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Production of Ascorbic acid from *Psoralea odorata* (Blatt & Halb) and *Glinus lotoides* L. *in vivo* and *in vitro* Tissue Culture

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Abstract: Investigation of Ascorbic acid contents from root, shoot, fruit and callus tissue of both selected plant species i.e. *Psoralea. odorata* and *Glinus lotoides* were carried out. Laboratory evaluations were made to assess the study of primary metabolites i.e. ascorbic acid of both plant species. Healthy and highly matured plants were collected from local areas of Bikaner region; root, shoot and fruit were separated from intact plants. Nodal part of plants raised and supplemented by frequent sub culturing on Murashige and Skoog's medium. The highest amount of Ascorbic acid from plant parts (52.40 mg/100gdw) reported in fruit of *G. lotoides* and minimum (41.35 mg/100 gdw) in root of *P. odorata*. In callus tissue culture highest amount of ascorbic acid investigated (10.00 mg/100gdw) from 8 weeks old tissue culture of *G. lotoides* while lowest (2.35 mg/100gdw) from 2 weeks old tissue culture of *P. odorata.*
Keywords: Production of Ascorbic acid from *Psoralea odorata* (Blatt & Halb) and *Glinus lotoides* L. *in vivo* and *in vitro* Tissue Culture.

Introduction

Both selected plant is considered as highly nutritious. *Glinus lotoides* (Molluginaceae) is short living perennial prostate herbs. The lipophilic compounds in the plant comprise fatty acid, glycosids of sitosterol, stigma sterol, flavonoid and waxes[1]. Despite the wide-spread use of *G. lotoides* by our community for the treatment of taeniasis. *Psoralea odorata* Blatt & Halb (Fabaceae) delile is an under shrub 30-60 cm. tall and widely distributed in tropical and subtropical region of India. *P. odorata* considered to be therapeutically active in the treatment of leucoderma of non-symphilitic origin. Primary metabolites are of prime importance and essentially required for growth of plants. Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites in of pharmaceutical compounds such as antipsychotic drugs[2]. Ascorbic acid also called as anti-scorbutic (Vitamin - C) is an important primary product and well known for its property as an electron donner in photophosphorylation. The role of ascorbic acid in plant growth and metabolism has been worked out by various workers[3-6]. The ascorbic acid concentration also been detected from arid zone plant species[7-9]. The present work estimated ascorbic acid from plant parts and callus tissue culture of *P. odorata* and *G. lotoides.*

Materials and Methods

Nodal segments of *P. odorata* were surface sterilized with 70% ethanol. These were sterilized for 2-3 min. in 0.1% $Hgcl_2$ solution, rinsed with sterile distilled water under laminar airflow. Nodal segment of plant

were cut into small pieces and aseptically placed on MS. Medium[10]. Supplemented with 4 mg/l BAP+1 mg/L 2, 4-D for *P. odorata.* All the media used throughout this study were supplemented with 3% sucrose and 7% agar. The pH was adjusted to 5.80with 1N NaOH or .1N Hcl before autoclaving at 121^{0}C and 15-lb psi for 20 min. The plant parts and eight weeks old callus tissue (Maximum growth index) of both plant species were used for estimation of ascorbic acid contents.

Estimation of Ascorbic Acid

Ascorbic acid was estimated using the protocol of Chinoy[11]. Dried plant parts 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.

Each of the 1 ml test solutions were mixed with 2ml of 5% MPA and kept for 30 minutes without stirring at room temperature. 5 ml of n-amyl alcohol and 3.2ml of dye (5mg/100ml, 2, 4-dichlorophenol indophenols) were added and air bubbled through the lower layer. Each of the test tubes was stoppered tightly, the mixture was shaken vigorously and the supper 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, 2ml of 5% MPA, 5ml n-amyl alcohol and 3.2ml distilled water (bland solution) before taking test samples. Ascorbic acid content present in 1ml of extract was measured by using the regression formula:

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

Where, Y = Concentration of ascorbic acid in mg, O.D. = Optical Density.
Ascorbic acid content per 100 gm dry weight was calculated as follows:

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

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.

Results and Discussions

Ascorbic acid (vitamin C) is a familiar molecule because of its dietary significance, it is not only an important antioxidant it also appears to link flowering time, developmental senescence, programmed cell death and response to pathogen through a complex signal transduction network. The present study shows that the highest amount of ascorbic acid observed in the fruit of *G. lotoides* (52.40 mg/100gdw) while lowest observed in root of *P. odorata* (41.35 mg/100gdw) as compared both plants species, *G. lotoides* shows highest production of ascorbic acid then another plant.

In callus tissue culture investigation shows that the amount of ascorbic acid was highest reported in 8 weeks old tissue culture (10.00 mg/100 gdw) of *G. lotoides* and it was lowest reported in 2 weeks old tissue culture (2.35 mg/100gdw) of *P. odorata*).

Table 1.1: Ascorbic acid from different plant parts of *P. odorata* and *G. lotoides* in mg/100gdw.

Plant species	Root	Shoot	Fruit
P. odorata	41.35	42.30	43.50
G. lotoides	44.25	50.28	52.40

Figure 1.1 : Ascorbic acid from different plant parts of *P. odorata* and *G. lotoides* in mg/100gdw.

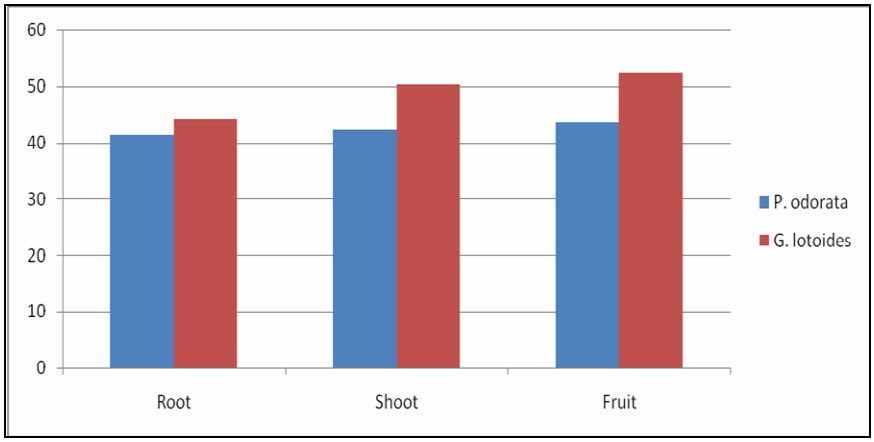
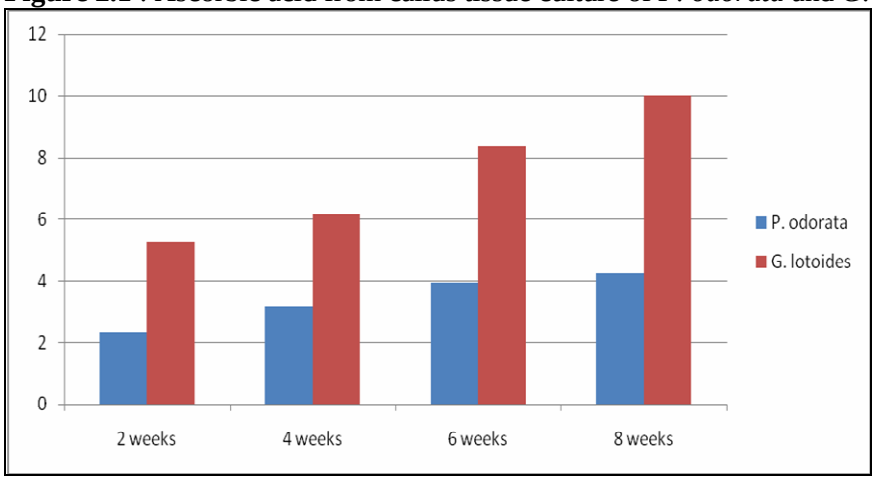


Table 1.2 Ascorbic acid from callus tissue culture of *P. odorata* and *G. lotoides* in mg/100gdw.

Age of callus	*P. odorata*	*G. lotoides*
2 weeks	2.35	5.25
4 weeks	3.18	6.15
6 weeks	3.95	8.35
8 weeks	4.23	10.00

Figure 2.1 : Ascorbic acid from callus tissue culture of *P. odorata* and *G. lotoides* in mg/100gdw.



Conclusion

The investigation shows that the both plant species and their cultures are good source for the production of ascorbic acid contents.

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