

Analysis of Ascorbic Acid Content in *Cocculus Pendulus*, *Tinospora Cordifolia* and *Moringa Oleifera*

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ABSTRACT. *Plant parts collected in summer and winters as well as unorganized cultures of some medicinal plants were analyzed for production of endogenous ascorbic acid. Unorganized cultures (calli) were initiated using various plant parts in selected plant species Cocculus pendulus, Tinospora cordifolia and Moringa oleifera, on MS Medium supplemented with different combinations and concentrations of hormones by frequent subculturing. Ascorbic acid production was compared quantitatively in all plant parts and unorganized cultures harvested at maximum growth index.*

Keywords: Ascorbic acid, growth index, medicinal plants, unorganized cultures

1. Introduction. Plants have been an integral part of traditional medicine across the continents since time immemorial. Medicinal plants have their values in the substances present in various plant tissues with specific physiological action in human body. Herbs like *Cocculus pendulus* and *Tinospora cordifolia* (family- Menispermaceae) and *Moringa oleifera* (family- Moringaceae) have been selected for present study of ascorbic acid estimation.

C. pendulus is quite common in plains upto 200 metres. The juice of leaves mixed with water has the property of coagulating in to a green jelly like substance which is taken internally with sugar as tonic. The shoots and the leaves are used in rheumatic pains. *T. cordifolia* is most versatile rejuvenating herb that has medical potency to keep the body free from illness. It acts as hepatoprotactant, protecting liver from hepatotoxicity. Skin diseases are cured with extracts of its stem. Bark of *T. cordifolia* has antiallergic, antileprotic, antipyretic, antispasmodic and anti-inflammatory properties. *M. oleifera* is considered as highly nutritious plant but along with nutritional values it has medicinal values also. Leaves are used in the treatment of fever, bronchitis, eye and ear infection, inflammation of mucous membrane, diarrhoea and gastric ulcer. Flower juice is useful in urinary infections. Roots and bark are used for cardiac and circulatory problems as tonic. Bark is appetizer and

digestive.

Ascorbic acid (vitamin C) a primary plant product is claimed as a 'cure all' for many human diseases and problems from cancer to common cold. It is readily absorbed and excreted in urine and apparently concerned with formation of cortical hormones as well as cholesterol metabolism. Deficiency of ascorbic acid causes scurvy disease. Endogenous ascorbic acid has been reported from various plant species as Cicer arietinum, Lycium chinense, Citrullus colocynthis and Corchorus depressus, Zygophyllaceous plants, Capparis decidua and Zizyphus mauritiana, Cucumis sativus, Arnebia hispidissima, Chenopodium sps. Chrozophora prostata, Euphorbia microphylla, Cassia angustifolia, Ailanthus excelsa, Adhatoda vasica and Barleria prionitis and Molluginaceae plants.

Presence of ascorbic acid, effect of C glucose on its production, in plant tissue culture has also been studied and compared with in vivo studies in Solanum nigrum, Abutilon pannosum, Calligonum polygonoides and Lasiurus indicus, Fagonia cretica, Lycium barbarum, Vigna aconitifolia, Withania somnifera, Capparis decidua and Zizyphus mauritiana, Ailanthus excelsa, Adhatoda vasica and Barleria prionitis.

2. Materials and Methods. Fresh plant parts of *C. pendulus*, *T. cordifolia* and *M. oleifera* were collected from local areas of Bikaner in summer and winter seasons. Plant parts of each plant species were separated, dried in shade and analyzed for estimation of endogenous ascorbic acid. Unorganized cultures with profuse callusing were established using seeds as explants, on Murashige and Skoogs medium supplemented with 2mg/L BAP +1mg/L 2,4-D in *C. pendulus*, 2mg/L BAP + 1.5mg/L IAA in *T. cordifolia* and 1.5 mg/L BAP+1.5mg/L 2,4-D in *M. oleifera*. These cultures were maintained for a period of six months by frequent subculturing at interval of 6 to 8 weeks at 26± 1 °C, 55% relative humidity and diffused light conditions (300 lux). The growth indices (GI) were calculated at different time intervals of 2,4,6,8 and 10 weeks using the formula given below. Cultures at the maximum growth indices were harvested, dried and analyzed for estimation of ascorbic acid.

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

Ascorbic acid was estimated by Chinoy (1962) [3] method. Dried plant parts as well as cultured tissue at the age of maximum GI, were weighed separately, crushed in a mortar in 2% Meta Phosphoric Acid (MPA) (100 mg tissue and seed sample in 1 ml of MPA) and allowed to macerate for one hour. These were then centrifuged separately at low speed (2500 r.p.m.) for fifteen minutes, the residues were discarded and the supernatants were used for the estimation of ascorbic acid following the procedure of Jensen (1962).

Each of the 1 ml test solutions were mixed with 2ml of 5% MPA and kept for 30 minutes without stirring at room temperature. 5ml of n-amyl alcohol and 3.2 ml of dye (5mg/100ml, 2,4-dichlorophenol indophenol) were added and air bubbled through the lower

layer. Each of the test tubes was stoppered tightly, the mixture was shaken vigorously and the upper layer was used for the estimation of ascorbic acid.

The Spectronic-20 colorimeter (Bausch and Lomb) was adjusted at wavelength of 546nm and set at 100% transmittance using a mixture of 1ml of the extract, 2 ml of 5% MPA, 5 ml n-amyl alcohol and 3.2 ml distilled water (bland solution) before taking test samples. Ascorbic acid content present in 1ml of extract was measured by using the regression formula:

$$Y = 0.1103 - (0.14 \times O.D.)$$

Where,

Y = Concentration of ascorbic acid in mg,

O.D. = Optical Density

Ascorbic acid content per 100 gm dry weight was calculated as follows:

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

Where,

A = Y = mg ascorbic acid / ml of original extract

V = total volume of the original extract (in ml)

W = weight of the plant tissue sample (in mg) used for analysis

3. Conclusion. The quantitative estimation of endogenous ascorbic acid was observed in order of root < stem < leaves < flowers < fruits among plant parts of selected plant species. Maximum amount was observed in fruits amongst all analysed plant parts with comparatively highest amount in *M. oleifera* fresh pods (77.67/100g.d.w.). Roots of all species showed comparatively minimum amount with lowest in *C. pendulus* roots (25.60 mg/100g.d.w.). Flowers also contained sufficiently high amount of ascorbic acid. *C. pendulus* showed comparatively minimum amount of ascorbic acid in all parts than *T. cordifolia* and *M. oleifera*. Similar results have been reported in other species also by various workers.

Goyal and Amardeep (1977) in *Abutilon pannosum*, *Lasiurus indicus*, *Ocimum americanum* and *Gossypium* cultivars, Goswami and Solanki (2004) [10] in *Arnebia hispidissima*, *Chenopodium* spp., *Chrozophora prostrata* and *Euphorbia microphylla*. Reddy (2005) in *Cassia angustifolia* reported same variation in amount of endogenous ascorbic acid in vivo. Nag et al. (1986) [7] reported increase from fruiting stage to rooting stage in plants like *Citrullus colocynthis* and *Corchorus depressus*. Mathur (1988) in *Bergia odorata* and *Eclipta alba* while Tyagi (2002) in moth bean cultivars reported decrease in ascorbic acid content from root to stem and increase from leaves to seeds.

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Plant parts of all plant species were collected and analysed in summer as well as winter seasons and it was observed that amount is little higher in summer than winter season except in roots of all selected plant species, as there is no effect of seasonal variation on roots.

Calli raised using seeds as explant in *M. oleifera*, nodal segment in *C. pendulus* and floral buds in *T. cordifolia* on MS medium supplemented with suitable combinations and concentrations of growth hormones were fragile and creamish white in colour. Growth indices in all plant species increased up to eight weeks but declined after that continuously upto twelfth week. However among all plant species *M. oleifera* showed maximum GI (8.34) and *C. pendulus* minimum (6.38). Highest concentration of ascorbic acid was achieved in eight weeks old calli but it was less than plant parts having maximum amount in flowers and fruits. Increased amount of ascorbic acid can be related to fast multiplication and high metabolic rate up to eight weeks while decline after that may be related with transformation of primary metabolites in to secondary metabolites.

Table 1: Growth indices (GI) 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	12 weeks
<i>C. pendulus</i>	0.67±0.08	1.24±0.06	4.01±0.11	6.38±0.26	6.02±0.44	5.34±0.12
<i>T. cordifolia</i>	0.88±0.09	1.62±0.04	4.87±0.09	8.03±0.31	7.81±0.39	6.49±0.15
<i>M. oleifera</i>	0.92±0.06	1.86±0.08	5.01±0.13	8.34±0.35	8.02±0.27	6.68±0.18

Table 2: Endogenous ascorbic acid concentration (mg/100g dry weight) in selected plant species (mean values of five samples)

Name of plant		<i>In vivo</i>					<i>In vitro</i>
		Root	Stem	Leaves	Flower	Fruit	Callus
<i>C. pendulus</i>	W	25.61±0.37	31.89±0.44	36.78±0.16	42.05±0.27	43.20±0.31	35.00±0.26
	S	25.60±0.39	32.02±0.36	36.80±0.29			
<i>T. cordifolia</i>	W	29.21±0.41	42.66±0.31	53.09±0.81	68.61±0.33	72.33±0.42	66.32±0.99
	S	29.22±0.40	42.73±0.11	53.21±0.68			
<i>M. oleifera</i>	W	35.52±0.25	39.27±0.50	58.45±0.46	71.82±0.46	77.67±0.29	68.77±0.23
	S	35.50±0.22	39.31±0.46	58.53±0.39			

W = winter season S = summer season

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