DEVELOPMENT AND QUALITY ASSESSMENT OF FERMENTED PEGAGA (CENTELLA ASIATICA) TEA

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UNIVERSITI SAINS MALAYSIA

2011

DEVELOPMENT AND QUALITY ASSESSMENT OF FERMENTED PEGAGA (CENTELLA ASIATICA) TEA

by

CHEW SHIO HEONG

Thesis submitted in fulfillment of the requirements for the degree of Master of Science

July, 2011

Dedicated to

Chen Shiao Yen

my parents

and

my brothers

With gratitude

ACKNOWLEDGEMENTS

I would like to express my gratitude and appreciation to my supervisor, Dr Fazilah Ariffin and my co-supervisor, Dr Nurul Huda and Prof Abdul Karim Alias for their supervision, guidance and encouragements throughout the whole research and writing of the thesis. With their value comments and recommendations, I learned a lot and managed to complete my research project successfully.

A special word of thank to the laboratory assistants from Food Technology Division for their help during the research. Moreover, I would like to express my appreciate to my friends, Tharindu, Aronal, Boni Ikhlas, Herpandi, Ummi, Tina, Nopi and those who had contributed for this research project either directly or indirectly. Special thank to Dr Aamir Bath for his contribution to the FTIR characterization.

Special thanks to Miss Chen Shiao Yen for her patient and support during my study. Acknowledgement goes to my parent for a continual encouragement and everlasting support.

Last but not least, special thanks to USM for providing me the opportunity and the fellowship which has made this research possible.

Chew Shio Heong

July, 2011

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LIST OF ABBREVIATIONS

Abbreaviation	Caption
*a**	Redness or greenness
*b**	Blueness or yellowness
C. asiatica	*Centella asiatica*
C. sinensis	*Camellia sinensis*
CAFF	Full fermented *Centella asiatica*
CANF	Non-fermented *Centella asiatica*
CAPF	Partially-fermented *Centella asiatica*
CE	Catechine equivalents
FAAS	Flame atomic absorbance spectrometry
FRAP	Ferric-reducing antioxidant potential
FTIP	Fourier Transform Infrared spectroscopy
DPPH	1,1-diphenyl-2-picrylhydrazyl
GAE	Gallic acid equivalent
HPLC	High performance liquid chromatography
*L**	Lightness
LC-MS	Liquid chromatography-mass spectrum
LOD	Limit of detection
ME	Mercaptoethanol
OPA	o-phatalaldehyde
RI	Refractive Index
T%	Percentage transmittance
TAC	Total proanthocyanidin content
TEAC	Trolox equivalent antioxidant capacity
TFC	Total flavonoid content
THF	Tetrahydrofurane
TLC	Thin liquid chromatography
TPC	Total phenolic content
TPTZ	2,4,6-tripyridyl-s-triazine
UV	Ultra violet

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PEMBANGUNAN DAN PENILAIAN KUALITI TEH PEGAGA (CENTELLA ASIATICA) YANG TERFERMENTASI

ABSTRAK

Centella asiatica merupakan herba yang terkenal dalam perubatan Ayurvedic dan mempunyai sejarah kegunaan yang panjang di seluruh dunia. Herba tersebut kaya dengan mikro dan makro nutrien yang menyumbangkan pelbagai sifat fitokimia. Dalam kajian ini, *C. asiatica* telah melalui beberapa peringkat fermentasi; tanpa fermentasi (0 jam, CANF), fermentasi separa (2 jam, CAPF) dan fermentasi penuh (24 jam, CAFF). Kajian menunjukkan bahawa teh *C. asiatica* harus disedia pada suhu 100 ℃ selama 10 minit untuk mendapatkan kandungan antioksidan yang maksimum. Daun teh *C. asiatica* digalakkan berulang guna selama 2-3 kali kerana masih terdapat jumlah antioksidan yang tinggi. Secara keseluruhan, proses fermentasi tidak mengubah nilai proximat CAPF tetapi merosot dalam CAFF. Kafein tidak dikesan dalam semua jenis teh *C. asiatica* yang dihasilkan. Kandungan tiamin, riboflavin, niasin dan asid askorbik yang tinggi turut dijumpai dalam teh *C. asiatica*, tetapi biotin hanya terdapat dalam CAFF. Proses fermentasi telah mengurangkan kepekatan niasin dan asid askorbik. Kajian menunjukkan bahawa sifat antioksidan dalam CANF dan CAPF tidak terdapat perbezaan signifikan tetapi berkurangan secara signifikan dalam CAFF. Kandungan fenolik dalam *C. asiatica* terdiri daripada asid gallik, naringin, asid klorogenat, catechin, rutin, asid rosmarinik, kuersetin, luteolin dan kaempferol. Dalam kajian penentuan kandungan glikosida triterpena, kandungan asiatikosida telah menurun secara signifikan selepas mengalami proses fermentasi. Kandungan medikasosida, asid medikasik, dan asid asiatik tidak terdapat perbezaan signifikan antara CANF dan CAPF, tetapi menurun secara signifikan

dalam CAFF. Isoleusin, asid aspartat, dan treonin merupakan asid amino utama yang terdapat dalam infusi *C. asiatica*. Secara umum, kandungan asid amino menunjukkan penurun selepas mengalami proses fermentasi, khususnya setelah fermentasi selama 24 jam. Fermentasi tidak mengubah kandungan mineral dalam teh *C. asiatica*, dan mempunyai kandungan kalsium, magnesium, dan natrium yang tinggi. Peningkatan kandungan gula yang jelas dikesan pada sampel-sampel CAPF. Profil kumpulan berfungsi FTIR untuk ketiga-tiga teh *C. asiatica* menunjukkan spektrum yang hampir sama menunjukkan tiada perubahan pada kumpulan berfungsi. CANF, CAPF dan CAFF masing-masing mengandungi 38, 27 dan 24 kompaun meruap yang dapat dikenalpasti. β-caryophyllene dan α-humulene adalah kompaun meruap yang utama dalam teh *C. asiatica*. Proses fermentasi ternyata menambah baik parameter warna (kecerahan, kehijauan dan kekuningan) teh *C. asiatica* tetapi meningkatkan kekeruhan di dalam infusi. Dalam penilaian sensori, CAPF memperoleh skor yang lebih baik berbanding dengan CANF. Namun, pemanjangan masa fermentasi tidak meningkatkan kualiti sensori. Secara keseluruhan, penurunan mikro dan makro nutrien dalam teh *C. asiatica* selama proses fermentasi adalah tidak dapat dielakkan. Namun, proses fermentasi telah meningkatkan nilai gizi tertentu seperti kandungan vitamin B, parameter warna dan qualiti sensori. CAPF didapati mempunyai gizi yang hampir sama dalam CANF tetapi mempunyai kandungan gula yang lebih banyak, infusi yang lebih gelap dan sifat sensori yang lebih baik berbanding dengan CANF. Pemanjangan masa fermentasi didapati bukan cara yang baik untuk menyediakan teh *C. asiatica* kerana mengurangkan nilai gizi dan sensori secara mendadak.

DEVELOPMENT AND QUALITY ASSESSMENT OF FERMENTED PEGAGA (CENTELLA ASIATICA) TEA

ABSTRACT

Centella asiatica is a famous herb in Ayurvedic medicine and has a long history of being consumed around the world. The herb was reported to enrich with micro and macronutrients that contribute to it numerous phytochemical properties. In this study, *C. asiatica* undergone various stages of fermentation as follow: no fermentation (0 hr, CANF), partial fermentation (2 hrs, CAPF) and full fermentation (24 hrs, CAFF). The present study suggested that *C. asiatica* teas should be prepared at 100 ℃ for 10 min to obtain the maximum antioxidant capacity. Reuse of *C. asiatica* tea leaves for 2-3 times is encouraged due to the relatively high amount of antioxidant which would still be present. In general, fermentation process did not affect the proximate results of CAPF, but decreased in CAFF. Caffeine was not detected in all kinds of *C. asiatica* teas produced. High thiamine, riboflavin, niacin and ascorbic acid contents were found in *C. asiatica* teas, but biotin was found only in CAFF. The fermentation process was found to reduce the concentration in niacin and ascorbic acid. Results demonstrated that antioxidant properties of CANF and CAPF was not significantly different but significantly reduced in CAFF. The phenolic compounds in *C. asiatica* teas consist of gallic acid, naringin, chlorogenic acid, catechin, rutin, rosmarinic acid, quercetin, luteolin and kaempferol. Triterpene glycosides, asiaticoside's content decreased significantly during the fermentation process. The contents of madecassoside, madecassic acid, and asiatic acid did not differ significantly between CANF and CAPF, but decreased significantly in CAFF. Isoleucine, aspartic acid, and threonine were the major amino acids present in *C.*

asiatica infusions. In general, amino acid content decreased during the fermentation process, especially after 24 h of fermentation time. Fermentation did not alter the mineral content in *C. asiatica* teas and were high in calcium, magnesium, and sodium. Apparent improvement in sugar content was found in CAPF after fermentation. The FTIR profiles for the three *C. asiatica* teas showed similar spectral patterns indicating no changes in the functional group. CANF, CAPF and CAFF contained 38, 27 and 24 volatile compounds that could be identified, respectively. β-caryophyllene and α-humulene were the major volatile compounds in *C. asiatica* teas. Fermentation process apparently improved color parameters (lightness, greenness and yellowness) in *C. asiatica* teas but increased the turbidity level in tea infusions. In sensory evaluation, CAPF obtained better score than CANF. However, longer fermentation time did not help to improve the sensory quality. From overall results, decreasing in micro- and macronutrient in *C. asiatica* teas during fermentation process is inevitable. However, fermentation process also helps to improve certain nutritional value such as vitamin B content, color parameter and sensory quality. CAPF had almost similar nutritional qualities as CANF, but had better sugar content, darker infusion and better sensory properties than CANF. Prolonged fermentation was found to be not a good practise to prepare *C. asiatica* tea as it greatly reduce the nutritional and sensory qualities.

CHAPTER 1

INTRODUCTION

1.1 Research background

In recent years, there has been an increased effort to identify foods and beverages with high antioxidant content and health-promoting properties. Herbs that were traditionally used in folk medicine have begun to attract consumer interest. One of the easiest ways to obtain the benefits from these plants is to prepare them as herbal tea. Herbal teas, or *tisanes*, have gained popularity throughout the world due to their antioxidant activity and fragrance, which are thought to have a calming effect on the mind (Aoshima, Hirata, & Ayabe, 2007).

Centella asiatica (L.) Urban, synonym *Hydrocotyle asiatica*, is locally known as "pegaga", and it belongs to the plant family Apiaceae (Umbelliferare). The herb is well known and has a number of names, such as "gotu kola", "Mandukaparni", "Brahmi", and Indian pennyworth. The herb is native to both tropical and subtropical countries, including China, India, South America, Madagascar, and Malaysia. *C. asiatica* is highly valued as a folk medicine. It is especially famous in Ayurvedic medicine for the treatment of leprosy, insanity, asthma, ulcers, eczema, skin tuberculosis, wounds, stomach aches, arthritis, varicose veins, and high blood pressure; it is also known as a memory enhancer (Hargono, Lastari, Astuti, & van den Bergh, 1999). In recent years, extensive studies of *C. asiatica* revealed that it has several therapeutic effects, including aiding in wound healing; antispasmodic and antipyretic properties; hypotensive and anti-stress activities; cosmetic effects; effects on tumors and stress-induced gastric ulcers; and depressing the central nervous system (Hargono et al., 1999).

C. asiatica contains a number of compounds that are useful to consumers. Triterpene glycosides (asiaticoside, madecassoside, asiatic acid, and madecassic acid) are the major active compounds in *C. asiatica*. The plant was also reported to have abundant phenolic compounds such as quercetin, catechin, epicatechin, rutin, luteolin, myricetin, kaempferol, naringin and naringenin (Bajpai, Pande, Tewari, & Prakash, 2005; Hussin et al., 2009; Mustafa, Hamid, Mohamed, & Bakar, 2010). The phenolic compounds present in the plant are believed to be responsible for its various pharmacological functions. Besides, *C. asiatica* is an important dietary source of micro- and macronutrients such as vitamins, amino acids, minerals, and sugars (Gupta, Jyothi Lakshmi, Manjunath, & Prakash, 2005; Hargono et al., 1999). Due to its promising nutritional value, *C. asiatica* is commonly used as porridge for feeding pre-school children in Sri Lanka to combat nutritional deficiencies (Cox, Rajasuriya, Soysa, Gladwin, & Ashworth, 1993). Previous studies also shown that *C. asiatica* may be a good source of antioxidant (Hussin et al., 2009).

C. asiatica for use in herbal tea is normally prepared by a simple drying technique. Infusions of *C. asiatica* by itself are light in colour, it is usually mixed with black tea (*Camellia sinensis*) to generate a darker colour. However, black tea has a high caffeine concentration, and caffeine withdrawal can lead to symptoms such as headache, fatigue, and muscle pain; on the other hand, a dose of more than 10 g (about 170 mg/kg body weight) can lead to death (Sinija & Mishra, 2009). In addition, caffeine contributes to bitterness and affects both the flavor and aroma of the tea infusion (Franca, Mendonça, & Oliveira, 2005). Thus, finding a way to improve the qualities of *C. asiatica* and eliminate the necessity of mixing it with caffeine-laden tea leaves would be very useful. Therefore, fermentation of *C. asiatica* leaves might be a feasible method, and the process is believed to retain the

nutritional value, at the same time enhance the quality of herbal tea in terms of colour, flavour, and taste (Du Toit & Joubert, 1998; Heck & De Mejia, 2007)

1.2 Research objective

The general objective is to develop fermented *C. asiatica* herbal teas using actual tea (*Camellia sinensis*) fermentation process. Therefore, this study aimed at achieving the following objectives:

i. To prepare *C. asiatica* herbal tea by using two stages of fermentation, namely partially fermentation and full fermentation, and no fermentation as a control.
ii. To study the optimum brewing condition for preparing *C. asiatica* infusion prepared from each fermentation stage in the function of time and temperature.
iii. To analyze the micro- and macronutrients, antioxidant properties, and functional group profile.
iv. To identify the volatile compounds on the *C. asiatica* herbal tea prepared from each fermentation stage using gas chromatography – mass spectrometry (GC-MS).
v. To evaluate sensory quality of *C. asiatica* herbal tea prepared from each fermentation stage.

1.3 Research outline

This study is focused on the effects of fermentation on the micro- and macronutrients, antioxidant properties and sensory quality of the *C. asiatica* herbal tea produced. Knowledge from tea (*Camellia sinensis*) fermentation process was applied to produce non-fermented, partially-fermented and fully-fermented *C. asiatica* herbal tea. TPC (total phenolic content) and FRAP (ferric-reducing antioxidant potential) of tea infusions were studied as a function of water temperature (60, 80 or 100 °C), the number of brewing time (1, 2 and 3) and the brewing time (1, 3, 5, 10, 15 or 20 min) to determine the optimum brewing condition. The best condition determined was used for further analysis.

Proximate and FTIR analysis were performed on dried tea leaves produced to study its proximate content and functional groups changed after fermentation. Total free amino acids, total free polysaccharides, water soluble vitamins, caffeine, antioxidant activities, triterpene glycoside, phenolic compounds, sugar, and minerals content were evaluated to observe the effects of *C. asiatica* upon fermentation. GC-MS was conducted to identify and observe the changes in volatile compounds on *C. asiatica* after fermentation. The tea infusions have been prepared to study its color parameters, turbidity level and acceptance by panelists.

CHAPTER 2

LITERATURE REVIEW

2.1 *Centella asiatica*

2.1.1 Botanical description

C. asiatica or 'pegaga' is a perennial, herbaceous creeper with kidney-shaped leaves (Figure 2.1). The leaves have 1.3 – 6.3 cm diameter, leaf stalks 2 – 5 cm long, peduncle about 6 mm and stem up to 2 m long (WHO, 1999). However, the form and shape of the *C. asiatica* plant can differ greatly depending on environmental conditions (James & Dubery, 2009). Flowers are in fascicled umbels with each umbel consisting of 3 – 4 white to purple or pink, sessile flowers.

Centella comprises approximately 50 species, native to both tropical and sub-tropical countries, including China, India, South America, Madagascar, and Malaysia (James & Dubery, 2009). The plant flourishes abundantly in shady, moist or marshy areas.

The taxonomic of *C. asiatica* is shown in Table 2.1.

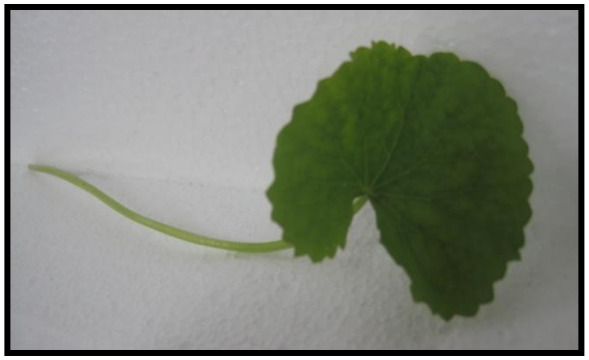


Figure 2.1: *Centella asiatica* plant

2.1.2 Synonyms

The plant has various synonyms such as *Centella biflora, C. coriacea, C. erecta, Hydrocotyle asiatica, H. biflora, H. eracta, H. lunata* and *Trisanthus cochinchinensis* (Hargono et al., 1999; WHO, 1999).

Table 2.1: Taxonomic hierarchy of *Centella asiatica*

Taxonomic Hierarchy	
Kingdom	Plantae
Sunkingdom	Tracheobionta
Divison	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Apiales
Family	Apiaceae/ Umbelliferae
Genus	Centella Linn
Species	*Centella asiatica* (Linn) Urban

Reference: USDA (n.d.)

2.1.3 Vernacular names

The plant is known by following vernacular names such as Ji xue cao, Chi hsueh tsao (Chinese); Asian pennywort, Indian pennywort, Marsh penny, Marsh pennywort (English); Gotu kola, Mandukparni, Mandookaparni, (Indian), Daun kaki kuda Pegaga and Pegagan (Malay) (Hargono et al., 1999; WHO, 1999).

2.1.4 Phytochemicals and chemical composition in *Centella asiatica*

Phytochemicals, or sometimes referred to as phytonutrients are any chemical or nutrient derived from a plant source which have a beneficial effect on health or an active role in the amelioration of disease (James, Abu, Wurochekke, & Oriji, 2007). There have findings suggested that the phytochemicals can replace some of the

commercial drug, which could bring to a reduction in toxicity and side effects of the later (Prabhakar & Doble, 2009).

C. asiatica is composed of various phytochemicals and nutrients beneficial to our health. Phytochemicals include terpenoids and phenolic compounds in plant not only playing important role in preventing cancer but also preventing various other diseases (Shibamoto, Kanazawa, Shahidi, & Ho, 2009). Nutrients such as sugars, lipids, and proteins, have been recognized to play an important role in preserving our health. Among the non-nutrients, vitamins, minerals and dietary fibers have been somewhat understood regarding their beneficial roles (Kanazawa, 2009).

2.1.4.1 Triterpene glycosides

Triterpene is derives from a basic molecular formulae of isoprene, with multiples of C_5H_8. Therefore, triterpene consist of six isoprene units and have the molecular formula $C_{30}H_{48}$. Glycosides are any group of organic compounds containing sugar (glycon) and nonsugar group (aglycon). In other definition, glycosides are also the accumulation of triterpene group of compounds includes sterols and triterpenes (James & Dubery, 2009). Triterpene glycosides are also known as saponins glycosides (Figure 2.2) in which saponins have an attached through an ester linkage (acyl glycoside) at C-28 (Figure 2.3) (Hostettmann & Marston, 1995). Saponins in common have the attachment of one or more monosaccharide chains to the aglycone. Glucose, arabinose, glucoronic acid and xylose are the monosaccharide most frequently attached directly to the aglycone (Hostettmann & Marston, 1995). On the other hand, saponins without glycone are also known as sapogenin. Sapogenin is classified according to their aglycone

skeleton. The first group consists of acid/non-steroidal saponins and the second group consists of the neutral/steroidal saponins.

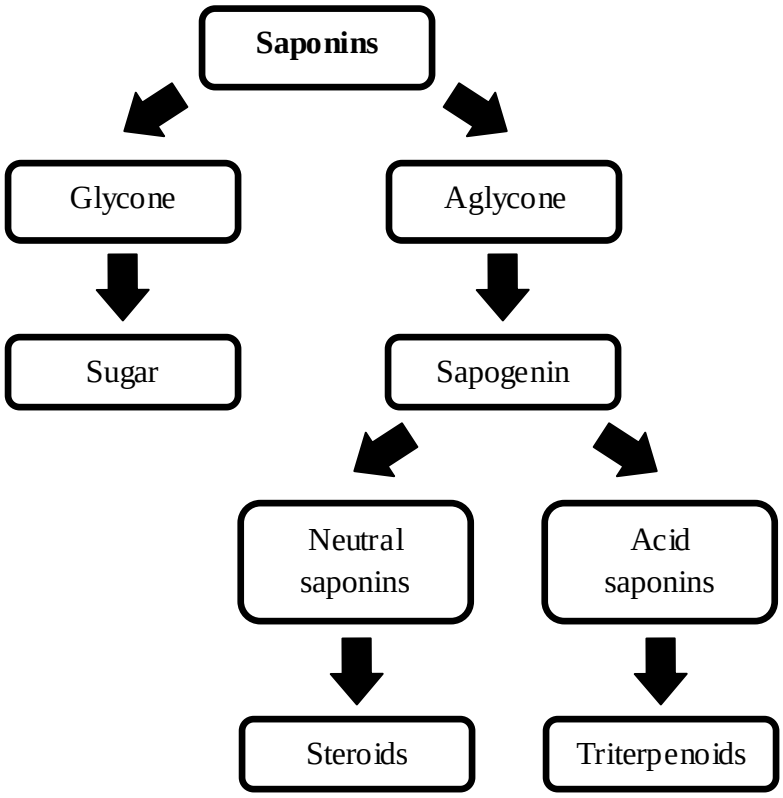


Figure 2.2: The group of saponin glycosides

C. asiatica accumulates large quantities of terpenoids, e.g. asiaticoside, centelloside, madecassoside, brahmoside, brahminoside, thankuniside, sceffoleoside, centellose, asiatic-, brahmic-, centellic- and madecassic acids (James & Dubery, 2009). The terpenoids are plant natural secondary metabolites that have an ecological role in regulating the interactions between plants and their environment (James & Dubery, 2009). However, the major active compounds in *C. asiatica* are asiaticoside, madecassoside, and their sapogenins; asiatic acid and madeccasic acid (Figure 2.3). The triterpene glycosides are biosynthetically constructed from α-amyrin unit (Hernandez-Vazquez et al., 2010). These compounds have been used for the standardization of this species as described in the European Pharmacopoeia, the

British Pharmacopoeia, the German Homeopathic Pharmacopoeia (GHO), and the Pharmacopoeia of the People's Republic of China and Indian Herbal Pharmacopoeia (Schaneberg, Mikell, Bedir, & Khan, 2003).

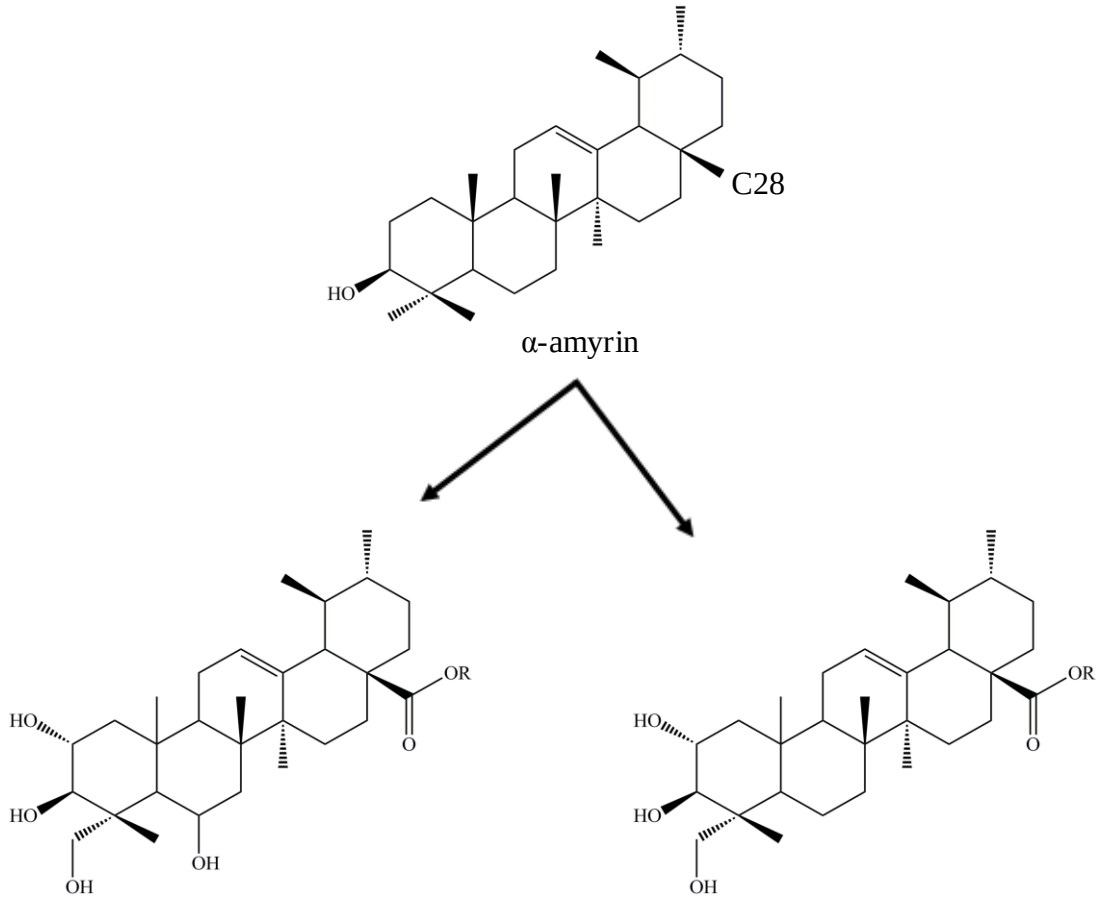


Figure 2.3: Structures of α-amyrin, the substrate for conversion, and the products asiaticoside, madecassoside, asiatic acid and madecassic acid found in *Centella asiatica* (Hernandez-Vazquez et al., 2010)

Depending on the origin of the plant material, these triterpene glycosides can account for 1 and 8% of the constituents (Brinkhaus, Linder, Schuppan D, & Hahn, 2000). Randriamampionona et al. (2009) and Randriamampionona et al. (2007)

reported that asiaticoside was the most abundant active compound in *C. asiatica* from Madagascar (up to 6.42% (dry basis) and 1.75%, respectively), followed by madecassoside (1.27% to 5.89%), madecassic acid (< limit of detection, LOD to 1.97%), and asiatic acid (< LOD to 1.89%). In China, Shen et al. (2009) reported *C. asiatica* contained highest amount of asiatic acid (10.2 to 14.6 mg/g), followed by asiaticoside (3.6 to 8.9 mg/g) and madecassoside (2.7 to 5.5 mg/g). In Nepal, the quantities of asiaticoside and asiatic acid from various altitudes ranged from 0.33 to 5.87% and from not detected to 0.66%, respectively (Devkota, Dall'Acqua, Comai, Innocenti, & Jha, 2010). Levels of madecassoside and madecassic acid were not measured in their work. Besides, asiaticoside and medecassoside were found to most abundant in leaves compared to the petiole and root (Aziz et al., 2007). The significant differences in triterpene glycoside content of *C. asiatica* originating from different countries is not surprising due to the fact that natural products are an unsurpassed source of bioactive compounds (James & Dubery, 2009). Aziz et al. (2007) also reported that differences between varieties in medicinal plants of the same species (chemotypes) are common and variation in secondary metabolites has been observed with identical phenotypes and growth conditions, depending on plant origin.

2.1.4.2 Health effects and triterpene glycosides

Triterpene glycosides from *C. asiatica* have been long recognized to their phytochemical properties and use as a health tonic. The medicinal uses of triterpene glycosides are shown in Table 2.2.

Table 2.2: Medicinal claims of *Centella asiatica*

Triterpene glycoside	Medicinal claim	References
Asiatic acid	- Dose: 25 mg/kg, reduced blood glucose levels significantly after streptozocin administration after 2 weeks; and increased serum insulin levels in mellitus diabetic rats - Dose: 30 ug/mL, control cell division in human hepatoma, colon cancer, breast cancer, melanoma cells and cytotoxic activity on fibroblast cells after 48 h - Dose: 20, 30, 40 50 ug/mL markedly inhibited cancer cell proliferation after 24 h. Apoptosis of SW480 human colon cancer cells was induced through increasing mitochondrial membrane permeability and cytochrome *c* release from mitochondria into cytosol, asiatic acid induced caspase-9 activity, which further activated caspase-3 and poly(ADP-ribose) polymerase cleavage resulting in irreversible apoptotic death in the tumor cells - Dose: 75 mg/kg reduced the infarct volume by 60% at day 1 and by 26% at day 7 postischemia and improved neurological outcome at 24 h postischemia, showed biological effects such as antioxidant, antiinflammatory, and protection against glutamate- or β-amyloid-induced neurotoxicity in a mouse model of permanent cerebral ischemia	Coldren et al., 2003; Krishnamurthy et al., 2009; Liu et al., 2010; Tang et al., 2009
Asiaticoside	- Dose: 0.2% solution, taking twice a day for 7 days produced 56% increase in hydroxyproline, 57% increase in tensile strength, increased collagen content and better epithelization in guinea pigs - Dose: 0.4% solution, increased hydroxyproline content, tensile strength, collagen content and epithelisation on punch wounds of streptozotocin diabetic rats	Shukla, Rasik, & Dhawan, 1999; Zhang et al., 2010

Table 2.2. Continued

	- Dose: 0.2%, topically apply twice daily for 7 days to excision-type cutaneous wounds in rats led to increase enzymatic and non-enzymatic antioxidants, namely superoxide	

	dismutase (35%), catalase (67%), glutathione peroxidase (49%), vitamin E (77%) and ascorbic acid (36%) in newly formed tissues. several fold decrease in lipid peroxide levels (69%) as measured in terms of thiobarbituric acid reactive substance - Dose: 20 mg/kg/d, showed hepatoprotective effects by decreased elevated aminotransferases, hepatocytes apoptosis and caspase-3, alleviation of mortality and improvement of liver pathological injury on lipopolysaccharide / D-galactosamine - induced liver injury	
Madecassoside	- Dose: 30 mg/kg, mice, showed anti-inflammatory effects by suppressing the clinical arthritis score and joints tissues pathological damage, reduced the proliferation of spleen cells, plasma levels of tumor necrosis factor alpha and interleukin-6, synovial tissues prostaglandin E_2 production and cyclooxygenase--2 protein expression in mice after 21 days - Dose: 40mg/kg, orally administered for 20 consecutive days alleviated infiltration of inflammatory cells and synovial hyperplasia as well as protected joint destruction, reduced the serum level of anti-CII IgG, suppressed the delayed type hypersensitivity against CII in ears, and moderately suppress CII-stimulated proliferation of lymphocytes from popliteal lymph nodes in CIA (collagen II (CII)-induced arthritis) mice	Li et al., 2009; Liu et al., 2008
Madecassic aicd	- Inhibited the LPS-induced expression of iNOS and COX-2 at the protein level and of iNOS, COX-2, tumor necrosis factor-α *(*TNF-α*)* interleukin-1β (IL-1β*)*, and IL-6 at the mRNA level in RAW 264.7 macrophage cells, suppressed the LPS-induced activation of nuclear factor-κB (NF-κB), abrogate inhibitory kappa B-α (IκB-α) degradation, and blocked p65 protein translocation to the nucleus. Madecassic acid more potently suppressed these inflammatory mediators than did madecassoside	Won et al., 2010

2.1.4.3 Phenolic compounds

Phenolics are compounds possessing one or more aromatic rings bearing one or more hydroxyl groups with over 8,000 structural variants and generally are categorized as phenolic acids and analogs, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, quinones, and others based on the number of phenolic rings and of the structural elements that link these rings (Table 2.3) (Fresco, Borges, Diniz, & Marques, 2006). These compounds play several important functions in plant products such as color, taste, and putative health promoting benefits (Boudet, 2007).

Many researchers have studied the phenolic compounds in the *C. asiatica* extract and results shown that *C. asiatica* is a promising source of phenolic compounds. In a recent study, Mustafa et al. (2010) showed that the methanolic extract of *C. asiatica* contained catechin, epicatechin, quercetin, myricetin, kaempferol and naringenin and that myricetin was found to be the most prominent compound. Hussin et al. (2009) reported a high content of naringin, quercetin, catechin, rutin and luteolin found in the methanolic extract of *C. asiatica*. At the same time, they also revealed that the catechin content in the extract was higher than that found in green tea. Bajpai et al. (2005) reported that a 50% methanolic extract of *C. asiatica* contained only quercetin and kaempferol, in which the quercetin content was the highest among the medicinal plants tested. Figure 2.4 shows the chemical structure of phenolic compounds reported to presence in *C. asiatica*.

The major phenolic compounds presence in *C. asiatica* observed from the previous studies are from flavonoids. Flavonoids are a group of more than 4,000 phenolic compounds that occur naturally in plants (Ren, Qiao, Wang, Zhu, & Zhang, 2003). These compounds commonly have the basic skeleton of phenylbenzopyrone

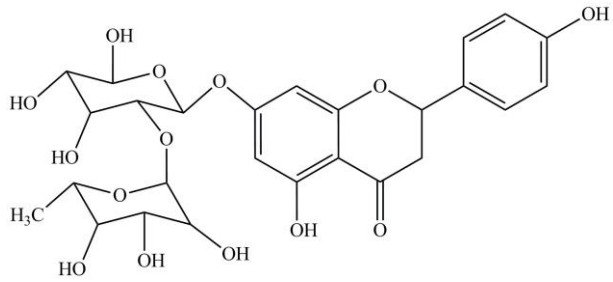
Table 2.3: The major classes of phenolic compounds in plants

Basic skeleton	Class	Examples
C_6	Simple phenols Benzoquinones	Catechol, hydroquinone, resorcinol
C_6-C_1	Phenolic acids	*p*-Hydroxybenzoic acid, Salicylic acid
C_6-C_2	Phenylacetic acids	*p*-Hydroxyphenylacetic acid
C_6-C_3	Cinnamic acids	Caffeic acid, Ferulic acid
	Phenylpropenes	Eugenol, myristicin
	Coumarins	Umbelliferone, aesculetin, scopolin
	Chromones	Eugenin
C_6-C_4	Naphthoquinones	Juglone
C_6-C_1-C_6	Xanthones	Mangostin, mangiferin
C_6-C_2-C_6	Stilbenes	Resveratrol
	Anthraquinones	Emodin
C_6-C_3-C_6	***Flavonoids***	
	Flavones	Sinensetin, nobiletin, tangeretin, isosinensitin
	Flavonols	Quercetin, kaempferol
	Flavonol glycosides	Rutin
	Flavanonols	Dihydroquercetin, dihydrokaempferol glycosides
	Flavanones	Hesperitin, naringenin
	Flavanone glycosides	Hesperidin, neohesperidin, narirutin, naringin, eriocitrin
	Anthocyanins	Cyanidin glycosides, cyanidin 3-glucoside
	Flavanols (catechins)	Catechin, epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate
	Chalcones	Arbutin, chalconaringenin, phloretin glucoside
	Isoflavones	Daidzein, genistein, glycitein, formononetin
$(C_6$-$C_3)_2$	Lignans	Podophyllotoxin, sesamin
$(C_6$-C_3-$C_6)_2$	Biflavonoids	Agathisflavone
C_6-C_7-C_6	Curcuminoids	Curcumin, tetrahydrocurcuminoid
$(C_6$-$C_3)_n$	Lignins	Pinoresinol
$(C_{15})_n$	Tannins	Casuarinin, chebulinic acid, gallotannin

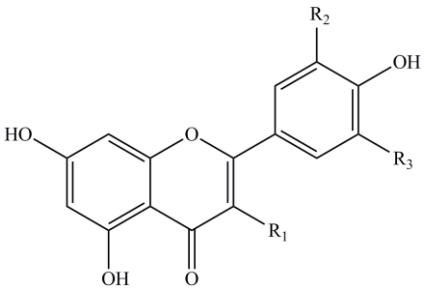
References:

1. Huang, Cai and Zhang (2010)
2. Robards, Prenzler, Tucker, Swatsitang and Glover (1999)
3. Velíšek, Davídek and Cejpek (2008)

structure (C_6-C_3-C_6) consisting of 2 aromatic rings (A and B rings) linked by 3 carbons that are usually in an oxygenated central pyran ring, or C ring (Cai, Luo, Sun, & Corke, 2004). Several classes of flavonoids are differentiated on the degree of unsaturation and degree of oxidation of the three carbon segment (Table 2.3).

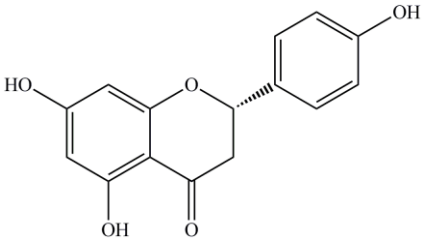


Naringin

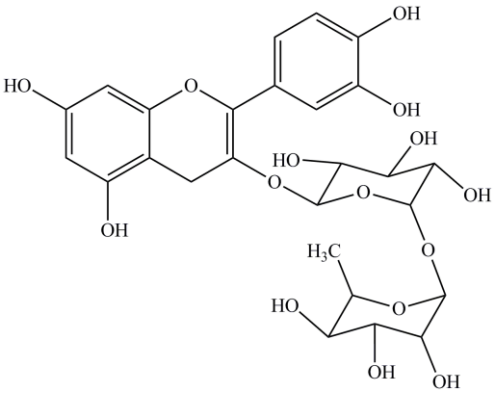


Flavones

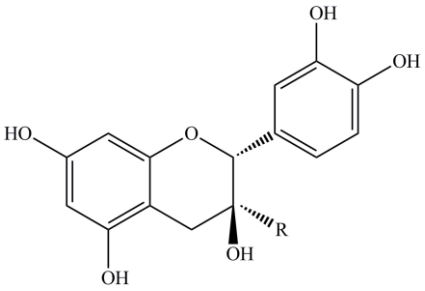
	R1	R2	R3
Kaempferol	OH	H	H
Luteolin	H	OH	H
Myricetin	OH	OH	OH
Quercetin	OH	OH	H



Naringenin



Rutin



	R
(+)-Catechin	H
(-)-Epicatechin	OH

Figure 2.4: Chemical structures of phenolic compounds present in *Centella asiatica* teas

2.1.4.4 Antioxidant activity and phenolic compounds

Generally, there are two categories of antioxidants, natural and synthetic. Recently, interest has increased in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity (Zheng & Wang, 2001). Plant materials may contain a wide variety of natural antioxidant compounds such as phenolic compounds (Table 2.3), nitrogen compounds (alkaloids, amines, betalains), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites, which are rich in antioxidant activity (Cai et al., 2004). However, phenolic compounds are the primary compounds that contribute to antioxidant activity (Parr & Bolwell, 2000).

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions which produce free radical reagents (Zheng & Wang, 2001). Numerous physiological and biochemical processes in the human body may produce free radical as byproducts. Overproduction of such free radicals can cause oxidative damage to biomolecules (for example lipids, proteins, DNA), eventually leading to many chronic diseases, such as atherosclerosis, cancer, cardiovascular disease, Alzheimer's disease, Parkinson's disease, diabetes, aging, and other degenerative diseases in humans (Cai et al., 2004; López, Martínez, Del Valle, Ferrit, & Luque, 2003). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role as reducing agent in adsorbing and neutralizing free radicals, quenching or scavenging reactive oxygen species (ROS), such as peroxyl radicals ($ROO^{\bullet}$), hydroxyl radicals ($HO^{\bullet}$), superoxide ion ($O_2^{-\bullet}$) and singlet oxygen ($^1O2$), and by complexing with pro-oxidant metal (Kähkönen et al., 1999; Zheng & Wang, 2001). Furthermore, epidemiological studies have shown that these

antioxidant compounds also possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral (Cai et al., 2004; Kähkönen et al., 1999).

Therefore, plant materials rich in phenolics compounds are increasingly of interest in the food industry. The importance of the antioxidant compounds of plant materials in the maintenance of health is also raising interest among scientists, food manufacturers, and consumers as the trend of the future is moving toward functional food with specific health effects (Kähkönen et al., 1999). Accordingly, research related to antioxidants represents nearly 70% of the studies focused on functional foods (Shibamoto et al., 2009).

2.1.4.5 Volatile compounds and fatty acids

C. asiatica is a weakly aromatic plant contains various volatiles and fatty acids. As discuss earlier, terpenoids are highly accumulated in *C. asiatica* is also the major component in essential oils. The essential oil of *C. asiatica* from Sounth Africa contains 11 monoterpenoid hydrocarbons (20.2%), 9 oxygenated monoterpenoids (5.46%), 14 sesquiterpenoid hydrocarbons (68.8%), 5 oxygenated sesquiterpenoids (3.9%) and 1 sulphide sesquiterpenoid (0.76%). Among the compounds, the predominant constitutes are α-hunulene (21.06%), β-caryophllene (19.08%), bicyclogermacrene (11.22%), germacrene B (6.29%) and myrcene (6.55%) (Oyedeji & Afolayan, 2005). On the other hand, 23 volatile compounds have been reported to found in Malaysia *C. asiatica* with dominant compounds are α-humulene (22%), γ-murolene (22%), β-cubebene (17%), α-caryophyllene (17%) and β-farnesene (4.8%) (Ali, 2008). Reports show that these essential oils have antibacterial and antifungal activity (Oyedeji & Afolayan, 2005; Ullah, Sultana, Haque, & Tasmin, 2009). The

major fatty acids in *C. asiatica* consist of palmitic, stearic, linoleic, linolenic and oleic acids (Khan & Abourashed, 2009; Khare, 2007).

2.1.4.6 Others constituents

The nutritional value of *Centella asiatica* is promising, as it is rich with various micro- and macro- elements (Table 2.4). As a result, the plant is commonly used as porridge for feeding pre-school children in Sri Lanka in order to combat nutritional deficiencies (Cox et al., 1993).

The proximate results show that *C. asiatica* from Malaysia contain high moisture content and moderate amount in protein, fat, carbohydrate and fiber. However, higher protein and fiber content was reported from India and higher fat content from South Africa (Table 2.4). Nevertheless, the plant was reported to contain high amount in calcium, phosphorus, iron, potassium and several vitamins.

Calcium is important in bone development and bone mass in humans, and its intake in adequate amounts help to prevent fractures and osteoporosis (Black, Williams, Jones, & Goulding, 2002). Magnesium is an essential element in many cellular reactions, involves in more than 300 enzymatic reactions such as glycogen breakdown, fat oxidation, protein synthesis, ATP synthesis, and the second messenger system, serves as a physiologic regulator of membrane stability and is involved in neuromuscular, cardiovascular, immune, and hormonal function (Ford & Mokdad, 2003). Phosphorus is another important element in bone health (Whiting, Boyle, Thompson, Mirwald, & Faulkner, 2002). Zinc is an essential mineral that plays an important functional role in a wide range of zinc-containing proteins, including a large number of zinc-dependent enzymes, needed for growth, normal development, DNA synthesis, immunity, neurosensory function and other important

cellular processes (Wood, 2000). Iron is crucial element in myoglobins, cytochromes, and iron-containing enzymes; plays roles in cellular processes such as the synthesis of DNA, RNA, and proteins, electron transport, cellular respiration, and regulation of gene expression; and iron homeostasis is critical for normal brain function, especially in learning and memory (Lieu, Heiskala, Peterson, & Yang, 2001).

There have two primary isomers of carotene, α-carotene and β-carotene which differ in the position of double bonds in the cyclic group at the end. However, β-carotene is the more common form found in plant. β-carotene is the main provitamin A carotene in food with conversion factor 12 μg β-carotene being equal to 1 μg retinol, and does not result in vitamin A toxicity even when ingested at high doses (Wang, Yin, Zhao, Russell, & Tang, 2004).Vitamin A is essential for vision, growth, reproduction, cellular differentiation and proliferation, and for the integrity of the immune system (Wang et al., 2004). Thiamine plays a key role in intracellular glucose metabolism which assist the conversion of carbohydrates into energy form as well as normal nervous system functioning (Bitsch, 2003). Riboflavin, act like thiamine in supporting the energy metabolism by converting carbohydrates, proteins and fats to energy into cells and is an essential element for healthy skins (Finglas, 2003). Niacin also involve in carbohydrates, proteins, and fats metabolism, behave as an important component for coenzyme Nicotinamide adenine dinucleotide (NAD) (Bender, 2003); and help to reduce the risk of arteriosclerosis (Finglas, 2003). Ascorbic acid is well known as natural antioxidant which act to against free radical damage and infection, enhance iron absorption, reduce symptoms of cold and flu, reduce risk of osteoporosis, against cardiovascular and eye diseases, and fight against

cancer by inhibiting the formation of carcinogenic *N*-nitroso compounds in the stomach (Ball, 2003; Finglas, 2003).

Table 2.4: Nutritional components in *Centella asiatica* from Malaysia, India and South Africa

Component in *Centella asiatica*	**Quantity (in 100 g)**		
	Malaysia[1]	**India[2]**	**South Africa[3]**
Moisture (g)	88.0	77.34	88
Protein (g)	2.0	4.76	3
Fat (g)	0.2	0.50	2.7
Carbohydrate (g)	6.7	4.24	3.81
Fiber (g)	1.6	8.11	1.92
Ash (g)	-	6.41	2.54
Calcium (mg)	171	425	2425
Magnesium (mg)	-	301	271
Phosphorus (mg)	32	59	327
Iron (mg)	5.6	19.56	18
Potassium (mg)	391	18	-
Carotene (mg)	2.7	-	-
Vitamin A (μg)	422	-	-
Vitamin B_1 (μg)	90	-	-
Vitamin B_2 (μg)	190	-	-
Niacin (μg)	100	-	-
Vitamin C (mg)	29	114	-

References:
[1] Saidin, 2000
[2] Baruah & Borah, 2009
[3] Odhav, Beekrum, Akula, & Baijnath, 2007

2.1.5 Traditional uses of *Centella asiatica*

C. asiatica has been widely used for long time in Asia region especially China and India. In China, the plant is used in traditional Chinese medicinal (TCM) as an

antipyretic, diuretic, and antidote in the treatment of icterus, heart stroke, diarrhea, ulcerations, eczema, and traumatic diseases (Tang & Eisenbrand, 1992). In Ayurvedic medicine from India, the plant is mainly used for central nervous system ailments including failing memory, insomnia, depression, stress and epilepsy (Ganachari, Babu, & Katare, 2004). In Sri Lanka, the plant is eat as vegetable and its extract is used in traditional medicine as a "galactagogue" (Hargono et al., 1999). The plant is also used to treat leprosy, wounds, cancer, fever and syphylli in South Africa and the extract has been used for many years to treat wounds among European (Oyedeji & Afolayan, 2005). Other folk medicine uses are for abscesses, blood purifier, headache, asthma, bronchitis, catarrh, convulsions, dysentery, hypertension, jaundice, pleuritis, rheumatism, spasms, tuberculosis, ulcers, urethritis, skin-related diseases, surgical lesions and cellulitis (Hargono et al., 1999; Hausen, 1993).

In Malaysia, the plant is boiled with green bean to treat dizziness; boil the leaves with onion to cure rheumatism; pound the leaves finely and apply to the forehead to treat typhoid; boil the roots to drink as tonic; and the roots is soaked for bathing by mothers after childbirth (Zakaria & Mohd, 1994). The plant is also commonly eaten fresh as salad or "ulam" among the Malay communities, which have beneficial effects in improving memory and in treating mental fatigue, anxiety, and eczema (Goh, Chuah, Mok, & Soepadmo, 1995).

2.1.6 Modern uses and studies of *Centella asiatica*

Due to its long history of being used in folk medicine for various kinds of treatments and diseases, the plant is become one of the most studied plant by scientist and researcher. According to a SciFinder Scholar search, not less than 1338 papers and patents were published and *C. asiatica* has continuously become the focus in

recent year (Figure 2.5). Although none of the claims listed in Table 2.5 have been evaluated by the Food and Drug Administration (FDA), positive investigations have been done which proven the medicinal effects of *C. asiatica* with modern medicinal theory.

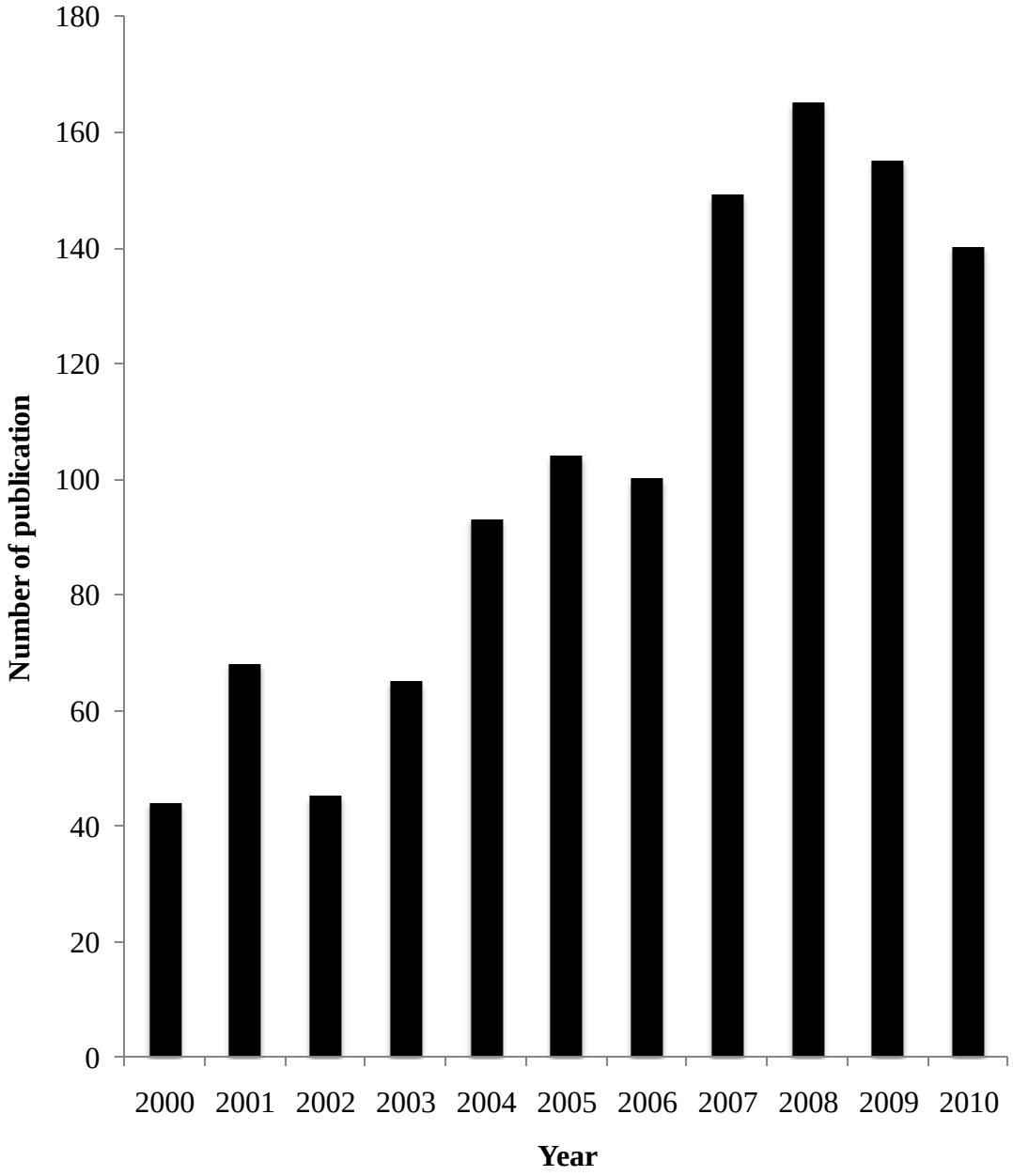


Figure 2.5: Number of research paper on *Centella asiatica* from 2000–2010. Data retrieved from SciFinder Scholar. Date of search: November 16, 2010.

Table 2.5: Medicinal effects of *Centella asiatica*

Medicinal effect	Experimental studies	References
Wound healing activity	- Ethanolic extract increased wound breaking strength, rate of wound contraction and epithelize faster in normal and dexamethasone-suppressed Wistar albino rats	Shukla et al., 1999
Antibacterial activity	- It's essential oil showed antibacterial activities against Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella sonnei*) organisms. Great to against Gram-positive bacterial - Crude extracts showed notable antibacterial and antifungal activity against sixteen microorganisms including 5 Gram-positive bacteria, 8 Gram-negative bacteria and 3 fungi	Oyedeji & Afolayan, 2005; Ullah et al., 2009
Anti-tumor activity	- Methanolic extract (100 µg/mL) showed 100% cytotoxicity to Dalton's and Ehrlich ascites tumour cells after 3 hour incubation at 37 ℃ - Aceton extraction fraction (3.5 & 8 µg/mL) inhibited the proliferation of mouse lung fibroblast cells after exposure 6-7 days at 37 ℃ - Crude and acetone extract reduced the development of murine solid tumors in mouse when administed simultaneously with tumor transplantations or given 10 days prior to tumor transplantation - Crude extract reduced ascites tumour growth and increased the life span of tumour bearing mice - 50 g/L aqueous extract showed promising activity against mouse melanoma ($B_{16}F_1$), human breast cancer (MDA MB-231) and rat glioma (C_6) cell lines, with IC_{50} values of 698.0, 648.0 and 1000.0 µg/mL, respectively	Babu, G., & J., 1995; Pittella, Dutra, Junior, Lopes, & Barbosa, 2009

Table 2.5. Continued

Neuropharmacology activity	- 100 mg/kg extract showed 50% protection while a higher dose (200 mg/kg) completely protected against pentylenetetrazol-induced convulsions in rats - 100-300 mg/kg aqueous extract prevent cognitive deficits in intracerebroventricular streptozotocin-induced cognitive impairment in rats after 14 days - 200 & 300 mg/kg aqueous extract reduced brain malodialdehyde levels and increased brain glutathione levels in rats after 21 days	Ganachari et al., 2004; Kumar & Guota, 2003; Russell, Michalek, Flechas, & Abraham, 1995
Antioxidant activity	- 1.3-40 mg/mL extract reduced the formation of lipid peroxidation product in brain homogenates from mice - Extract (0.3%) and powder (5%) reduced oxidative stress when given to H_2O_2-exposed rats for 25 weeks	Ganachari et al., 2004; Ullah et al., 2009; Wolfe, 1993
Anti-gastric ulcer activity	- Water extract reduced the size of acetic acid-induced gastric ulcers in rats at 7 days - Aqueous extract (0.005, 0.25 & 0.50 g/kg) inhibited ethanol-induced gastric lesions - 100, 200 and 400 mg/kg extract exhibited gastric mucosal protection, reduction or absence of edema and leucocytes infiltration of submucosal layer in rat	Abdull, AL-Bayaty, Younis, & Hassan, 2010; Cheng, Guo, Luk, & W., 2004; Cheng & Koo, 2000
Improve cognitive function	- Aqueous extract of whole plant (200 and 300 mg/kg) showed an improvement in learning and memory in both shuttle box, step through and step down paradigms in rats after 14 days. - Consume high dose (750 mg) of the plant extract once daily for 2 months enhanced working memory and increased N100 component amplitude of event-related potential in human trial.	Veerendra Kumar & Gupta, 2002; Wattanathorn et al., 2008

2.1.7 Products of *Centella asiatica*

Although *C. asiatica* is well known as medicinal plant, the plant is widely eaten as vegetable for many countries such as Malaysia, Indonesia, India, Sri Lanka and Madagascar. Therefore, the plant has been commercially cultivated in Malaysia (Figure 2.6) due to the fact that Malaysia has a tropical climate with high temperatures and rainfall all year, allowing the plant to flourish extensively throughout the year. *C. asiatica* can be harvested between 80-90 days and the yield can achieve as high as 25.6 t/ha. (MARDI, 2009). Nowadays, the plant not only can be found in local wet market, but also in hypermarket. In Madagascar, the plant has been massively collected by native people for local use and for export to fulfill the increasing demand of pharmaceutical and cosmetic industries (Randriamampionona et al., 2007). According to the report from the Export and Import Bank of India, *C. asiatica* is one of the important medicinal plants in the international market of medicinal plant trade (Paramageetham, Prasad Babu, & Rao, 2004).

Figure 2.6: *Centella asiatica* farm in Malaysia