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MiniReview
Centella asiatica in food and beverage applications and its potential antioxidant and neuroprotective effect

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Abstract: *Centella asiatica* L. is traditionally used as a medicinal herbs and alternative medicine in treating numerous kinds of diseases. The use of *Centella* in food and beverages has increased over the years. Its potential antioxidant and neuroprotective activity has been widely claimed in many reports and basically is very much related to its properties and mechanism of action of the plant's bioactive constituents namely the asiaticoside, asiatic acid, madecassoside and madecassic acid. As such, this review will cover the biological activity of the plant's active constituents in relation to its food and beverage applications. The plant cultivation and biotechnological approaches to improve the production of desired bioactive constituents by cultured cells will also be reviewed. In addition, the range of chemical compositions found in *Centella* and safety aspects are also included.

Keywords: *Centella asiatica*, food, beverage, antioxidant, neuroprotection

Introduction

Centella asiatica L. is important herbal medicinal plant used for various applications (James and Dubery, 2009) and used in Indian Ayurvedic medicine as a nerve tonic (Singh *et al*., 2008). Utilization of *Centella asiatica (Centella)* have been known for many years in treating all kind of diseases such as gastrointestinal disease, gastric ulcer, asthma, wound healing and eczema (Brinkhaus *et al*., 2000). The use of *Centella* in food and beverages has increased over the years basically due to its health benefits such as antioxidant (Hamid *et al*., 2002; Vimala *et al*., 2003; Ullah *et al*., 2009; Pitella *et al*., 2009), as anti-inflammatory (Duke, 2001), wound healing (Kimura *et al*., 2008) memory enhancing property (Subathra *et al*., 2005; Singh *et al*., 2008) and many others. The potential of *Centella* as an alternative natural antioxidant especially of plant origin and its protection against age-related changes in brain antioxidant defense system, have notably increased in recent years (Subathra *et al*., 2005). Free radicals have been claimed to play an important role in ageing process and capable of damaging many cellular components (Gulcin *et al*., 2003). These changes will affect the brain as it is particularly vulnerable to oxidative damage; as such many studies on its neuroprotection activity have been reported.

The *Centella asiatica* L. belongs to the family Apiaceae or Umbelliferae, a small creeping perennial herbal plant that flourishes in wet areas of Malaysia, Indonesia, India, and other parts of Asia including China. The herb is also known as pegaga in Malaysia, Indian pennywort and Gotu Kola in Europe and America, mandookaparni in India, pegagan or kaki kuda in Indonesia, Luei Gong Gen or Tung Chain in China (Tolkah, 1999). There are several types of *Centella asiatica* that can be found in Malaysia such as Pegaga Cina or Nyonya, Pegaga Daun Lebar, Pegaga Salad and Pegaga Renek (Anonymous, 2011a; Anonymous, 2011b).

Centella asiatica is used in Indian Ayurvedic medicine and in herbal medicine in Malaysia and China, and other part of Asia for hundreds of years (Brinkhaus *et al*., 2000). Besides being used as a traditional and alternative medicine, *Centella* is commonly used in these countries as vegetables and drinks as in tea or juice (James and Dubery, 2009).

Over the years, three reviews have reported the pharmacological studies on Centella (Jamil *et al*., 2007; Zheng and Qin, 2007; Pittella *et al*., 2009). However, there is still lack of information covers on antioxidant and neuroprotective effect of *Centella*. Thus, this review will present the advances made in the use of *Centella* plant in food and beverage applications as well as its potential as antioxidant and neuroprotection agents. In addition, the cultivation of the plant, its chemical composition and safety are also discussed.

Cultivation

Even though, its primary application has been in promoting wound healing, today it has become an important commercial plant due to many health beneficial effects (James and Dubery, 2009). The *Centella* plants are generally collected from the wild as well as in small scale cultivation. The plant is found in throughout tropical and subtropical regions,

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grown very well in areas with high moisture and humus rich soil as well as in sandy and clayey soils (Jamil *et al*., 2007). It grows in the lowland up to an altitude of 600 m. In Malaysia, it can easily grow naturally as weeds near streams, ponds, paddy fields, coconut and oil palm plantations, and other places throughout the country. With the increasing demand of *Centella* as herbal and medicinal plant, there have been an increased in interests to cultivate the plant in a large scale (Mohd Ilham, 1998). The plant is also consumed as a vegetable in Malaysia, China, Sri Lanka, India and Indonesia. In recent times, it has been grown as a cultivated vegetable in many developed countries. Cultivation of *Centella* is easy by way of vegetative propagation using the stolon, seed and roots, ready for harvesting in 3-6 months. In Malaysia, the plantation of *Centella* is carried out in the open farming or under a shade as well as by integrating with other commercial crops such as rubber, oil palm and coconut (Mohd Ilham, 1998). This commercial crop provides favorable condition to *Centella* growth as it protects the plant from direct sunlight, retain moisture and maintain artificial forest environment. For medicinal purpose and further processing, only the leaves and the aerial part of the plants are used (Brinkhaus *et al*., 2000; Zainol *et al*., 2008). In India, as an effort to conserve and cultivate the plant in scale applications, the government has taken some initiatives to select 60 accessions of the potential elite genotypes/chemotype of *Centella* containing high madecassoside and asiaticoside and grown in a Field Gene Bank (Thomas *et al*., 2010).

Due to the increased demand, source of *Centella* from natural habitat and propagation of *Centella* using the seed is no longer an economically viable proposition. Large-scale propagation by micropropagation tissue culture approach is the choice to meet the demand of the industry without sacrificing the efficacy and quality of the plants. In micropropagation technique using somatic embryogenesis, the callus derived from leaves (petiole and lamina) and internode explants demonstrated similar morphological characteristic as the parent plant (Nath and Buragohain, 2005). Whereas James *et al*. (2008) found the leaf tissues have higher triterpenoids content than the callus and cell suspension. By using precursor, elicitor and plant hormones, the plant can be manipulated to get the desired compounds. In the precursor–feeding studies, squalene at 0.16 mg/L increased madecassoside, asiaticoside and madecassic acid in callus culture, while squalene at 0.8 mg/L triggered higher production of asiatic acid and madecassic (Ling, 2004). The supplementation of amino acid and succinic acid at 3 and 4 mg/L also demonstrated the increase of triterpenes production in callus and cell suspensions, respectively. In another study, methyl jasmonate (MJ) as an elicitor found to enhance the production of asiaticoside in the hairy root culture (Kim *et al*., 2007). While using farnesyl diphosphate synthase (FPS) and MJ treatment, the *Centella* hairy root has successfully increased phytosterol regulation, and synthesis of asiaticoside and madecassoside (Kim *et al*., 2010). The addition of chitosan elicitation to the plant tissue culture further stimulated the production of secondary metabolites in *Centella* especially the flavonoids which possess strong antioxidative activity (Ong, 2008). This *in vitro* approach enables to increase the flavonoids content in *Centella* promising to provide as a continuous important nutraceutical supply of the active compounds.

Chemical composition

The chemical composition of *Centella* plant has a very important role in medicinal and nutraceutical applications and it is believed due to its biologically active components of triterpenes saponins (Loiseau and Mercer, 2000). The triterpenes of *Centella* are composed of many compounds including asiatic acid, madecassic acid, asiaticosside, madecassoside, brahmoside, brahmic acid, brahminoside, thankiniside, isothankunisode, centelloside,madasiatic acid, centic acid, and cenellicacid (Zheng and Qin, 2007). Among these triterpenes, the most important biologically active compounds are the asiatic acid, madecassic acid, asiaticosside, madecassoside (Inamdar *et al*., 1996). Due to their importance, they have been used as the biomarker components for quality assessment of *Centella* (Zheng and Qin, 2007). However, the content of *Centella*'s triterpene components can be affected by the location and diverse environmental conditions (James and Dubery, 2009). In Malaysia, there are 15 different accessions of *Centella* found and only 3 triterpenes (asiaticoside, madecassoside and asiatic acid) were identified (Table 1) with the leaves contain the highest amount of active components (Zainol *et al*., 2008). In addition to its bioactive components, it also contains high total phenolic contents which contributed by the flavonoids such as quercetin, kaempherol, catechin, rutin, apigenin and naringin (Zainol *et al*., 2004; Suntornsuk and Anurukvorakun , 2005) and volatile oils such as caryophyllene, farnesol and elemene (Qin *et al*., 1998).

Nutrient composition of *Centella* from Malaysia has been tabled by Tee *et al*. (1997). Total calories in 100 g of *Centella* are 37.0 Kcal. In general, *Centella* contained high concentration of potassium (391 mg) and calcium (171 mg), low in protein (2.0%),

Table 1. Average concentration of asiaticoside, madecassoside and asiatic acid in *Centella asiatica* obtained from Skudai and Pontian, Johor, Malaysia

Sample		Average concentration (µg/ml)		
		asiaticoside	madecassoside	asiatic acid
First accession (Skudai)	Leaves	1.14	0.71	0.00
	Petioles	0.17	0.15	0.00
First accession (Pontian)	Leaves	0.39	3.29	0.00
	Petioles	0.00	3.66	571.20
Second accession (Skudai)	Leaves	ND	ND	ND
	Petioles	ND	ND	ND
	Roots	ND	ND	ND
Second accession (Pontian)	Leaves	2.56	5.30	1142.67
	Petioles	0.49	0.00	2390.00
	Roots	0.00	1.57	3421.60

*ND = Not detected
(Source: Zainol *et al*., 2008)

carbohydrate (6.7%) and fat (0.2%). It also contains 87.7% moisture, 1.6% crude fiber, 1.8% ash, 32.0 mg/100 g phosphorus, 5.6 mg/100 g iron and 21 mg/100 g sodium. *Centella* 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 µg/100 g) and vitamin A (442 µg/100 g).

Food and Beverage

In Malaysia and Indonesia, *Centella* is commonly eaten fresh as vegetable (ulam and salad) especially among the locals Malay and Javanese populations (Huda-Faujan *et al*., 2007). The salads are eaten together with the main meal and can act as an appetizer. Beside eaten raw, it can be cooked as a part of a soup or as a main vegetable. Due to its mild bitterness it is always cook and served with the addition of coconut milk and/or shredded coconut and sometimes sweet potatoes and potatoes are added. Since the *Centella* is very popular as a vegetable, it is available everywhere in the wet markets and supermarkets in Malaysia. As a vegetable and therapeutic use, the whole plant including leaves, stem and root are consumed (Brinkhaus *et al*., 2000). It is used as health tonic and processed into cordial drinks and ready to drink juice (Mohd Ilham, 1998). The fresh plants are also blended to make drink and juice.

In Thailand, it is used as vegetable and tonic, drunk as a tea and juice (Punturee *et al*., 2004). The fresh prepared juice is popular and available in the restaurant and sold by many sellers by the road side. It is so common used as a drink by the Thai and Chinese people for thirst quenching purpose or cooling drink as well as reducing the "inner heat" to assist in healing and curing of aphthous ulcers (Wangrangsimakul, 1999). Aphthous ulceration is a condition affecting the mucous membrane of the mouth that causes pain and suffering to the sufferer. In Sri Lanka, the *Centella* leaves are used in their traditional curry called "mallung", and in their porridge known as "kola kenda". In India, it is one of the constituents of the Indian summer drinks "thandaayyee" and very important for brain tonic (Anonymous, 2011c).

Centella is also commonly used for making herbal tea whereby the infusion is made by pouring a cup of boil water over the *Centella* materials either using dried *Centella* or fresh materials, letting it brew a few minutes before drinking. The *Centella* herbal tea can be prepared either using a mixture of many different herbal plants or a single plant. It is believed the *Centella* herbal tea is considered as source of antioxidant activities and it has many beneficial effects (Naithani *et al*., 2006; Huda-Faujan *et al*., 2007).

It is a common practice to process *Centella* into many products. These *Centella* based products are available in many forms such as powder, infusion, soluble and extract of fresh and dried plants. One of the processed products is *Centella* herbal drink, whereby the demand for this product is on the rise due to its health benefits and the phytochemicals presence (Kormin, 2005). The study of heat treatment on herbal *Centella* drinks found that it still retain appreciable amount of madecassoside, madecassic acid, asiaticoside, asiatic acid and polyphenol compounds. Whereas the amount in untreated drink was 11- 17% higher than those in heat-treated *Centella* drinks. These phytochemicals are good sources of antioxidant. (Kormin, 2005). Another study reported that the fresh juice is better than the processed juice as it contains higher total concentration of volatile compounds and more volatile components (Wongfhun *et al*., 2009). A research conducted on different processing treatments of *Centella* juice namely high pressure processing (HPP), pasteurization and sterilization found a reduction in the total volatile concentration, except the HPP retained more volatile acyclic alcohols, aldehydes and oxygenated monoterpenoids (Apichartsrangkoon *et al*., 2010). Hence, HPP treatment gave better results than the sterilization and pasteurization process of the *Centella* juice.

In the food product development, using *Centella* as food ingredients is quite limited except in the new product development of herbal noodle from *Centella* extract (Zainol, 2004). By using response surface methodology (RSM) model, the formulation of the *Centella* herbal noodles was developed. The best formulation for the noodle was found to be 10% *Centella* extract, 5 g of salt and 5 g of sodium hydroxide. This formulation provides the best sensory acceptance and highest presence of flavonoids (cathechin, quercetin and rutin). The introduction of this product will enhance the multi-functional properties of *Centella* in food applications and an alternative consumption of natural products such as in cakes, ice-creams, deserts and puddings.

The essentials oil of *Centella* could provide an alternative source of synthetic antioxidant BHA. Comparison of antioxidant activity of the essential oil of *Centella* and butylated hydroxyanisole (BHA) in sunflower has shown strong antioxidant activity in lipid containing foods (Raza *et al.*, 2009). The antioxidant activity of the essential oil could be due to the terpenes and phenolic compounds (Oyedeji and Adayan, 2005).

Antioxidant capacity

Antioxidant is used by aerobic organism to protect the cells from oxidative damage by oxidants during oxygen metabolism. The main antioxidant agents such as superoxidase dismutase (SOD), catalase, glutathione peroxidase (GSH-Px), glutathione, ascorbic acid and tocopherol are important to protect the cells due to their ability in eliminating free radicals such as reactive oxygen species (ROS) (Young and Woodside, 2001). ROS or cellular damage is the main factors in cellular injury and aging process (Gulcin *et al*., 2003). ROS is responsible in the induction of some oxidative damage to lipids, nucleic acid, proteins and carbohydrates in which their damage cause aging, cancer and many other diseases (Aruoma, 1994). Recently, there is an increasing interest in the biochemical functions of natural antioxidants extracts from vegetables, fruits, flowers and medicinal plants such as *Centella* which can be an excellent candidate to prevent oxidative damage, hence promoting health benefits (Pitella *et al*., 2009). Hashim *et al*. (2011) reported that antioxidant in *Centella* (84%) is comparable to Vitamin C (88%) and grape seed extract (83%).

Studies on the extract of *Centella* reported to possess antioxidant properties. Recent studies reported that the optimum extraction condition is 40% ethanol for 60 min at 65°C whereas optimal solid-to-solvent ratio is 1:15 (Chew *et al*., 2011; Tan *et al*., 2011). The consumption of *Centella* is useful for the antioxidant effect as it offer an effective and safe way of increasing body immune system against free radicals (Rajadurai and Prince, 2006) and at the same time keep the oxidative stress in a state of balance (Kormin, 2005). Among the triterpenes isolated from *Centella* plant, asiaticoside is the most abundance and responsible to stimulate antioxidant activity in the early phase of the wound healing process (Shukla *et al*., 1999). *In vitro* studies, *Centella* leaves are reported to have high antioxidant in 3 pathways: superoxide free radical activity (86.4%), inhibition of linoleic acid peroxidation (98.2%) and radical scavenging activity, DPPH (92.7%) (Vimala *et al*., 2003). *In vitro* studies using linoleic acid model, ethanolic extract of *Centella* showed significantly higher antioxidant than the water and negligible amount in petroleum extract, whereas the roots of the plant demonstrated higher antioxidant activity than that of the leaves and petioles. (Hamid *et al*., 2002). Beside the triterpenes, the antioxidant protection effect of *Centella* is contributed by its enriched flavonoids and selenium contents to stimulate cell rejuvenation, improve physical and mental health (Ponnusamy *et al*., 2008). The scavenging activity of *Centella* water extract was an IC_{50} value of 31.2 μg/mL, whereas ascorbic acid and butylated hydroxytoluene (BHT) activity were IC_{50} values of 2.5 and 7.6 μg/mL, respectively (Pitella *et al*., 2009). In this context, *Centella* water extract has the capability to scavenging free radicals due to its phenolics (2.9 g/100 g) and flavonoids (0.36 g/100 g). Antioxidant activity of the crude extract of *Centella* leaves was comparable to the activities of rosemary and sage (Jaswir *et al*., 2004).

In vivo studies on antioxidant activity using lymphoma-bearing mice by giving oral treatment with 50 mg/kg/day for 14 days revealed that the *Centella* methanolic extract significantly increased the antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase), and reduced antioxidants like glutathione and ascorbic acid (Jayashree *et al*., 2003). In the administration of *Centella* aqueous extract on arsenic-induced oxidative stress in rats, it provided beneficial effect against delta-aminolevulinic acid dehydrase, gluthathione, superoxide dismutase and thiobarbituric acid reactive substance (Flora and Gupta, 2007). Hussin *et al* (2009) demonstrated a decrease in oxidative stress in rats after 25 weeks consumption of *Centella* extract and powder. Rats receiving *Centella* extract, powder and α-tocopherol have lower MDA values indicating low lipid peroxidation and decrease in the activity of superoxide dismutase in these rats. The result indicates the protective effect of *Centella* plant in combating oxidative stress undergone by the rats. In addition, Ullah *et al*., (2009) found high antioxidant activity (free radical scavenging activity by DPPH) in *Centella* extract with the IC_{50} value of 4.0 μg/mL in chloroform and 7.0 μg/mL in aqueous methanol extracts, compared to BHT and ascorbic acid (26.0 and 5.0 µg/mL, respectively).

At present, no studies on human have shown any side effect on consumption of *Centella*. However, a study done by Bjelakovic *et al*. (2007), reported that antioxidant supplements may increase the risk of mortality.

Neuroprotection effect

Centella extract has been used in Ayurvedic medicine as a nerve tonic. The micronutrients in the extract is reported to be responsible in retarding brain aging and assist in renewal of neural tissue, hence it is effective in enhance memory and revitalize the brain as well as increase attention span and concentration (Singh *et al*., 2008). Some of these improvement and stimulation effects have been proven experimentally. Oral supplement of *Centella* extract had shown effective protection from cognitive deficiency and oxidative stress in rats which resulted in strong memory enhancement effect (Kumar and Gupta, 2002; Subathra *et al.*, 2005). Neuroprotective effect of *Centella* extract was proven to protect monosodium glutamate-induced neurodegeneration (Ramanathan *et al*., 2007). Neuroprotective efficacy on standardized water extract of *Centella* (5 mg/kg body weight) for 10 days against 3-nitropropionic acid-induced oxidative stress in brain of prepubertal mice enhanced glutathione levels, thiols and antioxidant defenses in brain regions (Shinomol and Muralidhara, 2008; Shinomol *et al*., 2010). Besides triterpenes, flavonoids and selenium in *Centella* could contributeto its potent antioxidant to stimulate cell rejuvenation, improve physical and mental health (Ponnusamy *et al*., 2008).

In the study with elderly volunteers, *Centella* extract had shown positive modulation of cognition function and improvement of self-rated mood after 2 months of taking the extract at 750 mg daily (Wattanathorn *et al*., 2008). It is effective in the management of mild cognitive impairment and other related age-related problems of elderly people such as improvement of diastolic blood pressure, peripheral neuritis, insomnia and loss of appetite (Tiwari *et al*., 2008). The efficacy evaluation on *Centella* supplement ranging from 3 - 4 g according to body weight daily for 2 months to healthy middle age and elderly adult volunteers suggested the plant extract diminished the age-related reduction in cognitive function (Dev *et al*., 2009). The *Centella* aqueous extract also demonstrated very promising results in protecting the brain against neurodegenerative disorders such as Parkinson (Haleagrahara and Ponnusamy, 2010). The induced Parkinsonism in aged rats shown a decreased of lipid hydroperoxide and protein-carbonyl-content, increased the total antioxidant, and antioxidant enzyme levels (superoxide dismutase, glutathione peroxidase and catalase) in corpus striatum and hippocampus.

In Ayurvedic medicine and traditional Chinese medicine, *Centella* has been used for centuries to control anxiety, helps in relaxation and mental calmness (Wijeweera *et al*., 2006). Studies in human and animal models have reported that *Centella* possesses anxiolytic activity potential. Investigations of the pharmacology effect of *Centella* in a double-blind, placebo-controlled study have revealed that the 12 g orally intake of *Centella* extract effectively increase the acoustic startle response (ASR) after 30 and 60 min suggesting the extract potent anxiolytic activity in healthy human subjects which lead to calmness effect (Bradwejn *et al*., 2000). In animal studies, the water extract of *Centella* containing 84% asiaticoside possess anxiolytic activity by inhibiting phospholipase (PLA.sub.2) activities in rat cerebellum (Wijeweera *et al*., 2006). This group of enzymes has abnormal activity in the central nervous system in some neuropsychiatric diseases and *Centella* water extract is an excellent candidate as a potential therapeutic agent for neuroinflammation and oxidative stress.

There has not been any study of the negative effects of *Centella* on neurological systems. However, Sahelian (2011) reported that high dose of acetylcholine esterase enzyme, which can be found in *Centella* may cause side effects including nausea, vomiting, diarrhea, abdominal pain and dyspepsia.

Safety

Centella has been widely used in pharmaceutical industries and has shown good efficacy, performance and safety (Loiseau and Mercier, 2000). With a very low toxicity as attested by its long popular use as a natural product, the fresh *Centella* plants have been used in salads, vegetable and drink as juice (James and Dubery, 2009). It has been use for traditional Indian Ayurvedic and Chinese medicines for decades (Brinkhaus *et al*., 2000). The dried plants and leaves are used in tea and is recommended between 0.33 – 0.68 g three times daily (Anonymous, 2011c; Paocharoen, 2010). There are no known side effects associated with *Centella* within the recommended dosage. In oral application, asiaticoside 1 g/kg body weight has no proven toxic and good tolerance has been demonstrated by patients to *Centella* extracts or asiaticoside (Boely, 1975).

Conclusions

Centella asiatica is a very important herbal plant in food and beverages. Its potential as a natural antioxidant extract reflects its capability to become a candidate to prevent oxidative damage, hence promoting health benefits. Many studies especially in animal experiments and in human interventions have shown its wide pharmacological activities in brain improvement and neuroprotection effect. With a very low toxicity as attested by its long popular use as a natural product, *Centella* can be a potential herbal plant in many healthcare applications.

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