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Short Communication

Profiling of triterpenoid saponin content variation in different chemotypic accessions of *Centella asiatica* L.

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Abstract

Centella asiatica (L.) Urban is an important herbaceous medicinal plant with a worldwide distribution. The herb possesses a medicinal value and is used extensively in traditional systems of medicine. The medicinal properties of the herb are attributed to the presence of characteristic triterpenoids and their saponins in the leaves. The major triterpenoids are asiaticoside, madecassoside and their aglycones asiatic acid and madecassic acid, respectively, among others. The present study reports a remarkable qualitative and quantitative variability in secondary metabolites in different accessions of *C. asiatica* L. as determined by high-performance liquid chromatography (HPLC) analysis. The accessions analyzed in this study can be considered as the core set of discrete chemotypes of *C. asiatica*. Considerable and contrasting biochemical variations were observed in the terpenoid profiles of the chemotypes. From the basic and applied phytochemical utility, this chemotypic variability in the total content of triterpenoids is important and interesting.

Keywords: asiaticosides; *Centella asiatica*; chemotypes; triterpenoids

Introduction

Centella asiatica (L.) is a medicinally important plant of *Apiaceae* family and is commonly known as 'Indian Pennywort' or Gotu Kola. *C. asiatica* has a number of medicinal properties including nerve function improvement, inflammatory reduction, wound healing and memory enhancement (Arora *et al*., 2002). The plant is also used in cosmetic masks and creams to increase the synthesis of collagen and the firming-up of the skin (Sikareepaisan *et al*., 2011). Furthermore, *C. asiatica* is considered an effective anti-diabetic, anti-microbial and anti-proliferative herb (Roy *et al*., 2013).

C. asiatica has been reported to exhibit considerable variability in phytomolecules belonging to the triterpenoid class. Triterpenoids are biosynthesized via the mevalonate–1-deoxy-D-xylulose 5-phosphate (DOXP) pathway of isoprenogenesis (Chaurasiya *et al*., 2009). Plant phytochemicals/ bioactive molecules can be characterized and profiled by using efficient and sensitive techniques such as high-performance liquid chromatography (HPLC) to provide quality-related information for the identification of superior and elite chemotypes. Owing to the diverse medicinal uses of *C. asiatica*, qualitative as well as

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quantitative studies are conducted to evaluate phytochemical content of the plant. To assess natural and genetic variability, a comparative quantitative analysis of phytochemicals in the selected accessions of *C. asiatica* collected from different locations is reported (Thomas *et al*., 2010; Zhang *et al*., 2009; Joshi *et al*., 2012). Studies have also assessed the levels of asiaticoside and madecassoside in various parts of the plant with different phenotypes being observed *in vitro* and *in vivo* (Aziz *et al*., 2007; Singh *et al*., 2014). In an earlier report, all major phytochemicals in different phenotype-derived calli and cell suspension cultures have been analysed (James *et al*., 2008). The results of such analysis have provided valuable qualitative and quantitative information on the improvement and establishment of superior chemotypes for commercial and medicinal purposes. In the present study, selected accessions of *C. asiatica* were analysed to detect the variation in major individual phytochemicals as well as their total content. This study would help in the characterization and utilization of elite accessions and chemotypes that are rich in specific pentacyclic triterpenoid phytomolecule(s), leading to their utilization in plant improvement programmes.

Experimental

A total of 14 phenotypically distinct accessions of *C. asiatica* were sampled and used in the present study (coded as CA1–CA14). Fresh leaves (1 g) of all the chemotypes were chopped into pieces, finely ground and soaked in methanol for 24 h. The extract was collected by centrifugation and dried. The dried methanolic extract was dissolved in HPLC-grade methanol for HPLC analysis as reported earlier (Singh *et al.,* 2014). The HPLC analysis system consisted of a high-pressure constant flow pump (600E), an autosampler injector and the Waters 2996 Photodiode Array Detector, Waters Corporation, Milford Massachusetts, USA. Chromatographic separation was performed using a Waters C18 column, Waters Corporation, Milford Massachusetts, USA with a pore size of 4 μm. The chromatograms were developed at 216 nm. The HPLC analysis was performed in triplicate, and values were derived from the peak areas obtained from linear concentrations of authentic markers. The experiments were carried out in at least three replicates, and data were subjected to a one-way analysis of variance to assess the significant difference using Duncan's multiple range test ($P < 0.05$).

Discussion

Secondary metabolites of medicinal plants are of primary importance in their chemical profiling because these compounds are considered to be characteristic at the genus, species and individual levels (Sangwan *et al*., 2003). In this study, HPLC analysis of these compounds was performed in order to characterize them as chemotypes (Table 1). Our results revealed that the highest content of asiaticoside, asiatic acid and madecassoside was found in accession no. CA-9, and the highest content of madecassic acid was found in accession no. CA-11 (Table 1). There have been several reports on the profiling of secondary metabolites from the samples

Table 1. Quantitative estimation of major triterpenoids in 14 different accessions of *C. asiatica*[a]

S. no.	Asiaticoside (mg/g FW ± SD)	Asiatic acid (mg/g FW ± SD)	Madecassoside (mg/g FW ± SD)	Madecassic acid (mg/g FW ± SD)	Total content (mg/g FW ± SD)
CA1	0.652 ± 0.0158a	0.172 ± 0.002ab	0.337 ± 0.003a	1.846 ± 0.009a	3.007 ± 0.003a
CA2	1.810 ± 0.126b	0.277 ± 0.005abf	1.056 ± 0.006b	1.963 ± 0.040b	5.106 ± 0.177b
CA3	0.933 ± 0.115c	0.113 ± 0.005a	0.637 ± 0.004c	0.857 ± 0.041c	2.540 ± 0.155c
CA4	1.262 ± 0.003d	0.295 ± 0.006bf	0.868 ± 0.017d	0.826 ± 0.004c	3.251 ± 0.023d
CA5	1.837 ± 0.032b	0.733 ± 0.010c	1.027 ± 0.008b	1.896 ± 0.006d	5.493 ± 0.029e
CA6	0.875 ± 0.024c	0.639 ± 0.003c	0.169 ± 0.004e	1.851 ± 0.047ad	3.534 ± 0.026f
CA7	1.711 ± 0.011e	0.212 ± 0.006abf	1.147 ± 0.046f	2.072 ± 0.026e	5.142 ± 0.044b
CA8	0 ± 0f	0 ± 0d	0 ± 0g	0.275 ± 0.009f	0.275 ± 0.009g
CA9	4.606 ± 0.055g	2.530 ± 0.407e	2.548 ± 0.017h	1.350 ± 0.049g	11.03 ± 0.332h
CA10	1.640 ± 0.045e	0.293 ± 0.006abf	1.204 ± 0.097f	1.370 ± 0.054g	4.507 ± 0.053i
CA11	2.017 ± 0.015h	0.288 ± 0.0162abf	1.466 ± 0.030i	2.374 ± 0.022h	6.145 ± 0.037j
CA12	1.368 ± 0.033i	0.385 ± 0.005f	0.804 ± 0.014j	1.891 ± 0.004ad	4.449 ± 0.041i
CA13	0 ± 0f	0.116 ± 0.006abf	0 ± 0g	0.142 ± 0.005i	0.258 ± 0.004g
CA14	0 ± 0f	0.704 ± 0.007c	0 ± 0g	0 ± 0j	0.704 ± 0.007k

FW, fresh weight.
Mean values within a column with unlike letters are significantly different ($P < 0.05$; one-way ANOVA with Duncan's multiple range test).
[a] Data represent the values of three replicates of independent experiments.

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of *C. asiatica* collected from different locations, which involved phytochemical analysis of all four major triterpenoids (Randriamampionona *et al.*, 2007), minor triterpenoids (Zhang *et al.*, 2009), glycosides (Thomas *et al.*, 2010), aglycones (Joshi *et al.*, 2012) or selected glycoside and its corresponding aglycones (Devkota *et al.*, 2010). An extensive report on 60 different accessions of *C. asiatica* has been based on HPTLC analysis, wherein only triterpenoid glycosides have been profiled (Thomas *et al.*, 2010). There have also been reports on the quantitative estimations of the phytochemicals of *C. asiatica* using the HPLC method; however, a more efficient method with better resolution of chromatographically generated peaks belonging to triterpenoids is desirable (Rafamantanana *et al.*, 2009). The accessions used in the present study were grown under identical glass house conditions to minimize/avoid the effect of environmental conditions on the phenotype and chemo-composition(s). The reason for selection of these accessions was their distinct morphology with respect to leaf shape and size (Fig. S1, available online). The sensitive and high-resolution HPLC procedure afforded improved separation and thereby better quantification of all major triterpenoids (Fig. S2, available online). The sharp and well-resolved peaks for each of the triterpenoids of *C. asiatica* could be resolved in chromatographic profiles.

HPLC analysis of triterpenoid content in the cell and callus suspension of two morphologically different chemotypes of *C. asiatica* collected from South Africa has been reported to reveal the presence of varied accumulation of four major bioactive compounds (James *et al.*, 2008). The variation in the content of secondary metabolites might be attributed to their different geographical distribution as reported in the chemotypes of *C. asiatica* collected from different locations of Nepal (Devkota *et al.*, 2010). The variation in the concentrations of quercetin and kaempferol of two different chemotypes of *C. asiatica* collected from the regions of Maharastra and Gujrat also supported this hypothesis (Joshi *et al.*, 2012). On the contrary, asiaticoside content in *C. asiatica* obtained from three plantation areas in Indonesia did not vary substantially (Sondari *et al.*, 2011). *C. asiatica* grown at different locations also differed based on their essential oil content (Devkota *et al.*, 2013). Chemotypic variation is directly related to the environment or genetic variability of species (Sangwan *et al.,* 2013; Yadav *et al.*, 2014; Zhang *et al.*, 2012). This variation has revealed that not only growing conditions but also the genetic make-up of the plant is the contributing factor in relation to the biosynthesis of medicinally important triterpenoids. Thus, in the present study, we report a HPLC-based quantification of major triterpenoids that reveals chemotypic variability under identical environmental conditions, implying their genetic origin. This study provides useful information on the selection of *C. asiatica* chemotypes for the development of high-yielding plants.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S1479262114000860

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