized in Table 2. Generally, the herb is low in protein (2.4%), carbohydrate (6.7%), and fat (0.2%). *C. asiatica* has been reported to contain about 87.7% moisture, 5.4% insoluble dietary fiber, and 0.49% soluble dietary fiber, and 17.0 mg/100 g phosphorus, 14.9 mg/100 g iron, and 107.8 mg of sodium mg/100 g sodium. These values may considerably vary depending on the analytical method and biotic and abiotic factors.

Table 2 Macronutrient Percentage of Gotu Kola (%)
Protein Carbohydrate Fiber Moisture Fat Study

NM	6.7	1.6	87.7	0.2	Hashim (2011)
2.4	NM	5.92	84.6	NM	Joshi and Chaturvedi (2013)
9.94	51.92	18.33	84.37	NM	Das (2011)

NM, not mentioned.

Na	K	Ca	Mg	P	Fe	Study
21	NM	NM	NM	32	5.6	Hashim (2011)
107.8	345	174	87	17	14.86	Joshi and Chaturvedi (2013)
NM	NM	1.06	NM	370	32	Das (2011)

NM, not mentioned.

Values of the nutrient composition suggest that *C. asiatica* is a good source of dietary fibers which is a significantly important nutrient component. Dietary fiber intake provides many health benefits. A generous intake of dietary fiber reduces risk of developing coronary heart disease, stroke, hypertension, diabetes, obesity, and certain gastrointestinal disorders, and provides many other health benefits (Anderson et al., 2009).

Total calories in 100 g of C. asiatica are 37.0 kcal (Hashim, 2011). In general, C. asiatica contains a high concentration of potassium (345 mg) and calcium (171 mg). Potassium intake reduces the risk of stroke, kidney stones, renal damages, and many heart-related problems. Calcium is also an important structural component of bone. Adequate calcium intake throughout childhood and adolescence is needed to achieve maximum bone mass in young adulthood which is an important determinant of bone mineral status in later life. C. asiatica can be used as a nonexpensive nutritional source of both potassium and calcium. Results of mineral content in three different studies are from two different countries shown in Table 3. However, mineral content of the different morphotypes of C. asiatica can drastically vary. Chandrika et al. (2011) have reported the variations in mineral content in different morphotypes as summarized in Table 4.

C. asiatica is also rich in vitamin C (48.5 mg/100 g), B1 (0.09 mg/100 g), B2 (0.19 mg/100 g), niacin (0.1 mg/100 g), carotene (2649 μ g/100 g), and vitamin A (442 μ g/100 g) (Hashim, 2011; Joshi & Chaturvedi, 2013). Studies on vitamin content have been done in the last decade by various researchers and summarized in Table 5.

5. PHYTONUTRIENTS

C. asiatica contains a broad spectrum of phytonutrients that provide a range of beneficial effects. Generally, C. asiatica contains many classes of

Table 4 Mineral Contents of Different Morphotypes of *C. asiatica*Content (mg/100 g Dry Weight)

Morphotype	Calcium	Magnesium	Potassium	Sodium	iron	Copper	Zinc
G1 (heen/wel-1)	1150.9±711.3	240.7 ± 105.8	6295.0 ± 505.7	2200.2±98.6	40.4 ± 13.5	3.5 ± 2.0	11.3±0.7
G2 (heen/wel-2)	1583.3 ± 307.9	411.4 ± 60.2	4771.5 ± 180.0	1798.2±77.1	50.4 ± 15.8	2.8 ± 2.4	14.7 ± 1.0
G3 (salada)	2206.1 ± 996.1	426.9 ± 129.6	6165.0 ± 125.8	2597.1 ± 366.9	51.2±33.9	6.4 ± 4.1	19.4±7.2
G7 (bush)	1679.9 ± 345.2	541.8 ± 45.9	3079.0 ± 180.1	2099.5 ± 45.5	74.3 ± 34.1	4.6 ± 4.1	12.5 ± 1.3
G8 (giant-1)	1743.1 ± 373.8	841.3 ± 97.9	4705.9 ± 225.4	1114.2 ± 53.5	18.5 ± 5.4	2.6 ± 1.2	13.6 ± 1.3
G12 (giant-2)	1613.3 ± 160.7	640.7 ± 76.9	4950.9 ± 199.4	1511.1 ± 216.5	29.0 ± 6.2	3.3 ± 2.1	11.8 ± 1.1

Data presented as mean \pm standard error of the mean of five independent experiments (n = 5). Chandrika et al. (2011).

Vitamin A	Thiamine (B1)	Riboflavin (B2)	Niacin (B3)	Ascorbic Acid (Vitamin C)	β-Carotene	Reference
0.44	0.09	0.19	0.1	48.5	NM	Hashim (2011)
NM	0.04	NM	NM	11	3.9	Joshi and Chaturvedi (2013)
NM	NM	NM	NM	9.73	1	Das (2011)

NM, not mentioned.

phytonutrients such as triterpenes, carotenoids, glycosides, flavonoids, alkaloids, volatile oils, and fatty oils.

The major chemical constituents of the plants are as follows: terpenoid compounds, asiatoside, asiatic acid, madecassic acid, asiaticoside, brahminoside, brahmoside, centelloside, brahmic acid 0.097%, centellinic acid, isobrahmic acid, betulinic acid, stigmasterol, sitosterol, centellic acid, centic acid, indocentellic acid, centellose, thankuniside, thankunic acid, hydrocotylin, glycerides of oleic acid, linoleic acid, linolenic acid, palmitic acid and stearic acid, vallarine, pectic acid, aspartic acid, glycine, glutamic acid, α-alanine, phenylalanine, quercetin-3-glucoside, and kaempferol-3-glucoside.

6.1 Triterpenes

These are triterpenes and their derivative molecules which are the major chemical compounds found in *C. asiatica*. Most important constituents are asiatic acid (Fig. 7), asiaticoside (Fig. 8), madecassic acids (Fig. 9), madecosside (Fig. 10), thankuniside, brahmoside, brahminoside, and brahmic acid. Asiaticoside and madecassoside predominate mostly in leaves and lesser in roots (Das, 2011).

Asiaticoside isolated from *C. asiatica* has been studied for its wound-healing activity in normal as well as in diabetic animals (Shukla et al., 1999). Madecassoside and most other triterpenoids also possess these wound-healing activities (Liu et al., 2008). Triterpenes from *C. asiatica* reduce the immobility time and ameliorated the imbalance of amino acid levels confirming the antidepressant activity of *C. asiatica* (Chen, Han,

Figure 7 Asiatic acid.

Figure 8 Asiaticoside.

Figure 9 Madecassic acid.

Figure 10 Madecassoside.

Qin, Rui, & Zheng, 2003). C. asiatica has significant antibacterial activity and studies show that this is due to triterpene asiaticoside (Das, 2011).

Brahmic acid, isobrahmic acid, brahminoside, and brahmoside present in C. asiatica have shown some psychotropic, sedative, and anticonvulsant properties. It is also useful in dementia, mental disorders, and anxiety (Singh et al., 2010). Thus, "Mentat" a polyherbal formulation containing C. asiatica contributes to improvement of memory, attention, and concentration in children with learning disability (Upadhyay, Saha, Bhatia, & Kulkarni, 2002).

Different levels of triterpene compounds were observed at different maturity stages. The content of asiatic acid was not significantly different at all stages of maturity. However, the levels of madecassic acid, asiaticoside, and madecassoside were significantly different with advance in maturity. These compounds were higher when harvested at 60 days and decreased significantly thereafter. Thus, for the ratoon crop, it is recommended to harvest the plant at 60 days after ratooning since most of the bioactive compounds were observed to be highest at this stage of maturity (Rosalizan, Rohani, Khatijah, & Shukri, 2008).

6.2 Carotenoids

Carotenoids are one of the most important phytonutrients found in C. asiatica. Carotenoids, the colorful plant pigment, some of which the body can turn into vitamin A, are powerful antioxidants that can help prevent some forms of cancer and heart disease and act to enhance your immune response to infections.

Chandrika et al. (2011) have analyzed β -carotene, lutein, neoxanthin, and violaxanthin contents of six morphotypes of C. asiatica designated as G1, G2, G3, G7, G8, and G12 shown in Table 1. They also compared the proximate components and selected dietary minerals of these morphotypes. Their study showed that among these medicinally important herbs, less commonly consumed types contained higher levels of carotenoids (especially lutein and β -carotene) and minerals.

Hence, these six varieties of *C. asiatica* could be exploited as a good source of provitamin A and lutein to overcome vitamin A deficiency as well as age-related macular degeneration (AMD). The data on content of carotenoids and minerals could be helpful to create nutritional awareness among various communities on the importance of these different varieties of *C. asiatica* leaves. This is the first report on the individual carotenoid

composition, vitamin A activity, and mineral content of six different germplasms of medicinally important C. asiatica leaves grown in Sri Lanka. Results are summarized in Table 6. In vitro accessibility of β-carotene from cooked Sri Lankan GLVs and their estimated contribution to vitamin A requirement have been studied (Chandrika, Svanberg, & Jansz, 2006). This study showed one portion (100 g) of green leaves cooked without fat (coconut) only contributed from 14% to 18% of the recommended daily allowance, whereas C. asiatica cooked with scraped coconut (traditional malluma preparation) and coconut oil (stir fried) contributed 55% and 20%, respectively, showing that malluma preparation is the most suitable in terms of contribution to vitamin A requirement.

Lutein is also one of the major carotenoids in GLVs which show a marked antioxidant activity. It is a predominant carotenoid of human macular pigment known to benefit the eye by giving protection against AMD by preventing light-initiated oxidative damage to the retina, mediated by its ability to quench singlet oxygen and blue light in the retina. Carotenoid content and *in vitro* accessibility of lutein have also been carried out for C. asiatica and other selected GLVs from Sri Lanka using RP-HPLC (Chandrika, Basnayake, Athukorala, Colombagama, & Goonetilleke, 2010). This study showed that four main carotenoids, namely neoxanthin,

Table 6 Carotenoid Compositions of Different Morphotypes of *C. asiatica* Leaves Grown in Sri Lanka

Morphotype	All-trans- Neoxanthin (μg/g FW) ^a	<i>All-trans-</i> Violaxanthin (μg/g FW) ^a	<i>All-trans-</i> Lutein (μg/g FW) ^a	All-trans-β- Carotene (μg/g FW) ^a	RE ^b /100 g (FW) ^a
G1 (heen/ wel-1)	7.8 ± 2.0	20.5 ± 3.2	82.6 ± 10.2	42.5 ± 9.4	354.0
G2 (heen/ wel-2)	9.6±1.2	23.4±3.5	103.2±11.8	43.0 ± 4.5	358.0
G3 (salada)	9.4±1.8	13.2 ± 1.4	90.9±3.5	45.3±4.4	377.5
G7 (bush)	5.9±0.7	22.4±5.9	85.2±16.5	52.2±8.3	435.0
G8 (giant-1)	13.5 ± 1.4	26.3 ± 2.0	131.4±2.3	77.2±5.1	643.0
G12 (giant-2)	14.2 ± 2.8	26.1 ± 2.8	133.5 ± 10.1	73.7 ± 5.2	614.0

^{*}Number of samples analyzed (n=4).

Data presented as mean ± standard error of the mean.

Chandrika et al. (2011).

^b1 RE = 12 μg β-carotene (Institute of Medicine, 2001).

violaxanthin, lutein, and β -carotene, are present in C. asiatica leaves, and it was also evident that traditional cooking causes a reduction in lutein levels. The percentage in vitro accessibility of C. asiatica in malluma preparation and stir fried was 19% and 10%, respectively, showing that stir fired preparation is the most suitable in terms of lutein accessibility.

6.3 Glycosides

A glycoside is any molecule in which a sugar group is bonded through its anomeric carbon to another group by a glycoside bond. *C. asiatica* stores glycosides in inactive form. These glycosides are activated on hydrolysis. Asiaticoside, madecosside, and centelloside are the glycosides present within the plant. On hydrolysis, they yield trepene acids, asiatic acid, madegascari acid, and centellic acid, and all these are present in free form in the plant (Das, 2011).

6.4 Volatiles and Fatty Oils

The plant contains about 36% of volatiles and fatty oils. The fatty oil consists of glycerides of palmitic, stearic, lignoceric, oleic, linoleic, and linolenic acids. The major constituent present in C. asiatica oil comprises of terpenic acetate, while other prominent constituents were β -caryophyllene, farnesene, trans- β -farnesene, germacrene-D, α -humulene, bicyclogermacrene, sesquiterpene, and p-cymol (Das, 2011; Joshi & Chaturvedi, 2013).

6.5 Flavonoids

These are the yellow pigments, also collectively known as vitamin P and citrin. In C. asiatica is reported to contain flavonoids, 3-glucosylquercetin, 3-glucosylkaemferol, and 7-glucosylkaemferol. Apart from these, two new flavonoids named castilliferol 1 and castillicetin 2 have been isolated from the whole plant recently. Presence of several flavonoid derivatives such as quercetin, kaempferol, patuletin, rutin, apigenin, castilliferol castillicetin, and myricetin has been reported in C. asiatica (Das, 2011). Flavonoids play a major role in human body as an important antioxidant and they exert this effect by scavenging or chelating process (Schmitt-Schillig, Schaffer, Weber, Eckert, & Muller, 2005). According to Rahman et al. (2013), flavonoids have been analyzed as a quercetin equivalent.

6.6 Other Compounds

The plant is reported to contain tannins, inorganic acid, sugar, resin, and amino acids, namely aspartic acid, glycine, glutamic acid, α -alanine, and phenylalanine. Tannins act as an antioxidant (Rahman et al., 2013), thus provides a significant value for phytonutrient content.

6.7 Retention of Nutrients in C. asiatica on Dehydration

The GLV C. asiatica is seasonal and also highly perishable due to their high water content. There are heavy losses due to nonavailability of sufficient storage, transport, and proper processing facilities at the production point (Pande, Sonune, & Philip, 2000). There is a need to preserve the nature's storehouse of nutrients through convenient processing techniques. Dehydration seems to be the simplest technology for preserving GLV, especially when they are abundantly available.

In recent years, exhaustive efforts have been made for an improvement in the nutrient retention of dried products by altering processing methods and/or pretreatment. Blanching is a prerequisite for preservation of GLVs. It is necessary to prevent the formation of off-flavors, odors, and colors. However, it may cause partial destruction of some nutrients like ascorbic acid. Peroxidase activity is widely used as an index of blanching because peroxidase is the most heat-stable enzyme found in vegetables. Optimum conditions of blanching time and temperature are necessary to achieve the desired quality of dried products (Kadam, Samuel, Chandra, & Sikarwar, 2008). Retention studies of nutrients provide a clear understanding on the correct way of processing these GLVs. Gupta, Gowri, Lakshmi, & Prakash (2013) have analyzed the retention of some valuable nutrients in C. asiatica on dehydration shown in Table 7.

According to their study, the compositional changes that occurred on dehydration varied with the nature/type of the component. For example, the proximate principles were least affected, total iron and calcium content decreased slightly, but dialyzability of the minerals decreased significantly. Among the vitamins, the ascorbic acid shows the highest reduction with the dehydration process, total carotenes and β -carotene content also show reduction with the dehydration, while thiamine was retained moderately. Changes in the antinutritional factors were nonsignificant. The process of dehydrating concentrates the nutrients in C. asiatica, thus the dehydrated product is a rich source of dietary fiber which can find application in

Table 7 Proximate, Mineral, and Vitamin Composition of Fresh and Dehydrated Green Leafy Vegetables

Eculy Vegetables	Fresh	Dehydrated
Moisture (%)	85.7±0.59	7.9±0.29
Ash (g/100 g)	1.89 ± 0.01	2.00 ± 0.01
Iron (mg/100 g)	12.46 ± 0.00	13.97 ± 0.80
Calcium (mg/100 g)	193.4±6.99	178.9±11.97
Insoluble dietary fiber (g/100 g)	5.08 ± 0.00	4.17 ± 0.01
Soluble dietary fiber (g/100 g)	0.38 ± 0.01	0.51 ± 0.01
Ascorbic acid	13.8±1.69	1.9 ± 0.00
Thiamine	0.13 ± 0.04	0.06 ± 0.01
Total carotene	38.13 ± 2.00	27.78 ± 1.42
β-Carotene	5.46 ± 0.20	3.63 ± 0.54
Tannin	122.5 ± 7.16	146.9±7.17
Total oxalate	78.3 ± 5.31	59.3 ± 4.07
Soluble oxalate	414.4 ± 10.93	26.8 ± 6.72

Gupta et al. (2013).

development of high fiber and micronutrient-rich foods. Easy to preserve, feasibility, and convenience and off season availability are some of the advantages of incorporating dehydrated *C. asiatica* in products.



7. ANALYTICAL TECHNIQUES FOR IMPORTANT NUTRIENT COMPOUNDS

Many of the nutritional compounds found in *C. asiatica* can be analyzed according to the methods described in "AOAC Official Methods of Analysis—AOAC International." Some of the major and important components can be analyzed using methods described below.

7.1 Triterpenoids

The preliminary identification of madecassoside, asiaticoside, madecassic, and asiatic acid can be achieved by thin-layer chromatography (TLC) on silica gel plates and mass spectrometry, using a modification of the method described in the European Pharmacopoeia (2005). A combination of ethyl

acetate and methanol as the mobile phase was found to be successful in separating these compounds from the rest of the main components of the extract. The separated compounds are confirmed by MALDI-TOF mass spectrometry. High-performance liquid chromatography (HPLC) analysis can be done with a modifying procedure as described in Inamdar, Yeole, Ghogare, and Souza (1996).

7.1.1 Analysis by TLC

This method requires silica gel plates, ethyl acetate—methanol (60:40, v/v) as the solvent system and anisaldehyde solution as the spray detector. This system allows the identification of triterpenoid compounds of *C. asiatica* and their separation into saponins and sapogenins, and also can be used to prepare samples for HPLC analysis (Mangas, Moyano, Hernandez-Vazquez, & Bonfill, 2009). The separated compounds are confirmed by MALDITOF mass spectrometry. TLC of madecassoside, asiatic acid, and asiaticoside, and of hexane extract, ethyl acetate extract, and methanol extract was performed and compared with the standards of European Pharmacopoeia in Mangas et al. (2009).

7.1.2 Analysis by High-Performance Liquid Chromatography

The quantification of the four compounds madecassic acid, madecassoside, asiatic acid, and asiaticoside has been done by Mangas et al. (2006) by HPLC. The chromatographic analysis of *C. asiatica* had given the better results with a reversed phase using gradient elution. The purity of peaks can be obtained by using a photodiode array detector.

7.2 Carotenoids

7.2.1 Isolation of Carotenoid Standards by Open-Column Chromatography

Sesbania grandiflora leaves can be used as the source of standards because they are easy to extract from and have a high carotenoid content. To obtained the standards, the carotenoids are extracted (60 g) with cold acetone, partitioned to petroleum ether, concentrated in the rotary evaporator, and separated in open column of MgO:celite (1:1 activated for 2 h at $110\,^{\circ}$ C). The concentrations of the pure standards are determined spectrophotometrically using the A 1% 1 cm values (for β -carotene, 2592 in petroleum ether; for lutein, 2550 in ethanol; for violaxanthin, 2550 in ethanol; for neoxanthin, 2243 in ethanol).

7.2.2 Identification of the Carotenoids of C. asiatica Leaves

Identification of carotenoids can be carried out according to Rodriguez-Amaya (1999). This involved the combined use of the retention times, cochromatography with authentic samples, and the visible absorption spectra and, for xanthophylls, some chemical tests. The chemical reactions are acetylation with acetic anhydride of secondary hydroxy groups (as in lutein, violaxanthin, neoxanthin), methylation with acetic methanol of allylic secondary hydroxyl groups as in lutein, and epoxide–furanoid rearrangement of 5,6-epoxy groups (as in violaxanthin, neoxanthin) with diluted hydrochloric acid and iodine-catalyzed isomerization reactions to verify geometric configuration. Progress of the first two reactions is monitored on a thin layer of silica gel developed with 5% methanol in toluene, while the last two are carried out spectrophotometrically.

7.2.3 Reversed-Phase High-Performance Liquid Chromatography— Diode Array Detection Analysis of Carotenoids

Quantification are carried using high-performance liquid chromatography (HPLC) with diode array detection (HPLC-DAD) using an HPLC series 1200 (Agilent, Boeblingen, Germany) equipped with ChemStation software, degasser, quaternary gradient pump, autosampler, column oven, and diode array detector. The mobile phase consists of acetonitrile, methanol, and ethyl acetate containing 0.05% triethylamine used at a flow rate of 0.5 ml/min with DAD, using a monomeric C18 column (Agilent), 3 μ m, 4.6×250 mm². A gradient is applied from 95:05:00 to 60:20:20 in 20 min, maintaining this proportion until the end of the run. Purity of the peaks is confirmed using a UV-visible photodiode array detector. Quantification of carotenoids is carried out using external standardization. The β -apo-8'-carotenal can be used as an internal standard.

7.3 Determination of Mineral Content

Determination of mineral contents can be achieved with atomic spectroscopy (AAS) and inductive coupled plasma emission spectrophotometer (ICP). In AAS, the dried samples are weighed into a porcelain crucible and ashed in a muffle furnace at a temperature of 550 °C. The ashes are then dissolved into an acidic aqueous solution (HCl:HNO₃ 1:1) for analysis. This sample can be analyzed with atomic absorption spectrometer. Using this method, calcium, magnesium, iron, copper, and zinc can be quantified. Atomic emission spectrophotometer technique can be used to determine sodium and potassium contents. The results of chemical composition and

mineral content are expressed per 100 g in dry weigh (Chandrika et al., 2011).

In ICP method, the contents of minerals such as Na, Ca, K, Fe, P, Mg, and Zn are determined by pretreating the samples by dry ashing at 550 °C and dissolving them in nitric acid before injection into an ICP (Khatijah, 2001).

8. MAJOR HEALTH BENEFITS OF GOTU KOLA

The use of *C. asiatica* in food and beverages has increased over the years basically due to its beneficial functional properties. Its potential antioxidant, antimicrobial, cytotoxic, neuroprotective, and other activities have been widely claimed in many reports and is very much related to its properties and mechanism of action of the plant's bioactive constituents, namely the triterpenic acid (asiatic acid madecassoside acid), triterpenic saponin (madecassoside and asiaticoside), flavonoids, and other phenolic compounds (Seevaratnam et al., 2012).

Many studies have shown that *C. asiatica* has been effective against diabetes mellitus (Chauhan, Pandey, & Dhatwalia, 2010), depression (Chen et al., 2003), wound-healing activity, antibacterial activity (Das, 2011), neuroprotective activity (Lee et al., 2000), and in many other important ailments in the body.

It has been shown that the herb should be free of any nematode infestation in order to obtain the maximum nutritional properties. Meloidogyne incognita is a nematode pest of this plant which causes the root-knot disease. The symptoms of this infestation are basically restricted to underground parts where the root systems contain numeral galls/knots (Fig. 11) (Wijekoon, Salim, & Ekanayeka, 2002), while the above ground part will show the noncharacteristics symptoms such as yellowing and wilting only at a later stage. The infested plants are usually killed although at high nematode densities in soils they show wilting and yellowing symptoms are usually survived and with abundant knots they are usually seen in the market samples in Sri Lanka. The galled roots are inefficient in absorbing water and nutrients, while the poorly absorbed water and nutrients are inefficiently transported from roots to shoots due to the blocking of the transport pathway by the endoparasitic adult female nematodes. Wijekoon et al. (2002) showed that C. asiatica infested by the nematode with no apparent symptoms in aerial parts (leaves plus petioles) are significantly poor in their proximate values

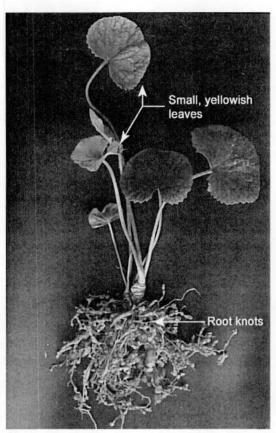


Figure 11 Meloidogyne incognita Infestation manifest as root-knot disease.

(Table 8). The response of different germplasm of *C. asiatica* to *M. incognita* has been shown in Table 9 (Degambada & Salim, 2011).

8.1 Antioxidant Activity of C. asiatica

Antioxidants are free radical scavengers that provide protection to living organisms from damage caused by reactive oxygen species. Although almost all organisms possess antioxidant defense and repair systems, these systems are insufficient to cope over entire damage. Therefore, dietary antioxidant supplementation is promising in strengthening the antioxidant defense and repair systems. Polyphenol, flavonoid, β -carotene, tannin, vitamin C, and DPPH (2,2-diphenyl-1-picrylhydrazyl) compounds are readily found in *C. asiatica* contributing to significantly higher antioxidant activity in the herb.

Table 8 Effect of the Infestation by *M. incognita* on Nitrogen, Phosphorous, and Potassium Contents in Shoots of *C. asiatica* 3 Months After as Determined by Kjeldahl Digestion, Spectrophotometric, and Flamephotometric Method, Respectively (Values Present per 100 g Dry Weight Basis)

Treatments	Nitrogen (mg)	Phosphorous (mg)	Potassium (mg)
T ₁ (control)	2008 a	79.8 с	4526 d
T ₂ (infested)	1596 b	79.6 с	3528 e

n=5.

Values followed by the same letter within a column are not significantly different at $p \ge 0.05$ in Student's *t*-test.

Table 9 Effect of Different Population Levels of *M. incognita* on Mean Na, Mg, Ca, and Fe Content in Shoots (Petiole+Leaf) of *C. asiatica*, 10 Weeks After Inoculation (Values Present per 100 g Dry Weight of Shoots of the Plant) as Determined by Atomic Absorption Spectroscopy

Treatment	Na (mg)	Mg (mg)	Ca (mg)	Fe (mg)
T ₀ (Control)	2479 a	578 a	1976 a	49 a
T ₁ (1000 eggs and J ₂ larvae)	2356 b	252 b	1805 b	41 b
T ₂ (2000 eggs and J ₂ larvae)	2225 с	220 с	1802 b	42 b
T ₃ (5000 eggs and J ₂ larvae)	1891 d	169 d	1739 с	38 c
T ₄ (10,000 eggs and J ₂ larvae)	699 e	160 e	1535 d	26 d

n = 10.

Values followed by the same letter within a column are not significantly different at $p \ge 0.05$ in ANOVA one-way test.

Antioxidant activity of *C. asiatica* is comparable to the activity of rosemary and sage and has been identified with high potential to be explored as a source of natural antioxidants (Jaswir, Hassan, & Said, 2004). Hashim et al. (2011) reported that antioxidant in *Centella* (84%) is comparable to vitamin C (88%) and grape seed extract (83%).

The degree of antioxidant activity varies with the type of tissues of the herb. According to Zainol, Abdul-Hamid, Yusof, and Muse (2003), C. asiatica leaves showed the highest antioxidant activity which also contains highest phenolic contents, when compared to other plant parts. This result suggested that phenolic compounds are the major contributors to the antioxidative activities of C. asiatica. On the other hand, Abdul-Hamid, Md Shah, Muse, & Mohamed (2002) reported that ethanol extract of root exhibits the highest activity though it was not significantly different from the leaves. The antioxidative activity of different parts of C. asiatica may be due

to the reduction of hydroperoxides, inactivation of free radicals, chelation of metal ions, or combinations thereof.

8.2 Antidiabetic Activity

Ethanolic and methanolic extracts of *C. asiatica* had shown significant protection and lowered the blood glucose to normal levels in glucose tolerance test carried out in the alloxan-induced diabetic rats (Chauhan et al., 2010). Nganlasom, Suttitum, Jirakulsomchok, and Puapairoj (2008) treated the wounds of the diabetic-induced male Sprague-Dawley rats with *C. asiatica* plant extract. The plant extract-treated wounds were found to epithelialize faster than control.

8.3 Cytotoxic and Antitumour Activity

A partially purified fraction of methanol extract of *C. asiatica* inhibited the growth of tumor cells with no toxic effects on lymphocytes. Water extract has a chemopreventive effect on colon tumorigenesis (Bunpo et al., 2004). Asiatic acid was found to have anticancer effect on skin cancer. Asiaticoside possesses good wound-healing activities because of its stimulative effect on collagen synthesis. It might be useful in cancer chemotherapy as it induces apoptosis and enhances antitumour activity of vincristine, an anticancer agent from *Catharanthus roseus* in cancer cells (Jamil et al., 2007). The *n*-hexane, carbon tetrachloride, chloroform, and aqueous soluble fractions of methanol extract of *C. asiatica* showed significant cytotoxic activities in the brine shrimp lethality bioassay (Ullah, Sultana, & Haque, 2009).

8.4 Neuroprotective Activity

The consumption of *C. asiatica* was useful to protect the cells from oxidative damage, to destroy excess free radicals, and to keep the oxidative stress state in balance. As a potent antioxidant, it exerted significant neuroprotective effect and proved efficacious in protecting the rat's brain against age-related oxidative damage (Subathra, Shila, Devi, & Panneerselvam, 2005). Asiatic acid exerted significant neuroprotective effect on cultured cortical cells by potentiation of the cellular oxidative defense mechanism. Therefore, it may prove efficacious in protecting neurons from the oxidative damage caused by exposure to excessive glutamate (Park, Bosire, Lee, Lee, & Kim, 2005). The plant accelerates nerve regeneration upon oral administration and contains multiple active fractions increasing neurite elongation

in vitro, suggesting that components in the herbs may be useful for accelerating repair of damaged neurons (Lee et al., 2000; Soumyanath et al., 2005).

8.5 Cardioprotective Activity

C. asiatica showed cardioprotective effect on antioxidant tissue defense system during adriamycin-induced cardiac damage in rats (Gnanapragasam, Ebenezar, Sathish, Govindaraju, & Devaki, 2004). The alcoholic extract of the whole plant was evaluated by Pragada, Veeravalli, Chowdary, and Routhu (2004) for cardioprotective activity against ischemia-reperfusion-induced myocardial infarction in rats, and their results strongly suggest the cardioprotective activity of the plant in limiting ischemia-reperfusion-induced myocardial injury.

8.6 Anti-inflammatory Activity

Extract of *C. asiatica* also exerted anti-inflammatory effects by reduction of acute radiation reaction in rats. The aqueous extract of the herb and its active constituent asiaticoside has an anti-inflammatory property that is brought about by inhibition of nitric oxide synthesis and thus facilitates ulcer healing (Guo, Cheng, & Koo, 2004). Crude extract showed anti-inflammatory activity in rats by prostaglandin E2-induced paw edema. Bioactive terpene acids such as asiatic acid and madecassic acid present in the crude extract may account for the anti-inflammatory activities (Somchit et al., 2004).

8.7 Antimicrobial Activity

Antimicrobial activity of C. asiatica also has been identified as a health benefit of this herb (Ullah et al., 2009). It is a practice in some local communities to use aqueous leaf extract for stomach aches especially for children. Although the exact scientific basis is not known, it had been shown that leaf extracts of C. asiatica possess antibacterial activity against Escherichia coli (Ullah et al., 2009) as well as against many other bacteria and fungi (Seevaratnam et al., 2012). The volatile extract exhibited a broad spectrum of antibacterial activities against both Gram-positive and Gram-negative organisms (Zheng & Qin, 2007). In addition to active compounds present in the leaf extract that are antimicrobial, there are several endophytic microorganisms associated with C. asiatica that have been reported to exhibit antimicrobial activity (Degambada & Salim, 2011). The plant-microbial synergistic effect with respect to antimicrobial activity greatly enhances the health benefit of C. asiatica.

8.8 Memory-Enhancing Activity

Aqueous extract of the herb showed significant effect on learning and memory and significantly decreased the levels of norepinephrine, dopamine, and 5-HT and their metabolites in the brain (Nalini, Aroor, Karanth, & Rao, 1992).

9. TOXICITY AND SAFETY

Except for few reports on contact dermatitis, no serious adverse effect of *C. asiatica* has been recognized (Izu, Aguirre, Gil, & Diaz-Pirez, 1992). Safety of consumption of dried plant has been proved in toxicity testing in which the median lethal dose of dried powder of *C. asiatica*, given orally into mice, was found to be higher than 8 g/kg (Chivapat, Chavalittumrongand, & Tantisira, 2011). In chronic toxicity study, Wistar rats of both sex receiving 20, 200, 600, and 1200 mg/kg/day of *C. asiatica* for 6 months showed no sign of significant alteration of body weight, blood chemistry, clinical chemistry, or histopathology in comparison to control group (Chivapat et al., 2004). In contrast, hepatic damage was reported in albino rats receiving oral administration of dried *C. asiatica* at the dose of 1000 mg/kg/day for 30 days (Oruganti, Roy, Kumar Singh, Prasad, & Kumar, 2010).



10. GAPS IN THE KNOWLEDGE AND FUTURE DIRECTIONS FOR RESEARCH

Several studies that have been carried out with *C. asiatica* provided detail information on many important compounds. Most of these studies have been carried out using various methodologies and nonstandard protocols for determination of the compounds. However, many classical techniques used in the past are now known to be less accurate. Also, some techniques are less precise and the authentication of the data is sometimes questionable. Liquid chromatography coupled mass-spectrophotometry (LC-MS), gas chromatography coupled mass-spectrophotometry (GC-MS) could provide much more accurate and precise analytical results for composition of the compounds present in *C. asiatica*.

It would be important to analyze compounds in *C. asiatica* grown under different climatic conditions, altitude, and soil conditions to determine the optimum growth conditions that provide maximum yields. Processing and

cooking methods are very important in retaining the nutritional compounds as these data are only available for carotenes (Chandrika et al., 2010, 2006). The effects of processing and cooking have not been carried out for many other important compounds present in *C. asiatica*.

It is possible that many varieties of *C. asiatica* are present in different countries; thus, variety-based studies on *C. asiatica* would be a promising effort in the future researches. Chandrika et al. (2011) have carried out studies on carotene and mineral contents of some commonly available Sri Lankan *C. asiatica* varieties/morphotypes.

11. CONCLUSION

According to many studies, it has been proven that C. asiatica possesses very valuable nutritional compounds when consumed with dietary food. Various studies has been done in the recent past has shown different functional properties of C. asiatica including antibacterial activity, antioxidant activity, antiulcer activity, antidiabetic activity, anti-inflammatory activity, cytotoxic activity, cardio, neuro, and skin protective activities, radioprotective activity, immunomodulatory effect, memory-enhancing activity, and wound healing effect (Jamil et al., 2007). Chemical investigations on C. asiatica have shown that it has diverse and complex chemical constituents. Overall, the chemical constituents of C. asiatica are well studied. Even though there were variations in methodology between the studies, the constituents examined, and the plant material used, triterpenes and carotenoids were the major constituents consistently identified. According to some researches, C. asiatica may lose some of its beneficial compounds during processing and we need to educate the public regarding the correct way of processing.

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