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Research Article

# Trampling and Pressure Conditions Affect *in vivo* Production of Asiaticoside in *Centella asiatica* (Linn.) Urban

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## Summary

Content of Asiatic acid and Madecassic acid increases in response to grazing by hoofed animals and simulated pressure conditions in *Centella asiatica* (Linn.) Urban. This is induced by tissue damage to leaves and petioles. However, genetic polymorphism coupled with environmental modulation is also suspected.

**Keywords:** *Centella asiatica;* Asiatic acid; Madecassic acid; Genetic polymorphism, Phenotypic plasticity.

## Introduction

It is known as Mandukparni in ayurveda in India. *Centella asiatica* (Linn.) Urban is a small medicinal herb yielding a number of preparations used for different ailments Dey (1980) [10]. Apart from being used as tonic, laxative, alterative, alexiteric, antipyretic, in urinary discharges, leucoderma, anemia, bronchitis, inflammations and scores of other ailments. Active compounds asiaticoside and madecassoside, the ester glycoside derivatives of triterpenic acid, Asiatic acid and madecassic acid have been isolated and purified [1]. Asiaticoside has been recognised as an anti-mitotic molecule. Hence, it is necessary to investigate its anti-mitotic effect for its promotion as a possible anti-tumour molecules. Phenotypic plasticity of some degree have been observed and reported in this plant without any change in chromosome number. Chemical variability is also indicated by variable colour intensity of the separated straw pink layer developed by HCL [1].

### Materials and Methods

Plant materials were collected from four types of habitat, (1) Fenced area, where practically no trampling does occur, (2) Accessible area where trampling occurs occasionally, (3) Apart, where stump of large logs of wood exert pressure condition and human trampling occurs frequently and (4) Heavily grazed ruderal area.

Total triterpene estimation was achieved by adding 10 drops of acetic anhydride into chloroform extract of leaves and petioles. Two drops of concentrated HCL was mixed to develop three partitioned coloured phases in which middle straw pink phase represents terpenes. Quantitative estimation of asiaticoside and madecassoside was in accordance with the protocol used previously [1]. Emery paper was used to create tissue damage in epidermal layer of leaves to see whether it induces content enhancement.

### Results and Discussion

Content of the biologically active compounds Asiatic acid and madecassic acid and their derived triterpene ester-glycoside, asiaticoside and madecassoside in centella asiatica (Linn). Urban increases in resonse to grazing in ruderal habitat. This increase might be practically induced by tissue damage caused by trampling and grazing by the hoofed grazing animals. However, it seems that trampling like pressure situation due to human activities might also mimic the conditions that could enhance the production and HMGR enzyme that plays key role in the synthesis of triterpenoids.

Genetic polymorphism induced by environmental modulation of terpenoid biosynthesis is suspected. Asiatic acid and madecassic acid are pentacyclic triterpenes which are synthesized from the C5 compound Isopentenyl Pyrophosphate (IIP) in cytoplasm [2]. IIP may be produced from three sources in plants i.e., from mevalonic acid [3], from deoxyxylulose [4] or from amino acids [5] (Tables 1 and 2).

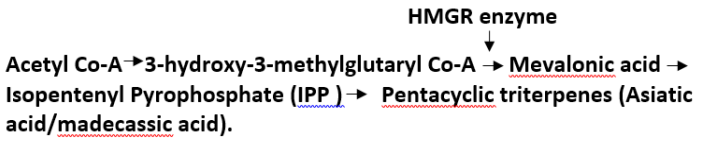
| Sample | Amount of asiaticoside (mg/gm of leaf & petiole) |
|---|---|
| 1. | 0.65 |
| 2. | 0.33 |
| 3. | 0.19 |
| 4. | 1.01 |

**Table 1:** Estimated contents of asiaticoside in plants of (1) a protected/fenced area (2) an accessible area where trampling by man occurs occasionally, (3) a park where sticks of large logs of wood create pressure condition and human trampling occurs frequently and (4) a heavily grazed ruderal habitat.

| Sample | Content (mg/gm of dried leaf & petiole). (Normal plant) | Content (mg/gm of dried leaf & petiole). (Tissue damaged specimen) |
|---|---|---|
| *Centella asiatica* | 2.30 | 3.10 |

**Table 2:** Content of asiaticoside in *Centella asiatica* (Linn.) Urban in normal state and tissue damaged condition (Specimen collected from natural habitat and garden in two groups side by side).

Mevalonate (MVA) is the favoured precursor for IIP when pentacyclic triterpenes are to be synthesized [6]. Mavalonate is synthesized from acetyl Co-A via the intermediate compound, 3-hydroxy-3-methylglutaryl Co-A. This conversion is catalysed by the enzyme HMG Co-A reductase (HMGR enzyme). It is coded by a specific gene of a multigene family [7]. Availability of HMGR regulates mevalonate incorporation into pentacyclic triterpenes. The substrate HMG Co-A gets converted into ketone bodies by the action of HMG Co-A lyase after being pushed into mitochondria if not converted into MVA [2].




Morphotypes have been reported in *Centella asiatica*. Dutta and Maiti (1968) [8] and Singh (1991) [9] reported some distinct morphotypes and biotypes from northern-eastern regions of India. Hence, high level of variability is expected in the amount of triterpenes in different biotypes or races. It is worth of investigation as higher amount of asiaticoside and madecassoside per gram of plant tissue would make the selected races commercially more viable. A molecular biology approach would help to identify rich strains of the plant from the abundantly distributed populations Figures 1 and 2.

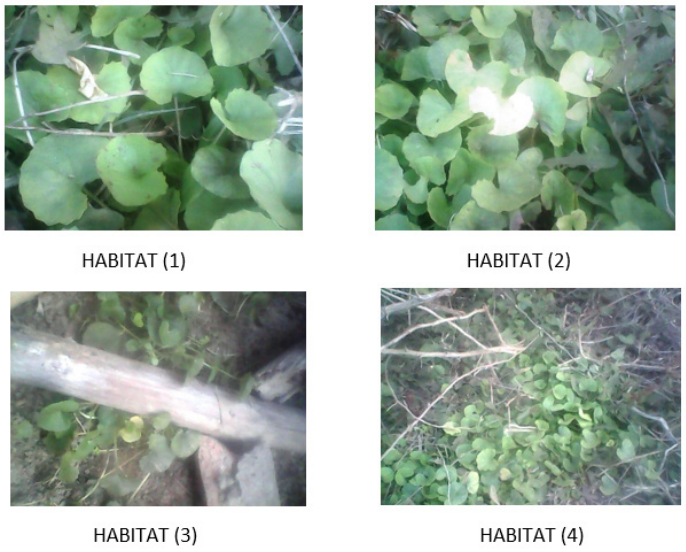



**Figure 1:** Showing plants of (1) a protected/fenced area, (2) an accessible area where trampling by man occurs occasionally, (3) a park where sticks of large logs of wood create pressure condition and human trampling occurs frequently and (4) a heavily grazed ruderal habitat.

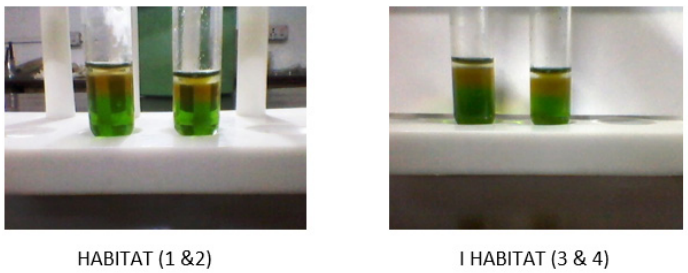



**Figure 2:** Showing images of three partitioned coloured phases of samples 1, 2, 3 & 4th respectively in which middle straw pink phase represents terpenes.

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