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 Aegle Marmelos and Moringa Oleifera and Effect of Growth Regulators and Salts on it in Vitro

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Abstract: ***Aegle marmelos and Moringa oleifera are two medicinally important plant species. Unorganized tissues of these plants were established on MS medium supplemented with 1.0mg/L BAP+2.0mg/L 2,4-D and 1.5mg/L BAP +1.5mg/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 2mg/L IAA, NAA and 10 mg/L NaCl and KCl in Aegle marmelos as well as Moringa oleifera.***

Keywords: ***Aegle marmelos***, ***Moringa oleifera***, antioxidant, growth regulators, salts

1. Introduction

Aegle marmelos* Corr.** commonly known as "**Bael**" is a medium sized slow growing deciduous, spiny, woody fruit tree of tropics. It belongs to family **Rutaceae**. Bael is a sacred tree , dedicated to Lord Shiva. It is extensively planted near Hindu temples for its leaves and wood used in worship and edible fruits in indigenous medicines. Pharmaceutically ***A. marmelos is having antibacterial, antihistaminic, anti inflammatory, anticonvulsant, anti stress, adaptogenic, antipyretic, antifertility, analgesic, hepato protective, insecticidal, hypoglycemic, cardiotonic, immune modulatory and wound healing activities.

M. oleifera commonly called "**Sigru**" belongs to family **Moringaceae**. All parts of ***M. oleifera*** tree such as root, root bark, leaves, flowers, unripe pods, seeds, seed oil are used in Ayurvedic system of medicine. Sigru is used externally as well as internally. Externally, the paste of its leaves and bark skin is applied in boils to subside the swelling and inflammation. Internally Sigru is used in vast range of diseases. Pods are recommended in loss of appetite. It is also beneficial in treatment of worms anorxia, ascites, tumors, abdominal pain, paralysis, joint pain and gout.

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 ***Aegle marmelos*** and ***Moringa oleifera*** were established on MS medium supplemented with 1mg/L BAP+2.0mg/L 2,4-D and 1.5mg/L BAP +1.5mg/L 2,4-D respectively (standardized MS media for both species). 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 (grown on Sd MS medium, Sd MS medium with all variations of growth regulators and salts in 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. marmelos*** and ***M. oleifera*** . Calli were harvested at maximum GI from all the samples separately in both plants and analysed 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 continuous from Sd MS medium to Sd MS medium fed with 1mg/L to Sd MS medium fed with 2 mg /L IAA and NAA but after that amount decreased in 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 2mg/L IAA and NAA in ***A. marmelos*** as wll as ***M. oleifera*** .

In calli fed with salts KCl and NaCl , the amount of ascorbic acid was increased from Sd MS medium to calli fed with 10 mg/L and then decreased from 10 mg/L to 20 mg/L upto 30 mg/L in both plant species. Maximum amount was calculated in calli fed with 10mg/L NaCl and KCl in ***A. marmelos*** as wll as ***M. oleifera***.

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

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Table 1.1: Effect of Growth Hormones on Ascorbic Acid Content (mg/100 g.d.w.) IN *A. marmelos* and *M. oleifera In Vitro* (At Maximum GI)

Name of Plant	*Sd MS Medium*	*Growth Hormones*					
		IAA/L			*NAA/L*		
		1mg	*2mg*	*3mg*	*1mg*	*2mg*	*3mg*
A.marmelos	0.68±0.04	0.71±0.03	0.74±0.05	0.65±0.04	0.71±0.03	0.73±0.04	0.63±0.05
M. oleifera	0.84±0.05	0.87±0.04	0.90±0.06	0.81±0.05	0.86±0.04	0.89±0.05	0.80±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. marmelos* and *M. oleifera In Vitro* (At Maximum GI)

Name of Plant	*Sd MS Medium*	*Salts*					
		NaCl/L			*KCl/L*		
		10mg	*20mg*	*30mg*	*10mg*	*20mg*	*30mg*
A.marmelos	0.68±0.04	0.71±0.05	0.67±0.06	0.64±0.05	0.70±0.05	0.66±0.04	0.62±0.03
M. oleifera	0.84±0.05	0.87±0.06	0.82±0.06	0.80±0.05	0.85±0.05	0.82±0.04	0.80±0.04

Values are mean of five replicates ± SD