Centella asiatica Morphotypes Differ Genetically as well as Macronutrients Content, Total Phenolic Content and Chemical Fingerprints of Leaves

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ABSTRACT

Purpose: *Centella asiatica* is an aromatic herb commonly grown in humid areas of tropical Asia. It has widely been used as a leafy vegetable and for medicinal preparations. Its medicinal and dilatory values have created a high demand for fresh consumption and value added products. Assessing morphological, biochemical and genetic diversity of existing germplasm is the essential first step in cultivation and breeding efforts.

Research Method: We analyzed leaf morphological characters, macro nutrient contents, total phenolic content and the high performance liquid chromatography (HPLC) profiles of five most commonly found *C. asiatica* morphotypes in Sri Lanka. The genetic diversity was assessed using inter simple sequence repeats (ISSR) regions. All morphotypes were collected and grown under controlled environmental conditions.

Findings: There were clear morphological differences among different morphotypes. Further, there was a significant difference ($P < 0.05$) among them in leaf macro nutrient content and total phenolic contents. The ISSR profiles and the HPLC profiles of Heengotukola are distinctly different from others.

Originality/value: Results show the significance of selecting superior morphotypes and for industrial applications for maintaining the standards.

Keywords: *Centella asiatica*, HPLC fingerprinting, Inter simple sequence repeats (ISSR), Total phenolic content, morphotypes

INTRODUCTION

Centella asiatica Urban belonged to family Apiaceae is an aromatic herb commonly grown in humid areas of tropical Asia. It is a popular leafy vegetable that is served as both raw and cook forms. Further, there are many value added products in the market including tea, cookies and drinks. It has widely been used in the traditional system of medicine and Ayurveda since ancient times. Different parts of the plant are used for their anti-aging, wound healing, anticancer, antimicrobial, antipyretic, diuretic and memory enhancing effects (Azis *et al.*, 2017; Hamidpour *et al.*, 2015; Jagtap *et al.*, 2009; James and Dubery, 2009; Pittella *et al.*, 2009; Zhang *et al.*, 2009). *C. asiatica* is

also useful in treating ulcer, lupus, varicose veins and mental retardation (Brinkhauset *al.*, 2000; Gray *et al.*, 2017).

Studies done over the last two decades identified certain phytochemicals found in different parts of the plant. For example, *C. asiatica* leaves consist of diverse secondary metabolic compounds including triterpenoids, volatile mono and sesquiterpene, flavonoids, vallarine and alkaloids (Chong and Aziz, 2011;

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James and Dubery, 2009;Polash *et al.*, 2017). Triterpenoids and their glycosides such as asiatic acid, madecassic acid, madecassoside and asiaticoside in leaves are mainly responsible for its characteristic properties (Hussinet *et al.*, 2009;Randriamampionona *et al.*, 2007). Few genes involved in triterpenoid biosynthetic pathway of *C. asiatica* have also been cloned (de Costa *et al.*, 2017;Kim *et al.*, 2005;Kim *et al.*, 2017;Sangwan *et al.*, 2013). Recently, *C. asiatica* leaf transcriptome was sequenced using Illumina next generation sequencing (NGS) strategy (Sangwan, 2013). This would speed up identification of relevant biosynthetic pathways and an optimization of production conditions.

C. asiatica is a diploid species with $2n=2x=18$ (Aziz *et al.*, 2007;Kaensaksiri, 2011) and cross pollinated through Lepidoptera insects (Duara and Kalita, 2013). This pollination behavior and other biological and environmental factors have led a high genetic diversity within the species. Previous genetic diversity studies carried out on *C. asiatica* using cytological, amplified fragment length polymorphism (AFLP), inter simple sequence repeats (ISSR) and simple sequence repeats (SSR) techniques have shown high genetic diversity among different accessions collected (Prasad *et al.*, 2016;Zhang *et al.*, 2012;Rakotondralambo *et al.*, 2012). This genetic diversity may reflect morphological and biochemical diversity among different accessions. In Sri Lanka, twelve morphotypes of *C. asiatica* have been identified based on morphological and morphometric characters (Jeewa & Wijekoon, 2005). Further, the local community believes that some morphotypes of *C. asiatica* are superior to the others and only those are used in traditional medicine.

Although there are studies on nutritional value of different morphotypes of *C. asiatica* (Chandrika *et al.*, 2011;Rosalizan *et al.*, 2008), there are no reports correlating the biochemical composition and morphology with genetic diversity. Identification of superior genotypes is the essential first step in any commercial scale cultivation and or for future breeding

efforts. Therefore, our work is focused on genetic, morphological and biochemical characterization of *C. asiatica* morphotypes in Sri Lanka. Of the available molecular fingerprinting techniques ISSR method was selected due to its availability and reproducibility (Ng and Tan, 2015).

MATERIALS AND METHODS

Plant Materials

Five different morphotypes of *C. asiatica*; G-1 (heen/wel), G-2 (wavy), G-3 (bush/pochchi), G-4 (medium) and G-5 (giant) were collected from farmers' fields and planted in pots 30 cm * 20 cm containing standard potting mix (Penas and Lindgren, 1990) and grown under controlled greenhouse. Three replicates from each green well-grown leaves at the harvesting stage were collected for the morphological and biochemical characterization.

Sample Preparation and Extraction

Leaf extract for total phenolic content and antioxidant activity determination were prepared using a standard method with minor modifications (Swain & Hillis, 1959). About 5 g of leaf mixed with 100 ml of 80% methanol, was homogenized using a mechanical blender, filtered through a Whatman filter paper No 1 and the filtrate was used for the analysis.

Biochemical Characterization

Total phenolic content (TPC); The total phenolic content of the methanol extracts was determined using Folin-Ciocalteu method with some modification (Singleton *et al.*, 1999). Twenty five microliter of the extract was mixed with 125 μ L of 0.1x Folin-ciocalteu reagent and kept at room temperature for 3 min. After 15 min incubation at room temperature with 125 μ L 6 % Na_2CO_3 (w/v) absorbance was measured at 600 nm wavelength using a UV-visible spectrophotometer (UV Vis 230 Spectrophotometer, Aurora Biomed) with distilled water as the blank. A standard curve

was constructed using gallic acid and TPC was expressed in milligrams of gallic acid equivalents per 100 g of sample (mg GAE/100 g of sample). Additional dilutions were performed if the absorbance value measured was over the linear range of the standard curve.

High performance liquid chromatography (HPLC) fingerprinting; *C.asiatica* leaves were ground into a fine powder with liquid nitrogen and dried until it reached to a constant weight at the room temperature. Hundred milligrams 100 of the powder was extracted with 1 mL of HPLC grade methanol under an ultrasonic conditions for 20 min. After centrifugation at 13000 rpm 10 min, the supernatant was filtered into an amber sample vial through 0.2 µm membrane filter. HPLC analysis was done in an Agilent 1200 series system with Agilent Zorbax SB-C18 column (4.6 * 250 mm, 5 µm) with a Phenomenx C-18 guard column (4.6 * 12.5 mm, 2 µm). The mobile phase consisted of 0.1% orthophosphoric acid (v/v, solvent A) and acetonitrile (Solvent B) in a gradient program of 50 % A and B for 0-20 min, 100% B for 20-35 min while maintaining column temperature at 30 °C. Flow rate and injection volume were 1mL/min and 10 µL respectively. Of the range of detection wavelength, 205 nm was selected as reported by Zhang *et al.*, (2009). MorphotypeG-2 was not incorporated in the HPLC analysis due to the lack of leaf materials.

Moisture Content;

One gram of the sample was dried in an oven at 105 °C to a constant and the moisture content was calculated by the difference in wet and dry weight.

Mineral Content;

Standard laboratory procedures were used to determine nitrogen, phosphorus, potassium and calcium levels of the leaf extracts (Black, 1965; Kirk, 1950; Koenig and Johnsen, 1942). Nitrogen, phosphorous and potassium values of potting soil and liquid fertilizer were also tested.

Morphological Characterization

Fourteen morphological characters were recorded for all five morphotypes. Among them, eight were quantitative characters (number of waves per leaf, number of leaves immersed per bush, stem length, runner length, stem diameter, leaf length, leaf width, leaf diameter) and the rest were qualitative (leaf color, leaf shape, visibility of waves, texture of leaf, stem color and runner colour).

Genetic Characterization

Genomic DNA was extracted from leaf samples following the method Doyle *et al.*, (1991) with some modifications. Seven ISSR markers (808, 818, 825, 841, 844, 846, 847) from University of British Columbia list (UBC) previously identified as polymorphic for *C.asiatica* were used in genetic diversity analysis (Zhang *et al.*, 2012). Amplification of DNA was carried out in a 25 µL reaction volume containing 1x PCR buffer, 1.5 mM MgCl₂, 200 µM dNTP (Promega, Cat No: U1515), 0.2 µM primer (Integrated DNA technologies), 50 ng of DNA, 0.8 µM spermidine and 1 Unit Go *Taq* Flexi DNA polymerase (Promega, Cat No: M8295). The PCR cycle consisted of 94 °C of initial denaturation for 5 minute, followed by 35 cycles of 94 °C for 1 minute, 55 °C for 30 seconds and 72 °C for 1 minute and final extension at 72 °C for 5 minute. Amplified products (3.5 µL) were electrophoretically separated using 8% polyacrylamide gel visualized under UV light after staining with Ethidium Bromide.

Statistical Analysis

All experimental measurements were carried out in triplicates and were expressed as mean ± standard error of mean. Experimental data were analyzed using Statistical Package for the Social Sciences (SPSS) 17.0 for Windows® (SPSS Inc.). Hierarchical cluster analysis of five morphotypes was performed based on the morphological characters and one-way ANOVA procedure followed by a Duncan test

was used to determine the significant difference ($P<0.05$) of means of each morphological characters between different morphotypes.

Dendrogram for macro nutrient content was constructed using SPSS (version 17) where principal Component Analysis (PCA) was conducted in order to identify contribution of each character to the variance.

In genetic characterization, the amplified fragments were scored as presence (1) or absence (0). In order to calculate polymorphic percentage, the total number of loci and polymorphic loci was counted. Binary data were converted into a distance matrix using dendro UPGMA program with 100 bootstraps (Garcia-Vallve and Puigbo, 2009). The resulted distance matrix was employed to construct a phylogenetic tree from an unweighted pair-group method with arithmetic means (UPGMA) using software package PHYLIP version 3.57 (Felsenstein *et al.*, 2002). Furthermore, software POPGENE32 was used to assess the genetic diversity of different morphotypes where Nie's genetic diversity was calculated.

RESULTS

There were clear morphological differences among different morphotypes even when they were grown in the same soil and environmental conditions (Figure 01, Table 01). Both

quantitative and qualitative characters were included in the cluster analysis and five studied morphotypes were grouped into two clusters at rescaled distance of 2.5 (Figure 02(A)). While G-3, G-4 and G-5 were grouped into cluster 1, G-1 and G-2 were grouped into another cluster. Within cluster 1, G-3 and G-4 belonged to one sub-group. All the qualitative characters, except leaf color were similar in all morphotypes of cluster 1 while all the qualitative characters except visibility of waves were similar in all morphotypes of cluster 2. The analysis of the variance (ANOVA) for quantitative characters showed no significant difference ($P>0.05$) in all the quantitative characters between G-1 and G-2 in cluster 2 and G-3 and G-4 in a sub-cluster within cluster 1.

When different morphotypes were harvested from different farmer fields, there was a significant ($P<0.05$) difference in their total nitrogen, phosphorous and potassium contents (Table 02). Therefore, all the morphotypes available were collected and grown under controlled greenhouse conditions for the study. Nitrogen, phosphorous and potassium contents of potting soil were 1.91 ± 0.05 mg/g, 100.49 ± 2.39 ppm, 0.84 ± 0.02 mg/g respectively, while liquid fertilizer had 0.0331 ± 0.00047 mg/L of nitrogen, 12.43 ± 0.65 ppm of phosphorous and 129.16 ± 7.21 mg/L of potassium content. Average temperature and relative humidity at 10.0 am. and 2 pm. were 28.0°C , 61.0% and 33.0°C , 80.0% respectively.

Figure 01: Different morphotypes of *C. asiatica* used for the study. G-1: heengotukola, G-2: wavy gotukola, G-3: pochchigotukola, G-4: medium sized gotukola, G-5: giant gotukola.

Table 01: Quantitative morphological characters of the five morphotypes

Characters	G-1	G-2	G-3	G-4	G-5
Leaf length	2.16 ^{ab} ±.16	1.93 ^a ±.06	3.16 ^{ab} ±.16	3.93 ^b ±.80	3.56 ^{ab} ±.23
Leaf width	3.33 ^{ab} ±.16	2.93 ^a ±.06	6.33 ^b ±.16	5.60 ^{bc} ±.40	4.33 ^c ±.16
Leaf diameter	8.33 ^a ±.33	9.66 ^a ±1.20	21.00 ^c ±1.00	19.00 ^{bc} ±1.52	14.66 ^b ±.33
Number of waves/leaf	Uncountable	12.00 ^b ±.57	25.66 ^{cd} ±.88	31.00 ^d ±4.93	16.00 ^{bc} ±1.73
Number of leaves immersed /bush	8.33 ^c ±.88	7.00 ^{bc} ±.57	4.00 ^{ab} ±.000	6.66 ^{bc} ±1.76	1.33 ^a ±.33
Stem length	5.66 ^a ±.66	6.00 ^a ±1.60	11.66 ^b ±1.20	7.66 ^b ±.88	17.76 ^c ±.37
stem diameter	.30 ^a ±.00	.30 ^a ±.00	.50 ^{ab} ±.057	.56 ^b ±.08	.50 ^{ab} ±.00
Runner length	8.33 ^b ±1.20	7.00 ^{ab} ±.28	15.00 ^c ±1.52	15.66 ^c ±.33	3.23 ^a ±.37

Data presented as mean ± standard error of the mean of independent experiments. Different upper case letters within a row refer to a significantly ($P < 0.05$) different values.

Figure 02: Dendrograms for morphotypes of *C. assiatoca* derived through UPGMA cluster analysis based on morphological (A), macro-nutrient composition (B) and Inter Simple Sequence Repeat profile (C) data.

The total nitrogen content of greenhouse grown morphotypes ranged from 26.25 to 35 mg/g showing significantly ($P < 0.05$) high values in G-2 and G-5 (Table 03). The phosphorous content ranged from 1.87 to 3.21 mg/g showing significantly high values in G-2 and G-5. The potassium content ranged from 15.28 to 21.72 mg/g showing the highest value of potassium content in G-5 and lowest in G-1 and the potassium was the most abundant mineral component in all morphotypes except G-3. The

highest calcium content was recorded in G-3 while the lowest in G-1. The moisture content ranged from 14.1 % to 15.4 % while it was significantly ($P < 0.05$) higher in G-5.

The TPC content of *C. assiatoca* was ranged from 3.9 to 12.0 mg of GAE/100 g and it was significantly ($P < 0.05$) different among all morphotypes tested while with the highest in G-5 (Figure. 03).

Table 02: Total nitrogen, phosphorous and potassium contents of different *C. assiatoca* morphotypes harvested from different farmer fields.

Field No	Nitrogen (mg/g)			Phosphorous (mg/g)			Potassium (mg/g)		
	G-1	G-4	G-5	G-1	G-4	G-5	G-1	G-4	G-5
Ff- 1	12.57 ^a ±0.90	14.64 ^a ±0.34	16.17 ^b ±0.75	1.35 ^a ±0.04	1.96 ^a ±1.57	1.53 ^a ±0.44	17.4 ^a ±1.09	19.34 ^a ±0.62	29.14 ^a ±2.51
Ff- 2	8.9 ^a ±.46	8.44 ^b ±.00	7.24 ^a ±0.34	1.57 ^a ±0.03	2.19 ^a ±0.02	2.12 ^{ab} ±0.03	20.81 ^b ±0.72	27.73 ^b ±0.72	23.19 ^b ±1.2
Ff- 3	8.24 ^a ±.00	10.44 ^a ±.00	6.9 ^a ±0.3	1.35 ^a ±0.07	2.99 ^a ±0.24	2.34 ^b ±0.13	15.35 ^a ±0.72	19.34 ^a ±0.63	18.08 ^b ±2.27

Ff- Farmer field. Data presented are ± standard error of three independent replicates. Different upper case letters within a column refer to significantly ($P < 0.05$) different values.

Table 03: Total nitrogen, phosphorous, potassium, calcium and moisture contents of different *C. asiatica* morphotypes grown in a greenhouse conditions.

Type	Nitrogen (mg/g)	Phosphorous (mg/g)	Potassium (mg/g)	Calcium (mg/g)	Moisture (%)
G1heen/wel	28.54±.01 ^b	2.23±.11 ^a	15.28±.28 ^a	12.73±.23 ^a	14.14±0.01 ^a
G2 wavy	33.49±.01 ^c	3.21±.08 ^b	18.25±.07 ^a	16.99±.17 ^{ab}	14.92±0.01 ^c
G3 bush/pochchi	26.25±.46 ^a	1.87±.03 ^a	16.84±.06 ^a	20.17±.94 ^b	14.53±0.01 ^b
G4 medium	27.66±.41 ^{ab}	2.17±.09 ^a	18.39±.01 ^{ab}	14.65±.12 ^{ab}	14.15±0.01 ^a
G5 giant	35.00±.01 ^c	2.34±.22 ^b	21.72±.57 ^b	17.10±.14 ^{ab}	15.42±0.01 ^d

Data presented as mean ± standard error of the mean of independent experiments. Different upper case letters within a column refer to significantly ($P<0.05$) different values.

Figure 03: Total phenolic content of different morphotypes of *C. asiatica*. Bars with different letters are significantly ($p<0.05$) different.

PCA done with nitrogen, phosphorous, potassium and calcium content showed that two principal components contributed to 82.75% of the total variance. The first principal component accounted for 51.54 % of the total variance. Nitrogen content contributed more to the variance (0.97), followed by potassium (0.80), phosphorous (0.73) and calcium (0.16). Characters that contributed to the second principal component included calcium (0.88) and potassium (0.39). Cluster analysis done with PCA grouped the five studied morphotypes into two clusters at rescaled distance of 10 (Figure 02 (B)). While G-1, G-3 and G-4 clustered together, G-2 and G-5 grouped into the second cluster. Within cluster 1, G-1 and G-4 belonged to one sub-group. There was no significant difference in ($P>0.05$) nitrogen, phosphorous, potassium and calcium content between morphotypes in sub-group of the cluster 1. However, potassium content of morphotypes in the cluster 2 were significantly different ($P<0.05$).

Chemical finger prints of methanol extracts obtained with HPLC identified eight peaks of which four 4,5,6 and 7 were present in all the morphotypes. Whereas peak 1 and 8 were unique to the G-3 and G-4 respectively (Figure. 04), (Table 04). Peak area percentages showed considerable variation of chemical constituents among *C. asiatica* morphotypes. Compound responsible for peak 6 is predominantly present in all the morphotypes while it was significantly high ($P<0.05$) in G-3 and G-4 gives an idea about quantity.

Amplified ISSR regions ranged from 100 to 1500 bp (Figure 05). The number of PCR bands per each primer ranged from 1 to 16, with an average of 5.2 per primer while 808 generated highest number of bands. Of the 85 loci amplified, 84 were polymorphic. ISSR markers 818, 825, 841, 844, 846 and 847 resulted 100 % polymorphic bands while 808 showing 94.14 % polymorphism with an average of 98.88 %

within population polymorphism (Table 05). The smallest genetic distance (0.0118) according to the Nei's genetic diversity values was recorded between G-2 and G-5 and the largest genetic distance was found between G-1 and G-2 (Table 06). The UPGMA dendrogram drawn on the basis

of genetic distances grouped five morphotypes into two clusters at rescaled distance of 0.75 (Figure. 02 (C)). Cluster 1 consisted of G-2, G-3, G-4 and G-5 while G-1 separated from main group.

Figure 04: HPLC chromatograms for methanol extracts of leaf samples of *C. assiatoca* morphotypes at 205 nm wave length.

Table 04: Comparative analysis of chemical fingerprints of *C. assiatoca* morphotypes.

Peak no	Peak Area %			
	G1	G3	G4	G5
1	.00±.00	2.14 ^a ±.06	.00±.00	.00±.00
2	.00±.00	5.23 ^a ±.11	4.72 ^b ±.05	.00±.00
3	.00±.00	4.14 ^a ±.11	4.11 ^a ±.03	.00±.00
4	1.26 ^a ±.12	7.58 ^b ±.16	6.19 ^c ±.07	.00±.00
5	1.18 ^a ±.04	2.16 ^b ±.07	1.97 ^b ±.04	1.15 ^a ±.01
6	9.90 ^a ±.05	15.53 ^b ±.33	15.73 ^b ±.18	5.24 ^a ±.06
7	6.86 ^a ±.05	7.68 ^b ±.21	8.49 ^c ±.05	2.99 ^d ±.10
8	.00±.00	.00±.00	2.88 ^a ±.04	.00±.00

Data presented as mean ± standard error of the mean. Different upper case letters within a row refer to a significantly ($P < 0.05$) different values.

Figure 05: *C. asiatica* morphotypes assessed with Inter Simple Sequence Repeat primers 808, 818, 825, 841, 844, 846, 847. G-1: heengotukola, G-2: wavy gotukola, G-3: pochchigotukola, G-4: medium sized gotukola, G-5: giant gotukola, L- 1 Kb molecular weight marker, promega - G5711.

Table 05: Inter Simple Sequence Repeat primers used and their characteristics

Primer code	Sequence 5' to 3'	Total amplified loci	Total polymorphic loci	Polymorphic %
808	AGAGAGAGAGAGAGAGT	17	16	94.14
818	CACACACACACACACAG	9	9	100
825	ACACACACACACACT	9	9	100
841	GAGAGAGAGAGAGAGACC	12	12	100
844	CTCTCTCTCTCTCTAC	10	10	100
846	CACACACACACACACAGT	11	11	100
847	CACACACACACACACAGC	13	13	100

Table 06: Genetic distances calculated by Nei's method. Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

Morphotype	G-1	G-2	G-3	G-4	G-5
G-1	1	0.3882	0.4353	0.6353	0.4000
G-2	0.9461	1	0.9059	0.5882	0.9882
G-3	0.8317	0.0988	1	0.5882	0.8941
G-4	0.4537	0.5306	0.5306	1	0.5765
G-5	0.9163	0.0118	0.1119		1

DISCUSSION

During ancient times, consumers, Ayurveda and traditional practitioners used the traditional knowledge for selecting superior genotypes for different purposes. For example, only heengotukola (G-1) is used in herbal medicinal preparations while all five morphotypes

are used in salads (Chandrika *et al.*, 2015). Consumers do not prefer G-5 in salads since it turns brown quickly after cutting into small pieces. Of the five, G-3 is the major morphotype under commercial cultivation. The current study provides scientific basis and significance of such selection.

Since it is a fast growing herb, soil fertility may play a significant role in plant growth, yield and the chemical composition. When the same morphotypes were grown in different farmer fields it showed significant differences in nutritional composition. This highlights the importance of conducting such experiments under controlled environmental and soil conditions.

Nutritional value is an important factor that determines the quality of a leafy vegetable. *C. asiatica* consists of substantial amount of nutrients for example, protein, vitamin C and minerals such as sodium, potassium, magnesium, ferrous, zinc, potassium and calcium (Chandrika and Prasad, 2015). Among them, few major ones were considered in the current study. Our results also support the previous findings that the potassium is a predominant mineral in *C. asiatica*. (Chandrika *et al.*, 2011). The TPC of the morphotypes reported here are lower than that of the previous studies (Hussin *et al.*, 2009; Zaino *et al.*, 2003). Differences may be due to the use of different plant parts, different extraction protocols and the genotypic differences. Further, distinctly different HPLC chemical profiles support the significantly different TPC values between each morphotype.

There was a considerable morphological variation among the morphotypes. Farmers, consumers and traditional practitioners differentiate them based on size, shape, petiole length, margin of leaves and growth habit of plant. Heengotukola (G-1) and wavy gotukola (G-2) clustered together in the dendrogramme built with morphological data. These two types share most of the morphological characters considered except the number of waves per leaf. However, heengotukola is closer to medium size gotukola (G-4) in biochemical and nutritional composition.

We used the same set of ISSR markers used previously for studying genetic diversity of *C. asiatica* (Zhang *et al.*, 2012). ISSR finger print of heengotukola (G-1) is considerably different

from other morphotypes sharing only few alleles with the others. High genetic diversity has been reported even in *C. asiatica* samples collected from a single site (Rakotondralambo *et al.*, 2012). Further, populations of *C. asiatica* with diploid as well as tetraploid have been reported (Kokubugata *et al.*, 1998). High genetic diversity is linked with sexual reproduction despite from vegetative multiplication. *C. asiatica* may also follow the natural phenomena that low level of sexual reproduction maintains a high level of genetic diversity in vegetatively propagated crops (Elias *et al.*, 2001).

Among the considered morphotypes, heengotukola is the preferred type for most of the herbal preparations while it is genetically different from the others and with significantly low TPC. Further, HPLC chemical profile of heengotukola is also clearly different from the others reflecting the genetic differences. Bush type (G-3) is the morphotype under commercial scale cultivations. It consists of the lowest total nitrogen and highest calcium content. Giant type G-5 is having the least consumer preference since it turns brown quickly after cutting. However, it consists of the highest total nitrogen, potassium and TPC. Therefore, it would be a better selection for dried herbal preparations. Further, it grows faster and rigorously than others (data not shown).

Here we only analyzed the leaf TPC, mineral content and HPLC fingerprints. However, both petiole and leaves are used in herbal preparations and some dishes. Therefore, further analysis with different plant parts is recommended. Further, TPC may not represent most of the active compounds present in *C. asiatica*. Probably, tri-terpenoids, volatile mono and sesquiterpenoids, vallerine and some alkaloids were excluded from the analysis. Further, peak area percentage was used as a method for quantification. Though it is an acceptable method (Abas *et al.*, 2014) further analysis with purified chemical standards will provide comprehensive data.

Nevertheless, the results show that morphotypes

are genetically different and show different nutritional and biochemical profiles. Therefore, selecting morphotypes for different purpose/s and maintaining the purity are important to achieve the industrial standards.

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Authors declare that there is no conflict of interest.

The datasets generated during the current study are available from the corresponding author upon request.

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