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A Review: Antibacterial activities, antioxidant properties and toxicity profile of *centella asiatica*

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Abstract

Centella asiatica (*C. asiatica*) has been widely used as traditional or alternative medicine for thousand years due to its capabilities to cure various kinds of diseases. This plant has been used widely to prepare numerous kinds of food and beverages in many countries due to its beneficial functional properties. Active compounds that contribute to its antimicrobial properties are madecassic acid, asiatic acid, madecassoside, and asiaticoside. *C. asiatica* extract can inhibit majorities of foodborne pathogenic and spoilage microorganisms. It also contained antioxidant properties and has been proven to have an insignificant toxicity effect on human consumption. Currently, there is an increase of interest in research development for natural antioxidants and antimicrobial agents to replace the synthetic types in the food industry. This review presents information on the antioxidant capabilities of *C. asiatica* and its function to inhibit, reduce or minimize microbial growth. The toxicity and safety aspects of consuming *C. asiatica* extract has also been highlighted to provide essential data for the development of natural preservatives.

Keywords *Centella asiatica*, chemical composition, antioxidant capacity, antimicrobial activity, toxicity

INTRODUCTION

Centella asiatica (*C. asiatica*) is a small creeping perennial herbaceous plant that belongs to the family of Apiaceae (Umbelliferae). It is known as 'pegaga' in Malaysia while Indian and European called it as Asian Pennywort and Gotu Kola, respectively [1,2]. *C. asiatica* is also a traditional medicinal herb that can be consumed either fresh or in dry form by local communities for certain countries. Stems and leaves are the most popular parts to be used as traditional drugs. Besides, it has also been claimed to have certain medicinal functions such as blood pressure reductions, cure kidney diseases, and wound healing [1-3]. There are a variety of antimicrobial agents and antibiotics drugs which act as the food preservative or additive in the current food industry. They have been used to improve food quality or food safety purposes during food preparation. Unfortunately, the usage of synthetic antimicrobial agents or antibiotics drugs can cause the evolvement of resistant microorganism strain towards certain types of medicine or drugs. Moreover, over-consumption of synthetic additives or preservatives will cause long-term or short-term side effects on our health.

Consumers nowadays emphasized more on their healthy diet and lifestyle. Synthetic additives have been restricted to be used in the food industry since they can be carcinogenic if overly-consumed [4]. Therefore, the usage of synthetic antioxidants or preservatives such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) has become a major concern for consumer health. Thus, natural preservatives should be encouraged to develop more by using natural plant extracts for consumer's health. Recently, researchers are highly interested in developing natural plant-based additives as a substitute for artificial additives [5]. Antioxidants compounds can be obtained from natural resources, basically available from any parts of plants like flowers, roots, fruits, and stems [4].

This review will discuss on antioxidant and antimicrobial properties of *C. asiatica* extract. Furthermore, the focus is given on suitable type of extraction solvent and concentration to achieve the optimum results for the antioxidant and antimicrobial test. In future studies, this information may help for the application of *C. asiatica* extract as an alternative food preservative to minimize the enzymatic browning issues on fresh-cut fruits and vegetables.

BOTANICAL DESCRIPTION OF *C. asiatica*

Based on the Malaysian Herbal Monograph, *C. asiatica* was defined as the herbaceous plant which consists of round apex leaves, with deeply cordate stipulate and petioles, usually at around 20 cm height [6]. *C. asiatica* belongs to Apiaceae family, also known as Umbelliferae. This kind of small perennial creeping plant was found indigenous to China, Australia, Madagascar, Southern United States, and Southeast Asian countries [1-2,7]. It can grow either in tropical or temperate swampy areas with attaining height from 15 to 25 cm (6-10 in). The roots grow vertically downwards with nodes. The plant has appeared either green or reddish-green colour. The plant has long green stalk with rounded apices which provide a smooth texture with leaves of palmately netted veins. The leaves are connected with 2 to 6 cm (0.79 in) long of pericardial petioles. The flowers are small (less than 3 mm, 0.12 in) with round umbels and each umbel contains three to four flowers. The flowers consist of white, red, pink, or purple colours [8,9].

The difference between terpenoid composition is highly depending on the different parts of *C. asiatica*. It was reported that the triterpene showed the highest amount on the leaves part, followed by roots and the least was the petiole [6]. In Malaysia, there are two phenotypes of *C. asiatica*, which are smooth leaves and fringe leaves [6,10]. The differences between both phenotypes are shown in Table 1.

Table 1 Differences between characteristics of *C. asiatica*, smooth leaves and fringe leaves phenotypes

Phenotypes	Smooth leaves	Fringe leaves
Stolon length	Shorter between the roots connections	Longer between the roots connections
Overall triterpene amounts	Higher compositions of the madecassoside and asiaticoside	Lower compositions of the madecassoside and asiaticoside
Triterpene amounts (roots)	Non-detectable on roots structures	Low content of asiaticoside on roots structures
Triterpene amounts (petioles)	Higher on leaves petioles	Lower on leaves petioles

The information was adapted from sources of [2] and [7].

Besides *C. asiatica*, *C. cordifolia* and *C. erecta* are two other related species, classified under *Centella* genus as well. Both of them are closely related species to *C. asiatica*. They can be found in tropical or subtropical regions of North and South of the United States, Africa, and Asia. These three species are difficult to differentiate morphologically without a higher level of taxonomy experience [11]. The differences between the three *Centella* species are categorized in Table 2.

Table 2 Morphological differences between *C. asiatica* and its closely related species, *C. cordifolia* and *C. erecta*

Species	*C. asiatica*	*C. cordifolia*	*C. erecta*
Stems growing behavior	Grow along the soil surface	Embedded very deeply in the soil	Embedded very deeply in the soil
Stems texture	Thin and fragile	Thick and hard	Thick and hard
Leaves surface area	6.78 to 7.83 cm^2	11.90 to 16.92 cm^2	22.40 to 42.20 cm^2
Leaves shape	Cordate blade-shaped	Cordate blade-shaped	Reniform shaped with a smooth glossy texture
Leaves margin	More dentate or crenate	More dentate or crenate	Less dentate

The information was adapted from the source of [11].

C. asiatica AS FOOD AND BEVERAGE

Other than having medicinal properties, *C. asiatica* is very useful in food and beverage industries because the whole plant units, from leaves to roots, are 100% usable and edible [1,11]. Sri Lanka people tend to use the plants to cook porridge and feed pre-school children as breakfast to deal with malnutrition from past generations. The porridge has been known as 'Gotu kola kenda' by the local community, a typical herbal mix of rice and greens [9,12]. 'Gotu kola sembola' is another Sri Lanka local food which can be prepared by cutting *C. asiatica* until finely then mixed with shredded coconut flakes, red onions, and seasoning spices [9].

In Malaysia and Indonesia, *C. asiatica* is commonly consumed in either fresh, cooked or dry forms. It is gaining popularity, especially in Malay and Javanese communities. It can be consumed as fresh vegetables or salad ('*ulam*'). The salad can be served as main meal or appetizer. Some people prefer to blend the leaves part into drinks or juices. Nevertheless, it can be cooked into soup or other main dishes. However, people are usually served together with coconut milk or shredded coconut due to the mild bitterness of *C. asiatica*. Some people prefer to add sweet potatoes or potatoes instead of coconut milk. In some local markets, the unprocessed plants can directly use to prepare herbal tea, tonic drinks, or blended into ready to drink fresh juices [1, 9].

Herbal tea is a local consumption practice by Thailand and India people. The herbal tea will be prepared by adding small pieces of dried and fresh leaves into boiled water [9, 12]. Such tea can retained an appreciable amount of madecassic acid, asiatic acid, madecassoside, and asiaticoside compared to other consuming methods [13]. However, non heat-treated herbal tea contained more active compounds (11 to 17%) than heat-treated herbal tea [12,13]. In addition, freshly prepared beverages were reported to contain a large amount of volatile antioxidants compounds rather than heat treated beverages [14].

C. asiatica AS TRADITIONAL REMEDY

C. asiatica also plays an important role as a traditional remedy to treat certain diseases. Consumption of the whole plant together with chickweed and creeping wood sorrel is able to combat dysentery. Other types of traditional remedies derive from *C. asiatica* are including leaf powder, medical ointment, and leaf syrup or juices, depending on the target sickness to be treated [9].

C. asiatica powdered leaves can be grounded into paste form for directly apply or used to make ointment paste and liquid extract. They can be used for both internal or external purposes. *C. asiatica*'s powder can be applied to cure ozaena, sore throat, ulcers, burns, leprosy, and scrofula by sprinkling the powder onto ulcers or sore throat area. Some people mix powder of *C. asiatica* with lime to treat sores for babies, with the suggested amount of three to five doses per day. Also, consuming around 1.5 g of dried leaves or powdered leaves per day can calm the nerves [9,15]. The ointment or poultice can be prepared by adding Vaseline or clarified butter into the leaves' powder. The ointment can be applied externally on the affected skin area to treat enlarged scrotum or elephantiasis. For the liquid extract, it can be consumed around one to five drops with three servings per day [9]. Furthermore, the leaf juices can deal with pain effectively by taking orally twice per day (10 mL per serving). Besides pain relief, leaves juice can combat skin irritation caused by heat prickles. Palm sugar can be added during leaf juice preparation as a tonic drink for woman after baby delivery [9].

C. asiatica's CHEMICAL COMPOSITION

According to the previous review, triterpene or terpenoids compounds are the main biologically active components to promote the antioxidant properties of *C. asiatica* [16]. The triterpene compounds contribute approximately 10% of the plant content. Besides that, the antioxidant activity in *C. asiatica* (84%) is comparable to ascorbic acid (88%) and grape seed extract (83%) [1]. It was discovered that *C. asiatica* can achieve 83.0% [17] and 65.7% [3] of free radicals scavenging inhibitor at a concentration of 1.00 mg/mL.

C. asiatica extract is known to contain significant amounts of asiaticoside (1.97 ± 2.65 mg/mL) and madecassoside (3.10 ± 4.58 mg/mL) [17]. However, the amount of asiatic acid (0.55 ± 0.89 mg/mL) and madecassic acid (0.55 ± 2.29 mg/mL) were pretty low. The enhancement of antioxidant activity of *C. asiatica* may contribute by asiaticoside content and flavonoid content [18]. In addition, there is no fixed triterpenes concentration due to the diverse planting locations and environmental conditions [1,4,19]. The ethanolic extract was also found to yield a significant amount of asiaticoside (42.85 mg/g) and madecassoside (18.86 mg/g) [3].

Previous authors had reported that the total phenolic contents of *C. asiatica* (expressed as gallic acid equivalents, GAE) was ranged between 98.50 mg GAE/g to 183.24 mg GAE/g [20,21]. The differences of total phenolic contents are probably due to several reasons: complexity of functional groups compounds, extraction method or solvent chosen, planting and harvesting location, parts used to conduct analysis, weather, storage condition, and plant species [19]. Moreover, total phenolic contents of *C. asiatica* had been found different from leaves (8.13 to 11.7 g/100 g), roots (6.46 to 10.5 g/100 g), and petioles (3.23 to 4.91 g/100 g) [4].

ANTIOXIDANT CAPACITY

Oxidative stress can happened due to an imbalance between production of antioxidants and oxidants, which results in cell damage by releasing reactive oxygen species (ROS). Several ROS examples include superoxide radicals, hydroxyl ions, peroxide, and nitric oxide radicals which caused pathophysiological states such as cancer, aging, inflammatory conditions, or cardiovascular diseases. Antioxidants agents are free radicals scavenging materials to avoid the human body from free radicals and ROS effects [11,18]. The polyphenol compounds and flavonoids are readily available in *C. asiatica* to perform a better antioxidant activity, especially the leaves since it contains the highest phenolic compounds [15].

Previous published report proved that antioxidative activity and total phenolic contents are strongly correlated (r^2=0.90) [4]. The results had indicated that phenolic compounds are positively contributed to the antioxidative of *C. asiatica*. The antioxidative properties of pineapples, apples, and vegetable juices were also discovered to be heavily dependent on the phenolic compounds [21, 22]. Antioxidant activity of Malaysia plants have superior free radicals scavenging activities if using ethanolic extracts and it is correlated to the total phenolic content of plants [23,24]. In addition, extraction methods and types of solvent may affect the level of *C. asiatica* antioxidant properties. Ethanol solvent was showing the highest antioxidant activities compared to water, chloroform, methanol, and light petroleum [23,25]. Recent studies have reported that the optimum extraction concentration for *C. asiatica* was by soaking the sample in 40% of ethanol for 60 min in a solid to solvent ratio of 1:15 [1,8,26]. The consumption of *C. asiatica* extract is safe and effective to improve the body’s immune system against the free radicals and maintain the oxidative stress in a balanced state. Among all triterpene compounds, asiaticoside is the most important to contribute to the early phase of wound healing. *C. asiatica* leaves are discovered to have an optimum antioxidative activity in three pathways, which consisted of superoxide free radical activity (86.4%), free radical scavenging activity, DPPH (92.7%), and linoleic acid peroxidation (98.2%) [1].

An in-vitro study which conducted using the linolenic acid model also revealed ethanolic extract of *C. asiatica* showed a significant antioxidant capability than water and petroleum extract [23]. The roots part has performed the highest antioxidative activities compared to leaves and petioles. Other than triterpenoid compounds, the antioxidant ability was contributed by flavonoids compounds to improve cell rejuvenation and physical health. The free radical scavenging activity for *C. asiatica* extract achieved IC_{50} value of 31.2 µg/mL, whereas ascorbic acid and BHT showed IC_{50} values of 2.5 µg/mL and 7.6 µg/mL, respectively [27]. Inhibitory concentration, (IC_{50}) value is defined as that the desired concentration of antioxidants required to achieve 50% inhibition of free radicals in the specific time given. The lower the IC value, the better the antioxidant activity of plant extract can be obtained [28]. The maximum IC_{50} value threshold to exhibit the best antioxidant activity must range between 10 µg/mL to 50 µg/mL. This proved that *C. asiatica* extract can scavenge the free radicals due to its phenolic (2.9 g per 100 g) and flavonoids compound (0.36 g per 100 g) [27,29-30].

Nevertheless, a research was conducted by using a lymphoma bearing mice and fed them with 50 mg/kg of *C. asiatica* methanolic extract for 14 days continuously. The production of the antioxidant enzyme (catalase, glutathione peroxidase, and superoxide dismutase) and reduced antioxidants (ascorbic acid and glutathione) was significantly increased. A decrease of oxidative stress was observed by feeding 25 weeks of *C. asiatica* powder. This indicated the reduction of lipid peroxidation and a decrease of superoxidase dismutase activities in these mice bodies. The protective effect by *C. asiatica* plant had successfully shown a reduction in oxidative stress in mice [15].

Furthermore, the total phenolic contents and antioxidant properties are the main factors for *C. asiatica*'s product to sustain for a prolonged storage period. There is an inconsistent decrease in total phenolic content was observed in pennywort and commercial ice creams throughout the 28 days of storage period. However, pennywort ice cream showed higher TPC values (172.31 to 44.03 GAE mg/100 mL) compared to commercial ice cream (170.02 to 38.15 GAE mg/100 mL) throughout the 28 days. ABTS antioxidant assay proved that pennywort ice cream was better than commercial ice cream and able to store longer throughout the 4 weeks storage periods [31].

ANTIMICROBIAL PROPERTIES

Antimicrobial compounds are important biological agents to inhibit and reduce the growth of microorganisms. Triterpene compounds are responsible for the antimicrobial activity of *C. asiatica*. Some of the foodborne bacteria such as *Listeria monocytogenes* and *Bacillus cereus* are still able to survive and grow under natural preservation such as freezing, salting, or pickling [32]. The previous studies demonstrated that the bacteria which inhibited by *C. asiatica* were *Listeria monocytogenes*, *Bacillus cereus*, *Salmonella enterica* serovar Enteritidis, and serovar Typhimurium, *Aspergillus niger*, and *Candida albicans* [2, 32-33].

Ethanolic extract of *C. asiatica* was reported to show the largest inhibition zone against *Aspergillus niger* (17.5 mm and 12.1 mm using 100% and 70% concentration of ethanol extract, respectively) and *Bacillus subtilis* (16.4 mm and 12.2 mm using 100% and 70% concentration of ethanol extract, respectively) via disc diffusion method [33]. Moreover, the ethanolic extract of *C. asiatica* could achieve 10.0 mm and 9.0 mm zone of inhibition against *Staphylococcus aureus* and *Escherichia coli*, respectively through disc diffusion method [2]. On the other hand, the ethanolic extract of *C. asiatica* was able to exhibit 7.2 mm and 8.2 mm of zone inhibition against *Aspergillus flavus* and *Candida albicans*, respectively.

C. asiatica has been promoted as a potential candidate for antimicrobial agents due to the presence of triterpenes polar compounds, which capable to combine with polyphenols adsorption on microbial membranes [33]. Microbial growth will be inhibited since their membrane has been disrupted and weakened, followed by dissolving the microbial cell wall [32,34-35]. It is also suggested that using 95% of *C. asiatica*'s ethanolic extract can exhibit the most desired antimicrobial capability against different enteric pathogens such as *Bacillus cereus* and *Listeria monocytogenes* under normal condition [31, 35]. The concentration of ethanolic extract is known as directly proportional to the antibacterial properties of sample extract.

SAFETY AND TOXICOLOGY ANALYSIS

Toxicity is an interaction between selective toxicants and living cells to determine the extent of adverse health effects on living organisms. The experimental screening of plant extract's toxicity is very crucial to ensure the safety and effectiveness of the samples. During the past 30 years, toxicity evaluations of plant materials are frequently detected via brine shrimp lethality assay (BSLA). BSLA was designed for toxicity testing of different concentrations of crude plant extracts [36,37]. Brine shrimp (*Artemia salina*) was selected for being simple, inexpensive, rapid, convenient, and required a small amount of test material. *A. salina* was extensively studied and represent over 90% of the BSLA studies among *Artemia* species. BSLA has been widely used in the screening of toxicity of plant extracts [29,37]. LD_{50} is known as the lethal concentration required to obtain 50% death of the test population with the BSLA. The extract with below 1000 µg/ml is found highly toxic, 500 to 1000 µg/mL as low toxic and non-toxic as LD_{50} is above 1000 µg/ml [37].

According to previous study, the cytotoxic activities of *C. asiatica* ranged between 500 µg/mL to more than 1000 µg/mL, indicating that *C. asiatica* have low or insignificant cytotoxic activity [37-41]. LD_{50} for *C. asiatica* using ethanolic extract was discovered to be found more than 1000 µg/mL at three different concentrations *C. asiatica* (100, 500, and 1000 µg/mL) against potassium dichromate (28.7 µg/mL) [38]. Previous findings also indicated all concentrations posed an insignificant toxicity level after 24 h of exposure. Besides, the mortality rate of brine shrimp was found to be 3.33%, 20.00%, and 40.00% at the concentration of 10, 100, and 1000 µg/ml of ethanolic extract of *C. asiatica*, respectively after 24 h exposure [39]. The LD_{50} calculated as 1926 µg/ml against etoposide (standard drug) showed value of 7.463 µg/mL. The maximum and minimum limit of toxic concentration was recorded as 60,822 µg/mL and 606 µg/mL, respectively, which indicated lower cytotoxicity level of *C. asiatica*. There was report showing that LD_{50} of 840 µg/mL and 765 µg/mL was found for *C. asiatica* using aqueous extract and chloroform extract, respectively, which showed a low toxic level of in *C. asiatica* [40]. This demonstrated that, there is a significant difference in obtaining LD_{50} results from different solvents' extracts. This happened due to, different solvents have different extraction potential for toxicity screening. In addition, some selective solvents are discovered as a poor medium to obtain specific bioactive compounds from plant samples which responsible for toxicity than other solvents [41]. The preparation of stock solutions and dilution factors of sample extracts may increase or decrease concentrations of sample solutions, which allows affecting the toxicity results of sample extracts directly during biological screening [37].

The efficacy, performance, and safety of *C. asiatica* has been witnessed and widely applied into traditional Indian, Asian and Chinese medicines, herbs or food and beverage, and pharmaceutical products [1]. According to the World Health Organization (WHO), the recommended oral intake of *C. asiatica* is 1.00 to 2.00 g per day for scar surface or wound healing while the recommended dosage for a dried plant is between 0.33-0.68 g per meal for tea or juice making [1,42]. To date, none toxic effects by *C. asiatica* intake being reported by the WHO [42]. The oral administration of *C. asiatica* extracts and asiatic acid were applied using experimental hamster and rabbit model. No toxicity effect has been observed after 1.0 mg/kg intake of asiatic acid [27] and 1 mg/kg of asiaticoside was fed via oral administration [43]. In fact, by giving asiaticoside of 1.00 g/kg of the patient's body weight has proven to be non-toxic in oral application of *C. asiatica* extract [1].

CONCLUSION

C. asiatica is regarded as an important traditional herbal medicine or remedies to cure people who were suffering from different kinds of diseases such as blood pressure reductions, treat kidney diseases, or wound healing. It is widely used for feeding sources for local communities. The natural extracts can prevent oxidative damage against the free radicals, hence maintained oxidative stress and promoted human health.

Several published data that had been highlighted regarding the potential role of *C. asiatica* as natural antioxidants and antimicrobial agents are discovered and recorded. The previous studies had reported that ethanolic extract of *C. asiatica* gave a favorable yield for important biological compounds which are madecassic acid, asiatic acid, madecassoside, and asiaticoside. As antimicrobial properties, *C. asiatica* ethanolic extract was able to inhibit most of the pathogenic bacteria and spoilage microorganisms. For antioxidant properties, the radical scavenging properties were correlated with high phenolic compounds for *C. asiatica*. Since the IC_{50} value of *C. asiatica* extract has achieved between 10 to 50 µg/mL, thus *C. asiatica* can prove as an effective antioxidant agent.

Based on the toxicology studies by using BSLA or other experimental animals, *C. asiatica* extract has shown its wide pharmacological activities and therapeutic effect. Also, the living organisms are secured from feeding or oral administration and LC_{50} value are between 500 to 1000 µg/mL, which indicate that it caused low or insignificant toxicity on the tested animals. Therefore, these data may provide essential information for future studies regarding the development of natural food preservatives by using *C. asiatica* extract as the replacement of synthetic additives which can secure consumers' health. However, more future studies need to be taken to understand the chemical and biological activities of *C. asiatica* in more detail.

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