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ENDOGENOUS ASCORBIC ACID FROM SOME MEDICINAL PLANTS - *IN VIVO* AND *IN VITRO*

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Four medicinal plants viz. *Adhatoda zeylanica, Barleria prionitis, Cassia angustifolia, and Ailanthus excelsa,* were selected for *in vivo* and *in vitro* comparative estimation of endogenous ascorbic acid. Plants of nearly same age were selected from three local areas and various plant parts were collected in flowering and fruiting season. Using seeds as explants unorganized cultures (calli) were initiated on MS medium supplemented with suitable concentrations of growth regulators for each plant species. Separated plant parts as well as calli harvested at maximum growth index (GI) were analyzed for endogenous ascorbic acid. Calli (*in vitro*) of all plant species have shown higher amount of ascorbic acid as compared to plant parts (*in vivo*); the highest amount was observed in *A. excelsa* fruits (*in vivo*) and calli (*in vitro*).

Keywords: Ascorbic acid; Growth index; Medicinal plants; Unorganized cultures.

Introduction

Ascorbic acid (vitamin C), a primary plant product, induces resistance to drought and salinity[1], hastens anthesis, delays ripening of seeds, enhances seedling growth and plays significant role in growth and metabolism in plants.[2] Differentiating organs show higher concentration of ascorbic acid as compared to other plant parts.[3,4] It is readily absorbed, excreted in the urine and apparently concerned with the formation of adrenal cortical hormones as well as cholesterol metabolism in human beings, while its deficiency causes scurvy disease. Presence of appreciable amount of ascorbic acid and effect of D-glucose on its production has been reported in several plants.[5-13] In the present study, an attempt has been made to analyse the production of ascorbic acid from different plant parts of some important herbs used in Indian System of Medicine, viz., *Adhatoda zeylanica* Medic. (common name Arusa, family Acanthaceae), *Barleria prionitis* Linn. (common name Jhinti, family Acanthaceae), *Cassia angustifolia* Vahl. (common name Senna, family Caesalpinaceae) and *Ailanthus excelsa* Roxb. (common name Ardu, family Simaroubaceae), as well as their *in vitro* tissue cultures.

Material and Methods

For each plant species, plants of same age group were collected from three nearby places of Bikaner region (60 to 70 km from city in different directions). Fresh plant parts (root, stem, leaves, fruits and seeds) were collected in flowering and fruiting seasons. Each part was separated, dried, powdered and used for analysis of endogenous ascorbic acid.

Using seeds as explants, cultures with profuse callusing were established on Murashige and Skoog's medium supplemented with 1.5 mg/l BAP + 1.5 mg/l NAA for A. zeylanica and B. prionitis, 2.5 mg/l BAP + 1.5 mg/l NAA for C. angustifolia, and 1.5 mg/l BAP + 1.0 mg/l NAA for A. excelsa. These cultures were maintained for a period of 6 months by frequent subculturing at an interval of 6 to 8 weeks at 26±1°C, 55% RH and light intensity at about 3000 lux. The growth indices (GI) were calculated at different time intervals of 2, 4, 6, 8 and 10 wk using the formula given below (Table1). Cultures at the maximum GI were harvested, dried, powdered and analysed for estimation of endogenous ascorbic acid.

$$GI = \frac{\text{Final fresh weight of tissue - Intial fresh weight of tissue}}{\text{Inital fresh weight of tissue}}$$

Ascorbic acid was estimated following the method of Chinoy[3]. Dried plant parts as well as dried cultured tissues of maximum GI were weighed separately, crushed in ice cold CO_2 -saturated water to make a definite volume. 3 mL of extract was mixed with an equal volume of buffered metaphosphoric acid at pH 3.6. Of this solution 2 mL was mixed with 5 mL distilled water and turbidity produced was adjusted to zero with spectronic-20 colorimeter (Bausch and Lomb). Another 2 ml was mixed with 5 mL of 2,6-dichlorophenolindophenol (prepared by dissolving 5 mg of dye in 100 ml of distilled water at 80°C)

Table 1. Growth indices of static cultures of selected plant species.

Name of plant	Growth indices at the age of				
	2 weeks	4 weeks	6 weeks	8 weeks	10 weeks
Adhatoda zeylanica	1.25±0.16	2.73±0.21	6.51±0.25	9.50±0.32	8.53±0.35
Ailanthus excelsa	1.51±0.12	3.17±0.20	7.10±0.19	10.12±0.44	9.31±0.39
Barleria prionitis	1.24±0.13	2.65±0.19	6.53±0.23	9.55±0.28	8.50±0.27
Cassia angustifolia	1.16±0.09	2.43±0.14	5.82±0.30	9.21±0.36	8.89±0.25

GI is mean of ten replicates ±SD

Table 2. Endogenous Ascorbic Acid (mg/100g dry weight) in selected four plant species.

Name of plant	Plant parts X± SD	*in vivo* Location 1 X± SD	Location 2 X± SD	Location 3 X± SD	*in vitro* Callus X± SD
Adhatoda zeylanica	Root	9.976±1.130	10.105±1.082	9.818±1.231	30.314±1.154
	Stem	12.587±1.189	12.229±1.242	12.741±1.204	
	Leaves	14.772±1.020	14.355±1.144	14.284±1.077	
	Fruits	28.376±1.140	28.588±1.113	28.691±1.296	
	Seeds	22.294±1.060	22.673±1.202	22.854±1.296	
Ailanthus excelsa	Root	18.456±1.211	18.196±1.098	18.656±1.149	36.322±1.340
	Rachis	19.739±1.042	19.811±1.256	19.934±1.103	
	Leaflets	20.598±0.971	20.402±1.051	20.613±1.201	
	Fruits	30.476±1.275	30.011±1.238	30.284±1.233	
	Seeds	29.465±1.209	29.783±0.909	29.957±1.012	
Barleria prionitis	Root	9.926±1.070	9.676±1.1258	9.503±1.276	31.559±1.130
	Stem	10.397±1.009	10.477±1.1704	10.578±1.144	
	Leaves	19.416±1.103	19.953±1.256	19.627±1.114	
	Fruits	29.672±1.135	29.779±1.191	29.949±1.355	
	Seeds	23.674±1.047	23.259±1.222	23.259±1.098	
Cassia angustifolia	Root	8.978±1.038	8.524±1.177	8.742±1.110	25.608±1.363
	Stem	11.525±1.079	11.114±1.196	11.333±1.001	
	Leaves	17.148±1.012	17.588±1.134	17.462±1.160	
	Fruits	23.818±1.176	23.543±1.175	23.615±1.259	
	Seeds	17.522±1.139	17.342±1.184	17.693±1.057	

The values are mean of ten replicates ± SD
Data were analysed by 2-way analysis of variance (ANOVA). Variation due to location is insignificant but variation between *in vivo* and *in vitro* is significant.

and optical density (OD) was measured. Absorbance of each of the sample was measured at 546 nm against blank. Ten replicates were taken for each plant part of each plant species from each selected location. Ten replicates of callus of each plant species were also analyzed for comparison. Ascorbic acid content present in 1ml of extract was measured by using the regression formula -

$Y = 0.1103 - (0.14 \times OD)$

Where Y = Concentration of ascorbic acid in mg, OD = Optical Density

Ascorbic acid content per 100 g dry weight was calculated as follows

Free ascorbic acid= $\frac{(A \times V)}{W} \times 1000 \times 100$

Where, A = Y = mg Ascorbic acid / ml of original extract, V = total volume of the original extract (in ml), and W = weight of the plant tissue sample (in mg) used for analysis

Result and Discussion

Analysis for plant parts showed that its amount of endogenous ascorbic acid was in the order of root < stem < leaves < seeds < fruits for each species from each selected location (Table 2). Roots of all plant species showed lower amounts of ascorbic acid as compared to other plant parts, while *C. angustifolia* showed the lowest concentration of ascorbic acid among roots. Higher concentration of ascorbic acid was shown in fruits of all four plants with the highest concentration in *A. excelsa.* The highest percentage of ascorbic acid in fruits can be related to the reservoir nature of fruits, while ascorbic acid content of leaves may be the result of their metabolic activity. Similar results have also been reported.

Initiation and establishment of callus of each plant species was initiated with many explants (leaf, nodal segment, shoot tip and seeds) on MS medium supplemented with various combinations of hormones (BAP, IAA, NAA and 2,4-D) with concentrations ranging from 0.05 mg/l to 2.5 mg/l. However, best results of callus establishment were obtained using seeds as explants for all plant species (data not shown). Suitable hormonal concentrations in MS medium were found to be 1.5 mg/l BAP + 1.5 mg/l NAA for *A. zeylanica* and *B. prionitis,* 2.5 mg/l BAP +1.5 mg/l NAA for *C. angustifolia* and 1.5 mg/l BAP + 1.0 mg/l NAA for *A. excelsa.* Calli of all plant species were fragile and creamish green in colour up to 8 wk (maximum GI). Later with decreasing GI, it started turning brown to black.

Calli harvested at maximum GI showed sufficiently high concentrations of ascorbic acid, which was even more than maximum amount observed in fruits (*in vivo*) (Table 2). Increased amount of ascorbic acid in tissue culture can be related to fast multiplication and high metabolic rate at the highest GI, i.e. 8 wk of growth.

The experimental data of the present investigation was analysed statistically by two-way ANOVA. The variation due to location was found insignificant but the variation between different plant parts (*in vivo*) and callus (*in vitro*) was significant.

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