# Tissue Damage Induces *in vivo* Production of Asiaticoside in *Centella asiatica* (Linn.) Urban

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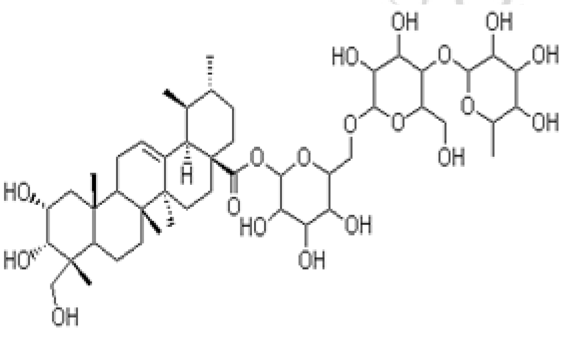
**Abstract:** ***The sub-erect plant Centella asiatica (Linn.) Urban was collected from two different areas. 7.5gm of leaves were collected from protected area where no trampling and grazing occurs and same amount of leaves from the heavily grazed ruderal area. The production of triterpene, Asiaticoside increases in response to grazing and trampling by hoofed animals. The production of Asiaticoside is also induced by tissue damage (artificially) in leaves and petioles of potted plants.***

**Keywords:** *Centella asiatica*, Asiaticoside, Tissue damage, Grazing, Trampling, Ruderal

## 1. Introduction

*Centella asiatica* (Linn.) Urban is a sub-erect plant from family Apiaceae (previously known as Umbelliferae) Known as Mandukparniin Ayurveda in India. Occurring in swampy areas, comprises some 50 species (James and Dubery 2009), inhabiting tropical and sub-tropical regions. Small medicinal herb yielding a number of preparations used for different ailments (Dey, 1980), and the plant leaves have also been used as powder for improving Memory (Kirtikar and Basu 1934). Active compounds asiaticoside and madecassoside (Kartnig, 1988; Farnsworth and Bunyapraphatsara 1992) are the ester glycoside derivatives of triterpenic acid. Asiatic acid and madecassic acid have been isolated and purified (Kumar et al. a, 2012). Schaneberg et al. (2003) reported six triterpenes - asiaticoside, madecassoside, asiatic acid, madecassic acid, terminolic acid and asiaticoside – B.

Structure of Asiaticoside



**Asiaticoside**

| | |
|---|---|
| Molecular Formula | $C_{48}H_{78}O_{19}$ |
| Molecular Weight | 959.12 |

Pentacyclic triterpenes are synthesized from the $C_5$ compound isopentenyl pyrophosphate (IPP) in cytoplasm (Isvett et al. 2002). In plants, IPP is produced from Mevalonic acid (Brown 1998), Deoxyxylulose (Rohmer et al. 1993) and Aminoacids (Koops et al. 1991). However, Mevalonate (MVA) is the favoured precursor for IPP. When pentacyclic triterpenes are synthesized (Akashi et al. 1994), phenotypic plasticity of some degree have been observed (Dutta and Maiti 1968 and Singh 1991) 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 (Kumar et al.,2012).

## 2. Material and Methods

Plant materials were collected from protected area where no trampling and grazing occurred and rom 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 represented terpenes.

7.5 g of leaves and petioles, air-dried for 15 hours, were powdered in mortar and pestle. MeOH-H2O gradient protocol was followed to isolate asiaticoside from the crude methanolic extract of the plant leaves and petioles through fractionation (Kumar *et al.* 2012a). Fraction 3 eluted with 60:40 MeOH-H2O was used to obtain crystals of Asiaticoside at $40^0$ C in a vacuum oven. Same protocol was also followed in artificially damaged leaves of *Centella asiatica* grown in the pots.

**Plate 1**

**Figure 1:** Heavily grazed (Ruderal habitat)

**Figure 2:** Protected Area

**Figure 3:** Normal Specimen (In pots)

**Figure 4:** Artificially Tissue

## 3. Result and Discussion

Investigation indicated that the Asiaticoside (Kumar *et.al.,* 2012) are produced in high amounts in leaves and petioles in heavily grazed ruderal area damage and also in artificially induced tissue damage condition. Table-1 shows the level of Asiaticoside was 1.5 mg/gm where the tissue damaged occurred due to heavy grazing of animals whereas in the protected areas were no trampling no grazing occurred it was 0.65mg//g. Hence, there was rise of 0.36 mg./gm in the production of Asiaticoside in heavily grazed area.

**Table 1**

| Sample | Conditions | Amount of asiaticoside (mg/gm of leaf & petiole) |
|---|---|---|
| 1. | Protected habitat where practically no trampling occurs | 0.65 |
| 2. | heavily grazed area | 1.5 |

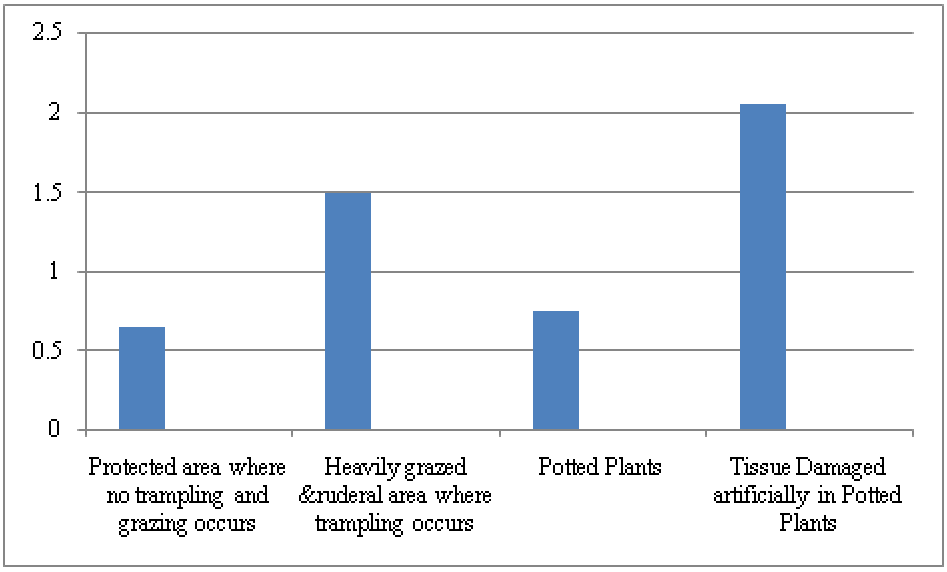
The impact of tissue damage conditions on the productivity of Asiaticoside in *Centella asiatica*

**Table 2**

| Sample *Centella asiatica* (Potted Plant) | Normal Specimen | Tissue damaged specimen |
|---|---|---|
| (mg/gm of dried leaf & petiole). | 0.75 | 2.05 |

**Content of the Asiaticoside in plants of *Centella asiatica* which was subjected to artificially induced tissue damage.**

**Table 3**




Histogram shows the production of Terpene, Asiaticoside increases during tissue damage due to heavily grazing and trampling or damaging artificially

The increasing trend in the production of Asiaticoside was also noticed in the potted plants, where the tissues of the leaves were damaged artificially by scrubbing with sand paper. 2.05 mg/gm of the terpene Asiaticoside was produced by the potted plant in response to the artificial tissue damage . This was 1.3 mg/gm higher than the asiaticosside produced in the potted plant (without tissue damage) . Higher production of defensive compound has been reported for several plants such as for *Trifolium repens* (white clover) where HCN is produced in response to herbivory grazers (Hayden and Parker, 2002).

## 4. Conclusion

It was discovered that the plant produced more useful terpene the Asiaticoside under conditions of tissue damage by herbivory animals in natural habitat. This was also noticed in the potted plants where artificially. induction of tissue damage also causes higher production of these useful compounds.

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