International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2014): 5.611

Ascorbic Acid (antioxidant) in *Ailanthus excelsa and Balanites aegyptiaca* and Effect of Growth Regulators and Salts on it *in vitro*

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Abstract: ***Ailanthus excelsa and Balanites aegyptiaca are two wild growing common plant species of desert, having medicinal importance. Unorganized tissues of these useful plants were established on MS medium supplemented with 1.0mg/L BAP+1.5mg/L 2,4-D and 1.5mg/L BAP +2.0mg/L 2,4-D respectively (standardized MS medium). Parts of established tissues were transferred to standardized (Sd) MS medium fed with various concentrations (1,2,3 mg/L) of growth regulators (IAA,NAA) and (10,20,30mg/L) salts (NaCl , KCl) separately. Tissues at the maximum GI (in all samples) were harvested, dried, powdered and analyzed for estimation of ascorbic acid. Maximum amount of ascorbic acid was calculated in callus fed with 1mg/L IAA, NAA and 20 mg/L NaCl and KCl both in Ailanthus excelsa and Balanites aegyptiaca.***

Keywords: Ailanthus excelsa , Balanites aegyptiaca , antioxidants, growth regulators, salts

1. Introduction

Ardu *(Ailanthus excelsa)* belonging to family **Simaraubaceae** is a large deciduous tree having a lot of medicinal uses. It is a native of India and Sri Lanka. The plant is recognized for its varieties of medicinal uses like contraceptive, post partum treatment, intestine tape worm, dysentery, epilepsy, heart troubles, asthma etc. The plant also having anticncerous, antibacterial, antimicrobial and antifungal activity. Two new tetracyclic triterpenes (ailexcelone and ailexcelol) were isolated from the heartwood of ***Ailanthus excelsa*** which are tested for their antifungal activity (Srinivas *et al,* 2006).

Balanites eagyptiaca **"Desert Date"** of family **Zygophyllaceae** is a common wild plant found in many kind of habitates, tolerating a wild variety of soil types from sand to heavy clay and climatic moisture levels. It is believed indigenous to all dry lands.

The root, stem bark, fruit pulp and kernel cake of ***Balanites eagyptiaca*** have medicinal properties. Fruit is used in whooping cough also in leucoderma and other skin diseases. It is used as an oral hypoglycemic and an antidiabitic . An aqueous extract of the fruit mesocarp is used in Sudanese folk medicine in treatment of jaundice. It is also used to treat dysentery and constipation. Fruit is used to treat liver diseases and as a purgative and sucked by school children as a confectionary in some countries.

Balanites eagyptiaca contains steroids (saponins, sapogenins, diosgenins) used as raw material for industrial production of contraceptive pills, corticoids, anabolisants and other sexual hormones. The saponins occurring in roots ,wood chips and fruits facilitate their use for washing clothes.

Antioxidant is simply a molecule that prevents another molecule from oxidizing. Since there are many processes in the body which result in oxidation . The intake of antioxidant is essential to counteract some of the negative results of the buildup of too many oxidized molecules in the body.

Primary metabolites are produced as a result of photosynthesis by which green plants utilize solar energy to yield the photosynthetic product-Carbohydrate. Besides this process some other primary synthetic processes also occur in plants which yield certain vital products such as proteins, amino acids, minerals and other nutritive contents, ascorbic acid, lipids, vitamins, nucleotide and energy compounds like alcohols, organic acids etc.

Ascorbic Acid or **Vitamin „C"** is claimed as a 'Cure all' for many human diseases and problems from cancer to common cold. Ascorbic acid is required in synthesis of collagen, neurotransmitters, steroid hormones etc. Vitamin C promotes the healing of wounds, bone fractures, bruises, hemorrhages, bleeding gums and forms the protective barrier between infections or disease and the surrounding healthy tissue. As an **antioxidant** it has many beneficial functions in combating many diseases and infections and also promotes proper calcium absorption. In plants, ascorbic acid is essential for photosynthetic activity via the detoxification of super oxide and hydrogen peroxide (H_2O_2) in chloroplasts in the absence of catalyses. Thus, it acts as a reducing agent in biological systems. It also assists in healthy cell development as well as normal tissue growth and repair. Vitamin C is a water soluble vitamin.

Free endogenous ascorbic acid production has been reported in tissue culture of ***Momordica charantia*** and ***Emblica officinalis*** (Mohan *et al.,*1974), ***Datura metel*** and ***Datura tatula*** (Nag *et al*., 1974), ***Trigonella foenum-graceum*** (Jain *et al.,* 1975), ***Ephedra foliata, Helianthus annus, Agave wightii*** and ***Tephrosia purpurea*** (Khanna *et al.,* 1977) ; ***Solanum xanthocarpum*** (Manot, 1977), ***Atropa belladona*** (Sharma, 1977), ***Papaver somniferum*** (Gaur, 1978, Khanna *et al.,* 1977),***Daucus carota*** (Sogani, 1978), ***Solanum nigrum*** (Rathore *et al*., 1979), ***Tribulus alatus*** and ***Zygophyllum simplex*** (Jit *et al.,* 1986), ***Lycium bararum***

(Nag and Grover, 1987),***Eclipta alba*** (Mathur,1988),***Seetzenta orientatis*** (Sethia,1988),***Calligonum polygonoides*** and ***Lasiurus sindicus*** (Bhojak,1991), ***Tinospora cordifolia*** (Goswami and Yadav,1994),***Lycium barbarum*** (Mukhi, 1995), ***Peganum harmala*** (Badia, 1999), ***Arabidopsis*** cell suspension cultures (Davery *et al.,* 1999), ***Ribes nigrum*** (Viola *et al.,* 2000),***Vigna aconitifolia*** (Tyagi, 2002), ***Capparis decidua*** and ***Ziziphus sp.*** (Chauhan, 2003),***Cassia angustifolia*** (Reddy, 2005), ***Balanitis aegyptiaca*** (Bedawat, 2006), ***Ailanthus excelsa*** (Rao, 2007), ***Adhatoda vasica*** and ***Barleria prionitis*** (Deepa, 2009), ***Cocculus pendulus*** and ***Tinospora cordifolia*** (Yadav, 2010), ***Moringa oleifera***(Talreja, 2010), ***Terminalia arjuna*** (Sharma,2012), three medicinal plants (Khandelwal *et al*., 2014).

2. Materials and Methods

Unorganized tissue of ***Ailanthus excelsa*** and ***Balanites aegyptiaca*** were established on MS medium supplemented with 1.0mg/L BAP+1.5mg/L 2,4-D and 1.5mg/L BAP +2.0mg/L 2,4-D respectively (standardized MS medium). Parts of established tissues were transferred to standardized (Sd) MS medium fed with various concentrations (1,2,3 mg/L) of growth hormones ((IAA, NAA) and (10, 20, 30mg/L) salts (NaCl , KCl) separately. GI was calculated in all samples. Tissues at the maximum GI (in all samples of both plant species) were harvested, dried, powdered and analyzed for estimation of ascorbic acid.

Extraction Procedure

Ascorbic acid was estimated by **Chinoy (1962)** 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 cultured tissue in 1 ml of MPA) and allowed to macerate for one hour. These were then centrifuged separately at low speed (2500 rpm) 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 2 ml of 5% MPA and kept for 30 minutes without stirring at room temperature. 5 ml of n-amyl alcohol and 3.2 ml of dye (5 mg/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 546 nm and set at 100% transmittance using a mixture of 1 ml of the extract, 2 ml of 5% MPA, 5 ml n-amyl alcohol and 3.2 ml distilled water (blank solution) before taking test samples.

Ascorbic acid content present in 1 ml of extract was measured by using the regression formula:

Y = 0.1103 – (0.14 X 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:

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

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. Results and Discussion

Maximum GI was observed at the age of eight weeks in standardized (Sd) MS medium and standaized MS media supplemented with various concentrations (1,2,3 mg/L) of growth hormones (IAA and NAA) and salts (NaCl and KCl at 10,20,30 mg/L) in ***A. excelsa*** and ***B. aegyptiaca*** . Calli were harvested at maximum GI from all the samples separately in both plants and analyzed for ascorbic acid content .

It was observed that amount of ascorbic acid was increased in callus fed with growth regulators IAA and NAA. Increase was from Sd MS medium to Sd MS medium fed with 1mg/L but after that amount decreased in Sd MS medium fed with 2 mg /L up to Sd MS medium fed with 3mg/L IAA and NAA separately in both plant species. The amount calculated in calli fed with 3mg/L IAA and NAA was even lower than amount of ascorbic acid present in callus grown on Sd MS medium. Maximum amount of ascorbic acid was calculated in callus fed with 1mg/L IAA and NAA in ***A. excelsa*** and ***B. aegyptiaca*** (Table 1.1).

In calli fed with salts, the amount of ascorbic acid was increased from Sd MS medium to calli fed with 10 mg/L KCl, NaCl and from 10 mg/L to 20 mg/L but decreased in 30 mg/L in both plant species. Maximum amount was calculated in calli fed with 20 mg/L NaCl and KCl in ***A. excelsa*** and ***B. aegyptiaca*** (Table 1.2).

Grotwh hormones showed positive response than salts as amount was comparatively higher in calli fed with growth hormones than salts in both plant species. ***B. aegyptiaca*** has higher amount of ascorbic acid than ***A. excelsa*** in all samples.

References

[1] Aberg, B. (1958). Ascorbic acid (Formation, storage, mobilization and transformation of carbohydrates). In: Encyclopedia of Plant Physiology. Ruhland, W. (Ed.) Springer Verlag, Berlin. 6:479-499.
[2] Badia, N. (1999). Influence of plant growth regulators and precursors on the production of primary and secondary metabolites in tissue culture of ***Peganum harmala*** Ph. D. Thesis, M. D. S. Univ., Ajmer, Rajasthan.
[3] Bedawat, S. (2006). Evaluation of ***Balanites aegyptiaca*** - an arid zone medicinal plant for its phytochemically important metabolites. Ph.D. Thesis, M.D.S. Univ. Ajmer, Rajasthan.
[4] Bhojak, S. (1991). Phytochemical investigation of some fodder plants of arid zone of Rajasthan *in vivo* and *in vitro*. Ph. D. Thesis, M. D. S. Univ., Ajmer, Rajasthan.

[5] Chauhan, N. (2003). Pharmaceutically important compounds from *in vivo* and tissue cultures of ***Capparis*** and ***Ziziphus*** growing in arid zone of Rajasthan. Ph.D. Thesis. M.D.S. University, Ajmer.
[6] Deepa (2009). *In vivo* and *in vitro* comparative studies of primary and secondary metabolites of two medicinally important plants belonging to family Acanthaceae.Ph. D. Thesis, M. G. S. Univ., Bikaner, Rajasthan.
[7] Davery, M. W.; Gilot, C.; Persiau, G.; Ostergaard, J.; Han, Y.; Bauw, G. C. and Van, Montagu, M. C. (1999). Ascorbate biosynthesis in ***Arabidopsis*** cell suspension culture. Plant Physiol. 121:535-543.
[8] Gaur, M. (1978). Production of primary and secondary products from *in vitro* plant tissue cultures. Ph. D. Thesis, Univ. of Rajasthan, Jaipur.
[9] Goswami, A. and Yadav, L. (1994). Seasonal evaluation of ascorbic acid contents of two plant species. Physiol. Res. 7 (1) 87-88.
[10] Goswami, A. and Solanki, S. (2005). Endogenous Ascorbic acid provided to the animals by Thar desert plants. Oikoassay 17 (1 & 2) 17-19.
[11] Goswami, A.; Deepa and Reddy, A. (2008).Endogenous ascorbic acid from some medicinal plants *in vivo* and *in vitro* Ind. J. Phyto. Res. 21 (2) 293-231.
[12] Jain, S. C.; Nag, T. N.; Mohan, S and Khanna, P. (1975). Ascorbic acid metabolism and growth in plant tissue cultures. Science and Culture 41: 292- 293.
[13] Jit, S.; Shekhawat, S. S. and Nag, T. N. (1986). Free ascorbic acid and growth of tissues from *in vitro* tissue cultures. Comp. Physiol. Ecol. 11(3)103-107.
[14] Khandelwal, S.; Rishi, A. and Khurana, S.M.P (2014). Estimation of primary and secondary metabolites from leaves of three medicinal plants. International Res. J. of Pharmacy. 5(10)783-785.
[15] Khanna, P.; Kamal, R. ; Taparia, R. and Jain, S.C. (1977). A quantitative estimation of free ascorbic acid and growth of tissue from *in vitro* tissue cultures. Vidya. B. Sciences. Vol. XX (1)1-6.
[16] Mathur, K. (1988). Primary and scondary metabolites from some arid zone plants of Rajasthan growing *in vivo* and *in vitro*. Ph. D. Thesis, Univ. of Rajasthan, Jaipur.
[17] Mohan, S.; Nag, T. N.; Jain, S. C. and Khanna, P. (1974). Free ascorbic acid from plant tissue cultures. Vidya B. Science 17: 8-13.
[18] Mukhi, B. (1995). Influence of plant growth regulators on product biosynthesis in tissue culture of ***Lycium barbarum*** Ph. D. Thesis, M.D.S. Univ., Ajmer, Rajasthan.
[19] Nag, T. N.; Jain, S. C.; Mohan, S. and Khanna, P. (1974). Ascorbic acid metabolism and growth in ***Datura*** tissue culture. Ind. J. Pharm. 36:49-50.
[20] Nag, T. N. and Grover, S. (1987). Free ascorbic acid from ***Lycium barbarum*** tissue culture. Ind. J. Bot. 10(1)14-16.
[21] Rathore, A. K.; Sharma, G. L. and Khanna, P. (1979). Free ascorbic acid from ***Solanum nigrum*** tissue cultures. Ind. J. Bot. 2:42-44.
[22] Reddy, A. (2005). Biochemical investigation of economically important plant of arid zone of Rajasthan- ***Cassia angustifolia*** - *in vivo* and *in vitro*. Ph. D. Thesis, M. D. S. Univ., Ajmer, Rajasthan.
[23] Sethia, M. (1988). Phytochemical analysis of some arid zone plants of Rajasthan growing *in vivo* and *in vitro*. Ph.D. Thesis. University of Rajasthan. Jaipur. India.
[24] Sharma,P.(2012). *In vivo* and *in vitro* phytochemacal studies of medicinally useful tree ***Terminalia arjuna***. Ph.D. Thesis, M.G.S. Univ. Bikaner, Rajasthan .
[25] Sogani, M. (1978). Production of primary and secondary products from *in vivo* and *in vitro* tissue cultures. Ph. D. Thesis, Univ. of Rajasthan, Jaipur.
[26] Tyagi, S. (2002). Comparative analysis of phytochemical in **Moth bean** cultivars growing in field
[27] and in tissue culture. Ph. D. Thesis, M. D. S., Ajmer, Rajasthan.
[28] Yadav, L. (2010). *In vivo* and *in vitro* phytochemical studies of two medicinally important plants belonging to family Menispermaceae. Ph.D. Thesis, M.G.S. Univ. Bikaner, Rajasthan .

Table 1.1: Effect of Growth Regulators on Ascorbic Acid Content (mg/100 g.d.w.) IN *A. excelsa* AND *B. aegyptiaca IN VITRO* (AT MAXIMUM GI)

Name of Plant	Sd MS Medium	Growth Regulators					
		IAA/L			NAA/L		
		1mg	2mg	3mg	1mg	2mg	3mg
A. exselsa	0.40±0.04	0.42±0.06	0.40±0.04	0.36±0.04	0.41±0.04	0.39±0.04	0.35±0.05
B. aegyptiaca	0.46±0.05	0.48±0.05	0.46±0.06	0.42±0.06	0.47±0.04	0.45±0.07	0.40±0.04

Values are mean of five replicates ± SD

Table 1.2: Effect of Salts on Ascorbic Acid Content (mg/100 g.d.w.) in *A. exselsa* AND *B. aegyptiaca IN VITRO* (AT MAXIMUM GI)

Name of Plant	**Sd MS Medium**	**Salts**					
		NaCl/L			**KCl/L**		
		10mg	**20mg**	**30mg**	**10mg**	**20mg**	**30mg**
A. excelsa	0.40±0.04	0.42±0.06	0.43±0.06	0.36±0.07	0.41±0.05	0.42±0.04	0.35±0.04
B. aegyptiaca	0.46±0.05	0.48±0.06	0.48±0.06	0.43±0.07	0.47±0.05	0.48±0.05	0.42±0.04

Values are mean of five replicates ± SD