Accession in *Centella asiatica*; Current Understanding and Future Knowledge

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*Centella asiatica* is described as a potential cure-all because of its wide usage. The accessions in *Centella asiatica* make standardisation important before using it. In Malaysia alone, there are more than 15 accessions of *Centella asiatica* with each having variation in their secondary metabolites. There are several reports from India to Korea, from Madagascar to Malaysia and from Thailand to South Africa about variations in chemical profiling of secondary metabolites and the reasons for these variations in *Centella asiatica*. Despite all these reports, there has been no attempt to bring all these variations together and discuss them under one heading resulting into scattered information about accession in *Centella asiatica*. In this review, we discuss the impact of accessions in *Centella asiatica* and what more need to be done to our knowledge of accession in *Centella asiatica*.

**Keyword:** *Centella asiatica*, Chemical Profiling, Accession, Environmental Factors, Genetic Diversity.

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Plant research is on the rise, this is evident from the 2015 Nobel Prize which was shared by an herbal scientist Youyou Tu, who isolated the semi-synthetic derivative artemisinin from the Chinese plant *Artemisia annua*. Plant like *Galega officinalis* which was used in the development of biguanides have also helped in the management of diabetes (Amin et al., 2013). Plant research has been around since antiquity, human beings have used plant to manage and treat illness since prehistoric time. The Ayurveda medicine has been used in the Indian subcontinent since the mid-first millennium before the coming of Christianity (Dikshith, 2008). The traditional Chinese medicine has been around for more than 2000 years ago in China and surrounding countries (Xue and O’Brien, 2003).

*Centella asiatica* (*C. asiatica*) is a plant known with the tropic and subtropic regions of the world because it loves to grow in wetland and area with altitude of about 1800 m (Kartnig and Hoffmann-Bohm, 1992). It is an oriental plant that has been described as an herb that can cure all illness (Gohil et al., 2010). *C. asiatica* has been used in the traditional Indian Ayurvedic and Chinese medicines for many centuries (Brinkhaus et al., 2000). Singh and Singh (2002) reported that the whole plant is used for medicinal purpose. Gohil et al., (2010) described *C. asiatica* as a perennial herbaceous creeper plant that belongs to the *Umbellifere* family. The creeping is a slender one with the stolon running up to about 2.5 m; the stolons grow horizontally connecting the plant to each other thereby giving way to new daughter
plants (Gohil et al., 2010). In the Malaysian Herbal Monograph (2003), *C. asiatica* was described as having a rounded apex leaves, deeply cordate stipulate base and petiole of height of about 20 cm. There are reports that there are up to 20 different species of *C. asiatica* with each having their peculiar features (Gohil et al., 2010). The plant is made up of many constituent and this confer it many abilities. The primary constituents are triterpenic acid sugar esters: asiaticoside, asiatic acid, madecassoside and madecassic acid (Singh and Rastogi, 1969); because of their abundance and importance, these constituents are used as biomarker to uphold quality assurance of the *C. asiatica* (Zheng and Qin, 2007). Several study have shown that the leaves of *C. asiatica* contain the highest amount of triterpene, Ling et al., (2000) explained that this is in response to the need for regulation of the pathways for biosynthesis of terpenoids compounds.

The list of the therapeutic functions of *C. asiatica* is exhaustive and that is why it is regarded as a potential cure-all herb (Gohil et al., 2010). In traditional medicine, *C. asiatica* is known to be an antioxidant (Ullah et al., 2009; Pitella et al., 2009), an anti-inflammatory agent (Nurlaily et al., 2012), wound healer (Kimura et al., 2008) memory enhancer (Subathra et al., 2005; Singh et al., 2008), leprosy healer (Zheng and Qin, 2007) and anti-hypertensive agent (Gnanapragasam et al., 2004). Formulations such as Madecassol, Emdecassol and Centellase are made from *C. asiatica*; they have been established clinically used for wound healing and skin smoothening (Brinkhaus et al., 2000). In Malaysia and neighbouring countries, *C. asiatica* serves as food where it is a major component of a local salad called Nasi Ulam, the leaves are also blended to make juice and dried to make tea (Hashim, 2011).

**Accession in Plants**

In herbal medicine, healers go to great length to collect herbal plants from exact locations so as to make sure the plants contain same properties. Many traditional healers recognise that getting plants from different locations could affect the composition of the plants; they often give different vernacular names to plants of the same species based on where they grow from. An example is seen in *C. asiatica*, until recently a type of the specie which grown in the swarm with cardate sinuated leaf margins was known as *Centella cordifolia* until it was changed to an accession of *C. asiatica* (James et al., 2008).

Panichayupakaranant (2011) explained that getting the optimal product from herbal plants is a big challenge because many factors affects the constituents of these plants thereby making phytotherapy a complex field of research as results varies from place to place. He listed those factors to include picking the highest yielding plant species/variety, genetic composition of the plant, conditions that affect growth, variations in climate, age of plant or period to harvest and the specific parts of the plants harvested for processing. Bourgaud et al., (2001) also explained that environmental conditions like type of soil, temperature, humidity, shading, altitude and location have been reported to have effect on the secondary metabolites of a plant. Collin (2001) explained that the secondary metabolites from plant can be manipulated by altering the photosynthesis, growth regulators type and concentration, composition and pH of the culture medium. The secondary metabolites of a plant are usually classified according to their biosynthetic pathways (Harborne, 1999). The metabolites include phenolics, alkaloids, terpenes and steroids (Bourgaud et al., 2001). Zainol et al., (2003) reported that accessions affect the composition of phenolic compounds in the leaf, root and petiole of *C. asiatica*. They also reported that two of the accessions (CA 05 and CA 01) have more antioxidant activities than other the two accessions (CA 08 and CA 11).

Patel (2015) reported that the type of soil affect the growing pattern in *C. asiatica* and concluded that sandy humus soil are the best soil for rapid propagation of *C. asiatica*. Harvesting time affect the composition of a plant. Sayeda (2006) reported that when *C. asiatica* was harvested on 45th day after planting, the yield was highest when compared with 35th, 55th and 65th days of planting. The author established it that after 45th day, the plant start shading and this reduce the yield of plant.

The climate condition of a location also affects the compound composition of a plant. Many plants change their composition during different time of the season which therefore affects the efficacy of the plant. Plants are known to migrate
and when they do so, their compound composition also become altered which make the compound composition more or less efficacious thereby making the compound more or less toxic. Rieger et al., (2008) reported that altitude, a component of climate affect the content of phenol in wild samples of Calluna vulgaris, Sambucus nigra, and Vaccinium myrtillus and the concentration of flavonoids was increasing in Calluna vulgaris and Sambucus nigra with increase in altitude; anthocyanins from Sambucus nigra and Vaccinium myrtillus was decreasing with increase in altitude.

The application of fertiliser is another factor that affects the compound composition of a plant. Siddiqui et al., (2011) reported that conjunctive usage of compost tea and inorganic fertiliser affects the growth, yield and triterpene composition of C. asiatica. Kolodziej (2006) reported that co-using organic and inorganic fertilisers increase the yields and chemical compositions of Plantago arenaria. Sayeda (2006) reported that fertiliser application affect the number of leaves in a pot and number of branch per pot in a study of thakuni (C. asiatica).

Sarkar and Bose (1984) reported that the genetic variability seen in rice (Oryza sativa) differ significantly by their single seed pattern of protein. Banerjee and Sharma, (1988) also reported that the diversity of gene influenced the rate of meristem in all cultivars of Musa. The different parts of Senna alata and Rhinacanthus nasutus, and time of harvesting have been reported to have effects on the content of active ingredient of Senna alata and Rhinacanthus nasutus (Panichayupakaranant and Intaraksa, 2003; Panichayupakaranant et al., 2006). Cartayrade et al. 1997 reported that the terpenes (ginkgolide and bilobalide) found in trees of Ginkgo biloba are predominant in the young seedlings and present in the roots and leaves but absent in the stems. While there are 15 accessions of C. asiatica in Malaysia (Zainol et al., 2008), there are about 40 accessions of clinacanthus nutans, a popular Malay and Thai plant known as Belalai gajah and Phaya yo respectively (Aslam et al., 2015).

Another natural product that have shown accession is propolis, this product is derived from resin of plants where they are collected by honeybees to serves as sealant to cover up the hives and draught extruder (Mahmoud, 2006). The composition of propolis depends on the hive, district, region, season and climate from which it is gotten (Toreti et al., 2013).

**Accession in Centella asiatica**

James and Dubery, (2009) observed that location of planting and the environmental conditions of that location affect the triterpene components of the C. asiatica. Accession in C. asiatica which could have resulted from geographical and seasonal variations often result into minor variation in the colour of the herbal product, the powders extracted from C. asiatica usually vary from pale brown to yellowish brown with characteristic odour which is dependent on the origin. Investigations from traditional healers in Malaysia reveal that depending on where the C. asiatica is planted, this determines the taste of the leaves. Those that grow in swampy area are bitter than those that grow on moist land and this also affect the size of leaves as seen in the work of Zainol et al., (2008).

**International Accession**

In a study to compare the C. asiatica that originate from India and Madagascar, there were significant differences in the triterpenic content of the two C. asiatica, it was discovered that the C. asiatica that originate from Madagascar have the highest content of asiaticoside (Rouillard-Guellec et al., 1997). Randriamampionona et al., (2007) compared the highest asiaticoside content gotten from Madagascar with that of Indian reported by Das and Mallick (1991), they observed the asiaticoside from Madagascar is 60 times higher than that of asiaticoside of Indian origin.

When the four secondary metabolites in the C. asiatica from South Africa was compared with Madagascar; in the leaf tissue of the South Africa strain, the ratio of the free acid to glycoside ratio was approximately 1:2.5 while in the leaf tissue of the Madagascar strain, the ratio of the free acid to glycoside ratio range from 1:5 to 1:30 (James et al., 2008). Randriamampionona et al., (2007) compared the highest asiaticoside content gotten from Madagascar with that of Indian reported by Das and Mallick (1991), they observed the asiaticoside from Madagascar is 60 times higher than that of asiaticoside of Indian origin.

James et al., (2008) reported that in the callus of C. asiatica of South Africa origin, asiaticoside and madecassoside was detected, similar to what Nath and Buragohain (2005) observed in the callus of Indian origin but different from what Kim et al., (2004) reported in the callus of C. asiatica of Korean origin.

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The scavenging and antielastase activities of the extract from Madagascar \textit{C. asiatica} were more potent than the India \textit{C. asiatica} (Rouillard-Guellec et al., 1997). In a comparative study that involved \textit{C. asiatica} from Indonesia, India, Madagascar and South America, \textit{C. asiatica} of Madagascar origin was the only one that contains Madasiatic acid (Kartnig and Hoffmann-Bohm, 1992).

### Intranational Accession

Zainol et al., (2008) mentioned that in Malaysia alone, there are 15 different accessions of \textit{C. asiatica}. In their study to investigate the triterpene content of four accession of \textit{C. asiatica} in Skudai and Pontian region of Johor Baru, they found that the leaves of the plant contain the most constituents when compared with the petiole and roots. The authors detected only 3 triterpenes namely asiaticoside, asiatic acid and madecassoside in the plants, this was different from the findings of Hashim et al., (2011) where 4 triterpenes namely asiaticoside, asiatic acid, madecassic acid and madecassoside were present in their own \textit{C. asiatica}.

In a study to evaluate the triterpenic constituent of \textit{C. asiatica} growing in seven different regions in Madagascar, there was a difference in the triterpene content of the different regions when they were analysed using HPLC. The highest content of triterpene (asiaticoside, madecassoside, asiatic acid and madecassic acid) was in the \textit{C. asiatica} of Mangoro region, an area known with high degree of humidity and elevated temperature, followed by the \textit{C. asiatica} from Beforona region, also an area in the eastern region of Madagascar known low altitude of about 508 m. The least triterpene content was in the Miaramasoandro and Anjozorobe in the plateau highland regions of Madagascar known with high altitude of 1316 m and 1197 m respectively (Randriamampionona et al., 2007).

The asiaticoside content in 5 accessions of \textit{C. asiatica} collected from different fields in India was variable with the mean content varying from 0.42 - 1.17\% (Gupta et al., 1999). Das and Mallick (1991) investigated the asiaticoside content from \textit{C. asiatica} grown from 10 ecotypic regions of India. The plants were grown in different location, soil types and altitude. The highest 3 asiaticoside content was found in the subtemperate Himalayan regions, the location and values of the asiaticosides was found as Rudraprayag 0.114\%, Shillong 0.105\% and Darjeeling 0.097\%; while the lowest values of asiaticoside was in the sandy loam plains of India, the location and values of the asiaticosides was in west Bengal 0.006\%, Calcutta 0.017\% and Sugar island 0.060\%. They concluded that altitude have an effect on the content of asiaticoside of \textit{C. asiatica}.

In a study in Thailand, \textit{C. asiatica} were collected from five different places namely Nakornsrithammarat, Phatthalung, Ratchaburi, Songkhla and Trang at different time of the year; March, July and December (Puttarak and Panichayupakaranant 2012). The total triterpene of the \textit{C. asiatica} extract collected from Trang and Songkhla were highest. When the \textit{C. asiatica} was harvested in March, the extract gotten from Trang was the highest but when the \textit{C. asiatica} was harvested in December, the highest was Songkhla. When the \textit{C. asiatica} was gotten in March and December in Phatthalung, the total triterpene was higher than July but lower than the \textit{C. asiatica} of Trang and Songkhla. Throughout the experimental harvesting time chosen, the total triterpene from Ratchaburi and Nakornsrithammarat was significantly lower when compared to other places. In a similar study that investigate the effect of planting time on the yield of \textit{C. asiatica} in Bangladesh, Sayeda (2006) reported that best yield was in \textit{C. asiatica} planted in July followed by August and then September. This can be attributed to the climate condition of Bangladesh at these months of the year (Puttarak and Panichayupakaranant, 2012). The compositions of asiaticoside and madecassoside and their corresponding genins were also different. The asiaticoside and madecassoside composition from \textit{C. asiatica} harvested in March and December was highest in Trang, Songkhla and Phatthalung while the corresponding genins of asiatic acid and madecassic acid was highest from those gotten from Nakornsrithammarat and Ratchaburi when harvested in same March and December. It was also reported that the plants from all provinces show high amount of asiaticoside and madecassoside when harvested in July. The authors concluded that the differences seen in the content of total triterpenes and the constituents in the different places at the different months may be
due to planting conditions and genetic compositions of the *C. asiatica* (Puttarak and Panichayupakaranant 2012).

Kartnig and Hoffmann-Bohm (1992) reported that Braminioside, Brahmoside and Brahminoside was only in *C. asiatica* of India origin and Thankuniside and Isothankuniside in the *C. asiatica* of Northeastern part of India. In *C. asiatica* of Malaysian origin, there were differences in the composition of the terpenoid and it was dependent on the part of the *C. asiatica*. The leaves contain highest content of triterpene followed by the roots while the petioles contain the lowest content of triterpene. The leaves of the phenotype with smooth leaf contain higher asiaticoside and madecassoside than the phenotype of the fringed leaf, the roots of the smooth leaf was not detectable while the roots of the fringed leaf contained a low content of asiaticoside, whereas the petioles of the smooth leaf contain higher content of asiaticoside and madecassoside when compared to the petioles of the fringed leaf. The phenotype with smooth leaf has the higher composition of asiaticoside and madecassoside in the leaves, roots and petioles when compared to the phenotype of the fringed leaf (Aziz et al., 2007).

In Nepal, there were great variations in secondary metabolite contents of *C. asiatica* (Devkota et al., 2010). The wild samples of *C. asiatica* from central Nepal contain more secondary metabolites (1.88%) than those from eastern and western regions of Nepal which have a mean value of 1.20% and 1.05% respectively. When environmental conditions were varied in the experiment; irrigation and occurrence of ecological disturbance didn’t have any significant effect on the secondary metabolites, other factors like light exposure, soil type and fertiliser application affect the triterpene composition (Devkota et al., 2010).

When the effects of plant growth regulators was studied in 4 accessions of *C. asiatica*, Tan et al., (2010) observed that accession named UPM03 attained the best biomass growth within a short time and highest content of individual flavonoids among the 4 accessions (UPM01, 02, 03 and 04) with growth promoters (luteolin and quercetin).

In a study by Kundu et al., (2015) to compared the bioactive compounds in 5 different habitat of *C. asiatica* in India, the total phenolic content in different accession of *C. asiatica* were in order: CA-4 > CA-5 > CA-3 > CA-1 > CA-2. The total flavonoid content was in order: CA-5 > CA-4 > CA-1 > CA-3 > CA-2; ascorbic acid content was in order: CA-4 > CA-5 > CA-2 > CA-3 > CA-1; reducing sugar was in order: CA-4 > CA-5 > CA-3 > CA-2 > CA-1. The antioxidant potentials and the antiradical activity of the different accessions was in order: CA-4 > CA-5 > CA-3 > CA-1 > CA-2 while the scavenging activity from the ethanolic extract of the accessions was in order: CA-4 > CA-5 > CA-3 > CA-1 > CA-2. The authors concluded that the variation in the major bioactive compounds was due to differences in geographical location, altitude and location of plants.

In a study on 14 phenotypically distinct accessions of *C. asiatica* done in India (named CA-1 to CA-14); for chemical profiling of the accessions. The highest content of the triterpenoids except madecassic acid was in the CA-9 while madecassic acid was highest in the CA-11 (Singh et al., 2015). The authors concluded that environmental factors and genetic make-up affect the triterpenoids content of *C. asiatica*.

*C. asiatica* was collected from 16 different geographical areas within India with noticeable variation in the morphological properties of the 16 accessions. Eighteen variability’s was identified among the accessions ranges from leaf colour, leaf margin and leaf shape to asiaticoside content (Mathur et al., 2003). They concluded that their result corroborate other results that show *C. asiatica* variation morphologically, cytologically and in parameters of growth.

In a study from China, Zhang et al., (2012) observed that when 14 accessions of *C. asiatica* were collected from different locations with different longitude, latitude and collection time, there was genetic diversity. The diversity was significantly high in *C. asiatica* collected from Jiujiang in the Jiangxi, Jinhua in the Zhejiang and Fuzhou in the Fujjiang Province. The diversity was significantly low in *C. asiatica* from Guang’an, in the Sichuan and E’mei in the Sichuan Province and Jiulongpo in the Chongqing Province. Using the inter-simple sequence repeat markers (ISSR), they reported that the diversity shown in the genetic structure and chemical composition is a product of the genetic make-up and environmental
factors. Similar results were observed using chemical fingerprinting and hierarchical clustering analysis (Zhang et al., 2009). They concluded that in picking population of plants to grow in China, *Centella asiatica* from provinces that show high diversity in genetic make-up and chemical composition must be considered.

Study on 60 different accession from South India and Andaman Island indicated that when the accessions were planted and collected under same location and conditions; when the environmental influences has been nullified and differences can only be genetically determined, there was still variation in the chemical profiling of asiaticoside and madecassoside (Thomas et al., 2010). After quantifying the asiaticosides, 7 accessions have asiaticoside greater than 1%, another 7 have asiaticoside less than 0.1%, 3 accessions have non-detectable asiaticoside; in the madecassoside, 5 accessions have madecassoside greater than 4%, another 8 accessions have madecassoside between 3 – 3.99%, 15 accessions have madecassoside less than 1% while 2 accessions have the lowest value of madecassoside, an accession 53/Ca 053 from Nanora in North Goa didn’t show both asiaticoside and madecassoside (Thomas et al., 2010). The results show that genetical factors play an important role in the biosynthesis of the triterpenes in *Centella asiatica* (Thomas et al., 2010).

In a rather different result, Gupta et al., (2014) reported that there is no significant difference in botanical descriptors, physical and chemical factors and secondary metabolites in *C. asiatica* gotten from the 2 accessions in Indo-Gangetic plains of India specifically the Varanasi state. The size of the leaves is not a determinant for the concentration of secondary metabolites. The reason for this difference could be because these authors used HPTLC while other used different separation techniques.

**Future Studies on *Centella asiatica***

*C. asiatica* is a medicinal plant which therefore makes exploitation and depletion from their natural environment common and rampant; this has make *C. asiatica* to be listed among the highly threatened species of plants by the International Union for Conservation for Nature (Pandey et al., 1993). There is a need for continuous production of *C. asiatica* on a large scale grown *in vitro* cultures to meet the demand of the herbal industry. Until recently, there have been few or little information on using *C. asiatica* in cell suspension cultures under different conditions so as to increase the biosynthesis of metabolites which are needed on commercial production.

Research on herbal product are always faced with using the appropriate amount of the active ingredient in the production of herbal medicines, the questions of which part of the plant and the appropriate time for harvesting needs more investigation. Further investigations into the physical and chemical factors that affect the composition of plants used in herbal medicines need more clarifications and efforts should be made by researchers to standardise their herbal product before using in an experiment and in the development of functional foods.

Further genetic characterization, by means of genetic fingerprinting is needed to further understand the diversity seen in the *C. asiatica*. This will involve understanding the genetic variability and heritability of *C. asiatica* thereby helping to improve the yield component of the plant. The present status of reproduction of *C. asiatica* which is by stem cutting instead of sexual reproduction is causing limited shuffling of genetic make-up. Much effort is needed to know if sexual or asexual reproduction is the best method to getting diversity in the genetic make-up because genetic diversity is the only way to provide the survivability and adaptability of *C. asiatica* against environmental factors. Likewise superior genetic fingerprinting methods like ultra-performance liquid chromatography should be considered in analysis. Liang et al., (2013) reported that ultra-performance liquid chromatography possesses superiorities over high-performance liquid chromatography because of time of analysis is shorter, efficiency of column is higher and less solvent is needed for quality control.

Studies to understand the biochemical pathways leading to the synthesis of triterpene in *C. asiatica* will help to understand how to manipulate and maximise the product from *C. asiatica* especially under in vitro study and bioreactor conditions.

In conclusion, after highlighting the accessions in *C. asiatica*, there is a need to identify high yielding accessions which will give optimal
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triterpenoids which are used as biomarkers. The information from all these accessions should be combined and used to develop *C. asiatica* that have fast growth, high biomass and triterpenoids needed in commercial quantities.

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