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Physical characteristics, nutrient contents and triterpene compounds of ratoon crops of *Centella asiatica* at three different stages of maturity

[Ciri-ciri fizikal, kandungan pemakanan dan sebatian triterpene tanaman ratun pegaga (*Centella asiatica)* pada tiga tahap kematangan]

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Key words: *Centella asiatica*, ratoon crop, physical characteristics, nutrients, triterpene compounds, maturity

Abstract

The physical characteristics, nutrient contents and triterpene compounds of *Centella asiatica* var. Nyonya were investigated at three different stages of maturity: 50, 60 and 70 days after ratooning. Physical characteristics of *C. asiatica* at each stage of maturity showed no statistical differences (p <0.05) in whole plant length, leaf width, root length and culm length. Moisture content was slightly higher (92%) when harvested at 50 days after ratooning, but the value decreased significantly to 88–89% when harvested at 60 and 70 days. The leaf colour intensity (C* values) was low in young plants but increased significantly as plants grew older. There was significant change in total soluble solids as the maturity period increased. The highest levels of chlorophyll and titratable acidity, and lower level of pH were observed at 60 days of harvest, while ascorbic acid content decreased significantly with advance in maturity. The plant contained significant amount of Na, K, Mg, Fe, Zn, P and Ca. With advance in maturity, the K and Ca levels increased significantly. Different levels of triterpene compounds were observed at different maturity stages. The content of asiatic acid was not significantly different at all stages of maturity. However, the levels of madecassic acid, asiaticoside and madecassoside were significantly different with advance in maturity. These compounds were higher when harvested at 60 days and decreased significantly thereafter. Thus, for the ratoon crop, it is recommended to harvest the plant at 60 days after ratooning since most of the bioactive compounds were observed to be highest at this stage of maturity.

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Introduction

Centella asiatica or pegaga is a plant which has been recognized as a very potent herb in various cultures. It is native to the warmer regions of both hemispheres. The plant is usually found in the swampy areas of India, Sri Lanka, South Africa and Southeast Asia such as Malaysia and Indonesia. The plant is also indigenous to China, the western South Sea Island, Australia, Madagascar, southern United States, and insular and continental tropical America. This slender and creeping herb is especially abundant in the tropical regions.

The other common names of the plant are Asiatic Pennywort, Indian Pennywort, Thickleaved Pennywort and Gotu Kola. The Chinese, Indians and Malays use this herb for various ailments ranging from treatment of mental disorders, immune system deficiencies, circulatory problems, skin problems, liver ailments, epilepsy, asthma, hair loss and tetanus. It is also used as a brain tonic (Indu Bala and Ng 2000). Among the Malays, *C. asiatica* is consumed traditionally as a salad vegetable. Today, the extracts of *C. asiatica* are widely used as active ingredient in many drugs and cosmetic preparations in Europe, USA and Japan.

The herb is a polymorphous, creeping plant, rooting at nodes, with cylindrical and glabrous stems. Nodes arise at regular intervals and the roots, flowers and leaves occur at the nodes. The leaves are glabrous and cordate (heart-shaped) or reniform (kidney-shaped) with long petiole (leaf stalk) ranging from 5–10 cm even up to 20 cm. Leaf margin may be serrated (saw-like teeth) or smooth. The rootstock consists of rhizomes, growing vertically down and stolons which grow horizontally, interconnecting one plant to another. The plant grows under a wide range of conditions, some races prefer light shade, while others do well in open sunny areas (Zainal Abidin and Kamarudin 2005). *Centella asiatica* is usually propagated using rhizomes/runners containing roots, stems and leaves. Harvesting of the main and ratoon crop is done at 80–90 days and 50–60 days after transplanting, respectively. The whole plants are harvested, i.e. when the leaves reached a maximum size of about 4.0–4.4 cm wide.

Centella asiatica contains active ingredients such as triterpenoids, glycosides, volatile oils, pectin, amino acids, alkaloids, calcium, iron, phosphorus and vitamins (Indu Bala and Ng 2000). Several triterpenoids compounds have been isolated from *C. asiatica*. The most important ones being asiaticoside, asiatic acid, madecassic acid and madecassoside (de Padua et al. 1999). They have been considered as pharmacologically active ingredients which are beneficial in improving human health. These compounds have been reported to exhibit significant wound healing activity (de Padua et al. 1999).

Studies have also shown that some of these compounds are potent scavengers of free radicals and, as such, are potentially useful in the prevention of arteriosclerosis, cancer, diabetes, neurodegenerative diseases, arthritis and others (Zainol et al. 2003). The presence of these compounds in *C. asiatica* is varied among cultivars and several other factors such as agronomic practices, time of harvest, stage of maturity and effective postharvest handling.

Variation in content of bioactive compounds in plants depends on both genetics and environment, including growing conditions, harvest and storage, processing and meal preparation (Jefferey et al. 2002). However, very little information was available on the influences of genetics, environment and postharvest handling on content of bioactive components of *C. asiatica*. Sharma (2000) reported that three months after planting, the herb contained about 0.8% asiaticoside, and the subsequent harvest yielded about 1.1%. Similar works done by Singh et al. (1999) showed significant differences in asiaticoside and madecassoside contents of *C. asiatica* collected at different regions of India. The

contents of asiaticoside and madecassoside were higher (0.54–4.42%) in materials collected from Udampur, the northern region of India.

The nutritional and mineral component is also an important factor in determining the quality of the herbs. Studies by Lee and Chichester (1974) indicated that the nutrient composition of the plants during maturation was influenced by factors such as genetics, agronomic practices, region and rate of growth, variety and climatic conditions. It was reported that 100 g of edible portion of fresh leaves of *C. asiatica* contains water (88 g), protein (2 g), fat (0.2 g), carbohydrate (7 g), fibre (1.6 g), Ca (170 g), P (32 mg), provitamin A (4.5 mg) and vitamin C (49 mg) (de Padua et al. 1999).

However, no research has been done correlating the chemical and nutritional contents of *C. asiatica* at different stages of maturity. As far as we know, there is no information available in the literature about changes in physicochemical characteristic of *C. asiatica* at different stages of maturity. The objective of this study was to determine the content of bioactive compounds of *C. asiatica* and its physicochemical characteristic at different stages of maturity.

Materials and methods

Plant materials

Centella asiatica var. Nyonya was obtained from a farmer's plot in Paya Rumput, Melaka. The plants were planted on soil beds measuring 3 m x 1 m and allowed to grow by applying standard agronomic practices (Zainal Abidin 2004). On each bed, five rows of plants were planted. The farmer was allowed to harvest 70% of the first crop at 80–90 days after transplanting. The remaining 30% of the plant population was allowed to grow as ratoon crops. They were harvested at 50, 60 and 70 days after the first harvest by pulling the whole plants out of the ground.

The herb was then transported to the Postharvest Laboratory, MARDI for further analysis. The whole plants were washed with running tap water and the chemical and physical analyses were done directly after harvest. At each stage, the plants were harvested in four replicates and each bed was considered a replicate. Immediately after harvest, the yield of the herbs from each bed was recorded. Ten clumps were then randomly selected per replicate for various analyses: physical, chemical, nutritional, mineral and triterpene contents.

Physical analysis

Six factors were analysed, namely the length of the whole plant, culm length, root length, width and colour of leaves as well as moisture content. The length of whole plant, culm length, root length and width of leaves were physically measured using a stainless steel ruler.

Leaf colour was measured on the leaf surface by randomly selecting 10 leaves per clump. Changes in colour were measured using a Minolta CR300 Chromameter which expressed colour in three numerical notation system as L*, a* and b* values. L* denotes the lightness and darkness of the colour while a* and b* denote the hues which represented two colour axes with a* the red-green axis and b* the yellow-blue. The chroma (C*) which indicates the intensity of the colour was also calculated using the formula $C^* = \sqrt{(a^2 + b^2)}$.

Moisture contents of the plant at different stages of maturity were determined using the air-oven method (AOAC 1984). The herb was homogenized in a blender and 10–15 g of the blended sample was dried in the oven overnight at 105 °C until a constant weight was obtained. The percentage of moisture content was calculated based on the amount of weight loss.

Chemical analysis

The pH was measured by blending the herb at room temperature and readings were taken using the HANNA digital pH meter model Ph 211. The percentage of total soluble solids (TSS) was measured using a digital refractometer (ATAGO – model

DBX 55). The total titratable acidity (TTA) was determined from a sample of extracted juice by titration with an alkaline solution (0.1 N NaOH) until pH 8.1 (Askar and Treptow 1993). Chlorophyll content of the leaves was measured as SPAD values using the Minolta Chlorophyll meter (Model SPAD 500).

Nutritional analysis

Analyses for protein, fat, ash and crude fibre were carried out according to the methods of AOAC (1984) and Tee et al. (1996). Carbohydrate was calculated by difference. Vitamin A was analysed by high performance liquid chromatography (Waters LC Module 1 Plus) according to Khatijah (2001). Vitamin C (ascorbic acid) was determined using the indophenol-dye titration method (Tee et al. 1996). The herb was blended with aqueous solution of metaphosforic acid and acetic acid mixture (15 g metaphosforic acid + 40 ml acetic acid + 200 ml water) and the filtrate was titrated with 2,6 dichlorophenolindophenol dye. The end point of titration is detected when the dye gives a rose pink colour in acid solution (Tee et al. 1996).

Mineral analysis

Minerals were acquired by pre-treating the samples by dry ashing at 550 °C and dissolving them in nitric acid before injecting into an inductive coupled plasma emission spectrophotometer (ICP) (Khatijah 2001).

Triterpene content analysis

Four triterpene compounds were identified as the pharmacological active ingredients in *C. asiatica*: asiatic acid, asiaticoside, madecassic acid and madecassoside. The extraction method used for determining the triterpene contents of *C. asiatica* was similar to the method reported by Nadarajah et al. (2001). Samples were washed and left to dry at room temperature before the fresh weight of the plants were taken. Samples were then dried in the oven at 40 °C for 4–5 days and then ground into fine powder before the extraction procedure. Five grammes of the powdered samples was extracted with 200 ml methanol for 16 h using soxhlet method. The crude extracts of *C. asiatica* were subjected to Thin Layer Chromatographic (TLC) analysis which was performed on commercially available Merck DC-Alufolien (20 x 20 cm) TLC aluminium sheet precoated with Kieselgel 60 P_{254} with 0.2 mm in thickness. The eluent used for TLC analysis was hexane and chloroform with ratio 2.5:7.5 respectively. This analysis would indicate the presence of the four compounds.

For quantitative analysis of the triterpene compounds, the *C. asiatica* extracts were analysed using the High Performance Liquid Chromatography (HPLC) equipment. The analysis was carried out with Waters HPLC system, comprising Waters 600E System Controller, Waters 2996 Photodiode Array Detector, a personal computer with Empower Pro software and Rheodyne injector. The column used was LiChrospher 100RP 18 5 µm, 125 x 4 mm. The detection wavelength was set at 205 nm. The mobile phase used for the separation was water (solvent A) and acetonitrile (solvent B) each containing 0.05% H_3PO_4 at a flow rate of 1.3 ml/min. The volume of injection was 20 µl. The buffers were degassed by flushing continuously with helium. At the end of each programme, the column was equilibrated with the initial solvent for 10 min.

Statistical analysis

A completely randomized design was used for the experimental set up with three treatments (days of harvest of ratoon crop – 50, 60, 70 days) and four replicates. Data were analysed statistically using analysis of variance (ANOVA) (Steel and Torrie 1980) and the differences among the means from four replicates were determined for significance at $p < 0.05$ using Duncan multiple range test (SAS Inst. 1990).

Results and discussion

Changes in physical attributes

During growth, there was not much change in the physical characteristics of the ratoon crops of *C. asiatica* var. Nyonya. At each stage of harvest, no statistical differences (p <0.05) were found in whole plant length, leaf width, root length and culm length (*Table 1*). At 50–70 days of harvest, the plant length varied from 40–45 cm, leaf width 4.5–5.6 cm, root length 9–11 cm and culm length 32–35 cm. Similarly, the yield was also not statistically different at different maturity stages (27–33 t/ha).

However, the moisture content was slightly higher in the young plant (92% at 50 days of harvest) and decreased significantly to 88–89% at 60 and 70 days of harvest (*Table 2*). This was probably due to loss of water from the leaves and increase in the dry matter content of the herb as the maturity stage increased. The intensity of the green colour of the leaves, C* values (chroma) was low in young plants but increased significantly to 35.12 at 70 days of harvest. The chromaticity coordinates a* and b* were higher in mature plants. These values showed the mature plants were greener as compared to young plants (*Table 2*). This was also reflected in the chlorophyll content which was higher when harvested at 60 days after ratooning.

Changes in chemical attributes

The total soluble solids (TSS) increased significantly (p <0.05) from 4.42 °Brix at 50 days of harvest to 6.94 °Brix as the maturity stages increased. The increment in the TSS probably due to rapid conversion of starch to sugar. The formation of organic acids during maturation was also observed by the correspondent increase in titratable acidity. Titratable acidity increased up to 0.18% at 60 days of harvest but declined thereafter (*Table 3*). Conversely, the pH was observed to be lower at 60 days of harvest and then increased to 5.95 at 70 days of harvest in accordance with the reduction in the total titratable acidity. The result indicated that ratoon crops harvested at 60 days are more acidic than younger or older plant.

Table 1. Yield and growth of ratoon crop of *Centella asiatica* harvested at different stages of maturity

Stages of maturity (days)	Yield (t/ha)	Length of whole plant (cm)	Culm length (cm)	Root length (cm)	Width of leaves (cm)
50	28.84 ± 0.43a	40.25 ± 3.71a	31.75 ± 3.34a	10.53 ± 1.01a	5.63 ± 0.44a
60	27.20 ± 1.41a	45.30 ± 2.78a	35.11 ± 4.27a	10.20 ± 1.77a	5.51 ± 0.41a
70	32.65 ± 0.18a	42.28 ± 4.08a	32.61 ± 4.98a	8.98 ± 1.84a	4.49 ± 0.50a
Mean	29.56	42.61	33.16	9.90	5.21
C.V.	14.19	8.36	12.69	16.04	9.09
Significant level	ns	ns	ns	ns	ns

Table 2. Moisture content and colour (L, a*, b* and C* values) of ratoon crop of *Centella asiatica* harvested at different stages of maturity

Stages of maturity (days)	Moisture content (%)	L value	a* value	b* value	C* value
50	92.1 ± 0.82a	43.3 ± 3.87b	–15.7 ± 2.60a	24.6 ± 5.92b	29.19 ± 1.74
60	88.3 ± 0.94b	44.1 ± 0.2b	–14.8 ± 0.24a	21.3 ± 0.23b	25.91 ± 0.56b
70	88.6 ± 0.52b	46.4 ± 0.94a	–19.4 ± 2.0b	29.4 ± 3.31a	35.12 ± 3.76a
Mean	89.67	44.60	–16.63	25.10	30.07
C.V.	14.49	1.66	6.56	9.27	8.23
Significant level	0.05	0.05	0.05	0.05	0.05

Changes in ntrients and minerals

Centella asiatica has substantial amount of nutrients such as protein and vitamin C, and minerals such as Na, K, Mg, Fe, Zn, P, and Ca. The plant contained about 3–3.5% protein with very little fat in the plant tissues. There is no significant difference in the amount of protein at the various stages of maturity. However, the ascorbic acid content decreased significantly at $p < 0.05$ with advance in maturity. The highest level of vitamin C content (15.33 ± 0.35 mg/100 g) was observed in the young plant (50 days of harvest) and began to decline as the maturity increased (*Table 4*). The decrease in the level of vitamin C during maturation is probably due to biochemical oxidation. Vitamin C is easily oxidized in the presence of oxygen by both enzymic and non-enzymic catalysts (Mapson 1970). Seung and Kader (2000) reported that conditions favourable to water loss after harvest result in rapid loss of vitamin C especially in leafy vegetables. This finding can be correlated with the decrease in moisture content of *C. asiatica* as the maturity stage increased (*Table 2*).

The results in *Table 5* indicate that the plant was rich in minerals particularly Ca and K. It was also a good source of K with level ranging from 342– 433 mg/100 g. The highest level of K was found in samples harvested at 60 days after ratooning. There was no significant increase in the K content by delaying the harvest time. The Ca content increased significantly ($p < 0.05$) at 60 and 70 days of harvest. The presence of high levels of these elements indicates that the plant could provide alternative sources of K and Ca in our diets. The levels of Na, Mg, Fe, Zn and P also increased significantly ($p < 0.05$) as the maturity stages increased (*Table 5*).

Changes in triterpene contents

Development of triterpene compounds was affected by the harvest dates. This was observed in the contents of madecassic acid, asiaticoside and madecassoside. Their levels were significantly different at $p < 0.05$ with

Table 3. Chemical attributes of *Centella asiatica* harvested at different stages of maturity

Stages of maturity (days)	pH	Titratable acidity (%)	Total soluble solids (°Brix)	Chlorophyll content (SPAD value)
50	5.52 ± 0.08b	0.11 ± 0.63b	4.42 ± 0.02c	34.9 ± 1.10ab
60	5.22 ± 0.12c	0.18 ± 0.48a	5.21 ± 0.01b	37.4 ± 1.48a
70	5.95 ± 0.07a	0.12 ± 0.61b	6.94 ± 0.01a	33.84 ± 0.61b
Mean	5.56	0.14	5.52	35.38
C.V.	1.67	9.38	10.71	6.49
Significant level	0.05	0.05	0.05	0.05

Table 4. Protein, fat, ash and vitamin C of *Centella asiatica* harvested at different stages of maturity

Stages of maturity (days)	Protein (g/100 g)	Fat (g/100 g)	Ash (g/100 g)	Vitamin C (mg/100 g)
50	3.23 ± 0.35a	0.23 ± 0.01a	1.31 ± 0.03b	15.33 ± 0.35a
60	3.25 ± 0.39a	0.20 ± 0.01b	1.27 ± 0.03b	13.30 ± 0.12b
70	3.53 ± 0.36a	0.19 ± 0.01b	1.58 ± 0.15a	13.92 ± 0.29ab
Mean	3.33	0.21	1.39	14.18
C.V.	1.23	5.43	6.91	7.96
Significant level	ns	0.05	0.05	0.05

Table 5. Mineral contents (mg/100 g) of *Centella asiatica* harvested at different stages of maturity

Stages of maturity (days)	Sodium	Potassium	Magnesium	Iron	Phosphorus	Calcium	Zinc
50	5.76 ± 0.68b	341.67 ± 11.38b	12.11 ± 1.72b	3.67 ± 0.52b	16.27 ± 0.88b	59.6 ± 3.77b	0.89 ± 0.1b
60	8.17 ± 2.73ab	432.85 ± 46.5a	16.77 ± 3.25a	5.15 ± 1.68b	21.51 ± 1.39a	74.68 ± 12.1a	1.24 ± 0.82a
70	10.93 ± 1.83a	418.32 ± 73.89a	17.56 ± 1.53a	11.49 ± 1.98a	24.10 ± 3.47a	86.57 ± 4.22a	1.29 ± 0.67a
Mean	8.29	397.61	15.48	6.77	20.63	73.62	1.14
C.V.	21.24	10.98	14.88	22.04	8.83	10.98	15.74
Significant level	0.05	0.05	0.05	0.05	0.05	0.05	0.05

advance in maturity *(Figure 1)*. However, the amount was higher at 60 days of harvest and decreased significantly at 70 days of harvest. The decline is probably due to degradation of terpenoid content as the plant matured. The only triterpene compound not affected by the harvest date was the asiatic acid content. This compound was not significantly different at all stages of maturity (*Figure 1*). Its amount stabilized at 0.03–0.04 mg/100 g from 50 to 70 days of harvest.

Conclusion

Nutrient and terpene contents of *C. asiatica* were influenced by stages of maturity. Among the triterpene compounds, asiaticoside, madecassoside and madecassic acid were found highest at 60 days of harvest as compared to those harvested at 50 and 70 days. Furthermore, the plant was rich in potassium when harvested at 60 days after ratooning. Other nutrients such as sodium, phosphorus, magnesium, zink and iron increased with advance in maturity. The plant also contained substantial amount of protein and very little fat at all stages of maturity, but the amount of vitamin C

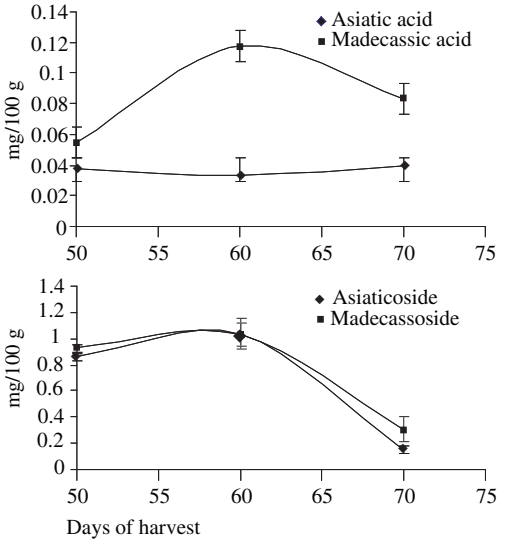

Figure 1. Changes in asiatic acid, madecassic acid, asiaticoside and madecassoside contents of ***Centella asiatica*** *at 50–70 days of harvest*

decreased as the plant matured. The effect of maturity stages on physical quality of *C. asiatica* was not significantly different except for colour of the leaves. Thus the best time to harvest and consume the plant is 60 days after ratooning since most of the biologically active compounds were observed highest at this stage of maturity.

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Abstrak

Sifat fizikal, kandungan zat pemakanan dan sebatian triterpene *Centella asiatica* var. Nyonya telah dikaji pada tiga tahap kematangan: 50, 60 dan 70 hari selepas tumbuh semula atau ratun. Sifat fizikal *C. asiatica* seperti panjang keseluruhan pokok, batang dan akar serta lebar daun tidak menunjukkan perbezaan yang signifikan (p <0.05) pada tempoh kematangan yang berbeza. Walau bagaimanapun, peratus kandungan lembapan didapati tinggi (92%) apabila dituai pada 50 hari selepas ratun dan peratusannya menurun sehingga 88–89% apabila dituai 60 hari dan 70 hari selepas ratun. Keamatan warna daun yang diukur dengan nilai C* adalah rendah pada daun muda tetapi meningkat apabila matang. Perubahan kandungan pepejal larut meningkat secara signifikan apabila pokok semakin tua. Tahap kandungan asid tertitrat dan klorofil paling tinggi pada tuaian 60 hari selepas ratun tetapi bacaan pH adalah terendah pada tahap ini. Kandungan asid askorbik menurun secara signifikan apabila tempoh kematangan meningkat. Pokok mengandungi Na, K, Mg, Fe, Zn, P dan Ca secara signifikan. Kandungan K dan Ca meningkat dengan signifikan apabila pokok semakin matang. Sebatian triterpene didapati berbeza pada tahap kematangan yang berbeza. Kandungan asid asiatik tidak berbeza secara signifikan pada tempoh tuaian yang berbeza. Namun begitu, kandungan asid madekasik, asiatikosida dan madekasosida berbeza secara signifikan apabila tempoh kematangan bertambah. Kandungan sebatian bioaktif tersebut tinggi 60 hari selepas ratun dan berkurangan 70 hari selepas ratun. Oleh itu, dicadangkan tempoh masa yang sesuai untuk menuai pegaga ratun ialah 60 hari selepas ratun memandangkan kandungan bioaktifnya paling tinggi pada kematangan tersebut.

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