Int J Pharm Bio Sci 2013 Oct; 4(4): (B) 1269 - 1276

Research Article **Biochemistry**

International Journal of Pharma and Bio Sciences

ISSN
0975-6299

MORPHOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL STUDY OF *In vivo* AND *In vitro* RAISED *Centella asiatica* (L.) Urban

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ABSTRACT

Centella asiatica (L.) Urban is commonly used for curative rationale and food relevance. In the present study, certain morphological, physiological and biochemical parameters of *in vivo* and *in vitro* raised plants were compared. *In vitro* grown plant exhibited high level of chlorophyll (1.360 g/ fresh wt.), increased leaf area (2.5-3.5× 3.9-6.6 cm), low amount of phytic acid (2 mg/100mg), low ascorbic acid (10.95 mg/100g) and low SOD activity (428.52 unit/g) whereas *in vivo* grown plant exhibited reduced amount of chlorophyll (0.930 mg/g fresh wt.),decreased leaf area (1.8-2.7× 3.6-4.3 cm), increased level of phytic acid (3 mg/100g), ascorbic acid (12.44 mg/100g) and high SOD activity (491.22 unit/g). Study of these parameters restrengthens the potential of plant as nutraceutical for its food value as well as medicinal applications.

KEYWORDS: ***Centella asiatica, in vivo, in vitro*, biochemical, nutraceutical, physiology.**

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INTRODUCTION

In nature, there are many underutilized green which nurture the human inhabitants and are resilient, adaptive and tolerant to undesirable climatic state. They are underutilized due to lack of consciousness and popularization of technology. *Centella asiatica* (L.) Urban is one of the underutilized green leafy vegetable, eaten by Malay and Javanese people[1] which constitute requisite component of individual nutrient regime in terms of a vital source of vitamins, minerals, carotenoids, dietary fibre and ascorbic acid, and also prove its efficacy in alleviating problem of micronutrient malnutrition, stimulating cell rejuvenation and improving physical and mental health[2]. *In vitro* cultivation of plant is necessary pace for conception of virus free material, genetic transformation[3], micropropagation and for production of secondary metabolites. Plant resulting from tissue culture practices can result in variations moreover at cellular or morphological level[4]. Assessment of processes that appear in cells under *in vitro* ecosystem with that of integral plant give some perceptive of the mechanism involved. Therefore, the present study was undertaken with the objective of scrutinizing morphological, physiological and biochemical status of *in vivo* and *in vitro* raised plants of *C. asiatica*.

MATERIALS AND METHODS

C. asiatica was collected from MRDC, Pantnagar and were washed with sterile water. The sterile explants (leaf and stem) were then inoculated aseptically on culture media supplemented with different phytohormones for *in vitro* establishment of plant.

Morphological observations

In vivo and *in vitro* raised plants were dissected under a dissecting microscope to critically examine morphological characters.

Physiological observation

Total Chlorophyll:

Chlorophyll content of *in vivo* and *in vitro* raised leaves of *C. asiatica* was determined by measuring the absorbance[5].

Biochemical parameters

Phytic acid

Phytic acid was estimated by measuring the intensity of complex at 500 nm form by wade reagent and phytic acid present in the sample[6].

Ascorbic acid

Determination of ascorbic acid was done by measuring the ability of plant sample to decolorize 2-6 dichlorophenol indophenol solution[7].

Superoxide dismutase activity

SOD activity was determined in fresh leaves by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT)[8].

RESULTS AND DISCUSSION

Morphological description

Perennial, creeping herb with long stolons, young parts hairy, reddish brown. Leaves long petioled, rosulate, reniform or orbicular, crenate-dentate, glabrous. Peduncle of umbel hairy, much shorter than petioles; umbels solitary or few together in axils of leaves, one or few flowered. Bracts ovate, small, hairy embracing flowers. Flowers sessile; calyx teeth obsolete. Petals ovate, rounded, reddish; Stamens 5, smaller than or as long as petals; Carpels oblong, compressed with many ridges; stylopodium depressed. Fruit ovate to orbicular, 2- notched, glabrous or hairy, 7-9 ribbed (Figure 1 and 2). Leaves were larger in *in vitro* raised plants compared to *in vivo* grown plants. Leaf area was also found to be higher under *in vitro* raised plants compared to *in vivo* grown *C. asiatica*. Leaf shape was found to be crenate in *in vivo* raised plants while it was dentate under *in vitro* grown plants. Flower size was found to be smaller (3 mm) compared to *in vivo* (5 mm) raised plant. Bracts were found to be absent under *in vitro* raised plants though they were present in *in vivo* grown plants (Table 1).

Table 1
Morphological characters of *in vivo and in vitro* raised plants of *Centella asiatica* (L.) Urban.

Morphological Parameters	*In vivo*	*In vitro*
Leaf shape	Crenate	Dentate
Leaf size	Smaller	Larger
Leaf area	1.8-2.7× 3.6-4.3 cm	2.5-3.5× 3.9- 6.6
Floral character:	Bract present	Bract absent
Flower size	5 mm	3 mm
Peduncle	glabrous	hairy

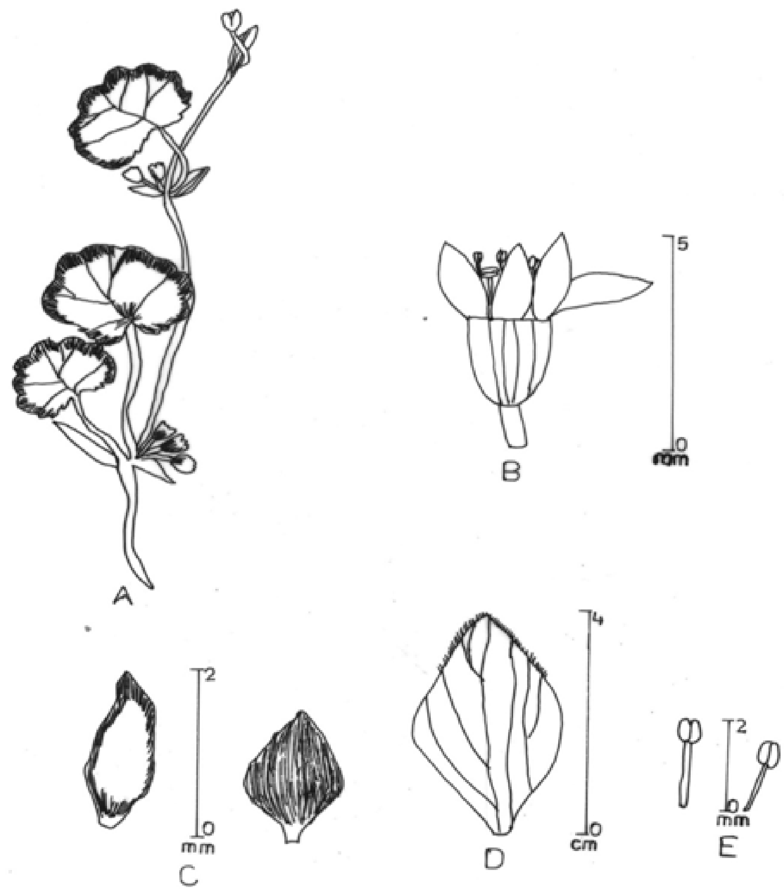

Figure 1
Morphology of in vivo raised plant of C. asiatica (A) Plant; (B) flower; (C) petals (D) bract; (E) stamens.

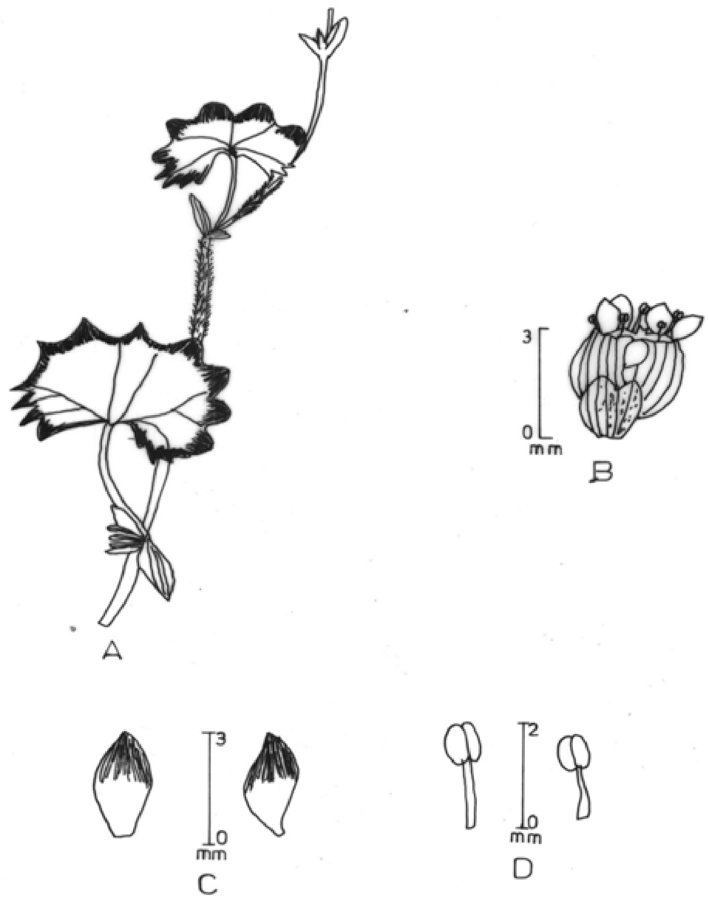

Figure 2
Morphology of in vitro raised plant of C. asiatica (A) Plant; (B) flower; (C) petals; (D) stamens.

Physiological parameters
Chlorophyll and leaf texture

The total chlorophyll content in both *in vivo* and *in vitro* raised leaves of *C. asiatica* was determined. Generally the plants are able to modulate their growth, development and physiology according to varied environmental specification. The present study revealed that leaf chlorophyll content, a measure of photosynthetic activity was found to be higher under *in vitro* raised plant (1.360 mg/ g fresh wt.) compared to *in vivo* grown plant (0.930 mg/g fresh wt.) showing that the nutrient status of the plant is better under *in vitro* conditions (Table 2). This finding is in accordance with other study[9] where they reported increased cholorphyll content in differentiating maize callus compared to *in vivo* conditions. Similar studies[10] also proposed increased chlorophyll content in *in vitro* cultures of *Pinus radiata* cotyledons. This can be attributed to the reason that *in vitro* leaves resemble shade leaves in several aspects[11]. According to others[12-15], chloroplast of sun leaves possess much lower amounts of light harvesting complex as compared to shade leaves. In contrast, chloroplast of shade leaves possess higher and broader grana thylakoid stack and primarily invest in the pigment antenna and thus shade leaves exhibited higher chlorophyll compared to sun leaves. The other reason attributed to the above mentioned cause can further be explained by the fact that shade leaves possess higher light capturing capacity due to the larger antenna size of the photosystem II and lower rates of light saturated photosynthesis due to lower amount of photosynthetic enzymes such as Rubisco and thus have high concentration of chlorophyll[16]. Higher chlorophyll content in *in vitro* grown leaves can also be explained due to classical effect of exogenous cytokinin on chlorophyll content[17] or due to the presence of exogenous carbohydrates[18, 3]. The leaf area of *in vivo* and *in vitro* grown leaves of *C. asiatica* was determined (Table 1). The results of *in vivo* and *in vitro* grown plants of *C. asiatica* indicated that the leaf area increased in *in vitro* conditions (2.5- 3.5 × 3.9- 6.6 cm) compared to *in vivo* conditions (1.8- 2.7 × 3.6- 4.3 cm). In addition to this, leaves were found to be thin in *in vitro* raised plants compared to *in vivo*. This finding is in accordance with other study[19] that sun exposed leaves were relatively thicker. It can be correlated with leaf area that thick leaves are less proficient in increasing leaf area than those of thin leaves.

Biochemical Parameters

Phytic and Ascorbic acid

Since *C. asiatica* (L.) Urban is utilized as green leafy vegetable and is known to contain certain antinutritional factors that seize adverse nutritional and physiological consequence among individuals and animals with manifestable toxicity impeding metabolic processes in a manner that negatively manipulate growth and bioavailability of nutrients[20, 21]. Phytic acid (hexaphosphate of inositol) is one of the antinutritional factors having the capability to form chelate with di and trivalent metal ions such as Cd, Mg, Fe and Zn to form poorly soluble compound that are not promptly captured by gastrointestinal tract. Human small intestine has a very restricted competence for hydrolysing phytic acid owing to be deficient in endogenous phytate decomposing enzymes thus decreasing the bioavailability of nutrients[22]. The present finding indicated less amount of phytic acid in *in vitro* raised plants (2 mg/100g) compared to *in vivo* (3 mg/100g). In the present study phytic acid content was found to be lower than reported for other tropical vegetables grown in Southeast Nigeria[23], where phytic acid content ranged from 34.70-68.50 mg/100g. Likewise, in case of non conventional leafy vegetables namely *Hibiscus cannabinus* and *Haematostaphis barteri*, phytic acid content was found to be (17.80-19.78 mg/100g), consumed by rural dwellers in Adamawa state of Nigeria[24]. In a similar study[25], antinutritional content of *Amaranthus asper* was found to be 9.25 mg/g. Thus the present finding indicated that lower phytic acid content in the scrutinized vegetable will bestow enhanced bioavailability of minerals thus promoting the plant for use as a green leafy vegetable apart from its use as a medicinal herb (Table 2). Ascorbic acid, a natural antioxidant was found to be higher under *in vivo* (12.44 mg/100g) raised plants compared to *in vitro* (10.95 mg/100g) (Table 2). The present finding is in consistence with other study[26] where they reported that sun exposed leaves possessed substantial increase in ascorbic acid content compared with shade leaves. Increased level of ascorbic acid content in sun exposed leaves emphasized its importance in photoprotection[27]. The amount of ascorbic acid during the present study was found to be higher than earlier studies on this plant[28, 29]. This proves the efficacy of the plant in diminishing the probability of cancer of stomach, bladder and colon[30] by obstructing the configuration of N- nitroso compound.

Superoxide dismutase activity

The present study revealed significantly high SOD activity under *in vivo* raised plants (491.22 unit/g) compared to *in vitro* (428.52 unit/g) (Table 2). This is in conformity with others[19], who reported that sun exposed leaves exhibits increased SOD activity compared to shade leaves. This can be explained by the fact that leaves under full sunlight cannot incinerate all the photosynthetically active radiation resulting in excess excitation energy[31]. This directs to the production of reactive oxygen species which consecutively results in subsequent mutilation of the photosynthetic organization[32]. Thus plants have developed shielding mechanism to evolve reactive oxygen species. SOD is one of the antioxidant system which catalyses the dispropotionation of $2O_2^-$ radicals to hydrogen peroxide and oxygen[33, 34].

Table 2
Physiological and biochemical parameters of *in vivo and in vitro* raised *Centella asiatica* (L.) Urban.

Parameters	*In vivo*	*In vitro*	t- value
Chlorophyll (mg/g fresh wt.)	0.930 ± 0.01	1.360 ±0.03	14.895*
Phytic acid (mg/100g)	3 ± 0.04	2 ± 0.02	1.549^{ns}
Ascorbic acid (mg/100g)	12.44 ± 1.03	10.95 ± 0.40	1.349^{ns}
SOD activity (unit/g)	491.22 ± 13.27	428.52 ± 14.62	3.176*

Values expressed are means of three replicates. * indicate significant at 5% and ns indicate non significant.

CONCLUSION

In India, assorted category of underutilized foods is accessible, but is not devour to the degree they should be, inspite of their high nutritive value. The present communication reported lower phytic acid and increased level of ascorbic acid content which is an interesting finding for the reason that though phytic acid relegate Fe accessibility but its effect is neutralized by higher ascorbic acid content which keeps iron available for amalgamation by promoting acidic condition in the stomach and intestine, thus creating optimal conditions for Fe assimilation.Looking into the predominance of elevated level of micronutrient malnutrition among vulnerable sector, underutilized green leafy vegetable can serve as the promising approach to permeate nutritional disarray.

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