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Effect of Precursor β-phenylalanine on Production of Flavonoids of *Maytenus emarginata in vitro*

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Abstract: ***Medicinally important Maytenus emarginata is an ever green tree of desert useful against various diseases. Flavonoids have been reported in its plant parts. In present study unorganized tissue raised on MS medium supplemented with1.5 mg/L BAP +1 mg/L NAA, harvested at maximum GI, were analysed for qualitative and quantitative estimation of flavonoids by Subramanian and Nagarajan (1969) method. These tissues were fed with different concentration of dl β-phenylalanine (25, 50, 75, 100 mg/100ml) to observe the effect on GI and quantitative production of flavonoids. Maximum GI and amount of flavonoids (leutiolin, kaempferol, quercetin and total flavonoid) was estimated in eight weeks old tissue fed with 75 mg/100ml phenylalanine.***

Keywords: *Maytenus emerginata,* β-phenylalanine, flavonoids, medicinal plant, GI

1. Introduction

Maytenus emerginata* (Willd.)** is an ever green tree that tolerates various types of stresses of the desert and is found in drier parts of central, south –western and north western India. ***Maytenus have been used for fever, asthma, rheumatism and gastrointestinal disorders, carcenoma and leukemia, gastrointestinal troubles etc. Medicinal plants are rich source of secondary metabolites, biosynthetically derived from primary metabolites but restricted to specific taxonomic genera of plant kingdom and specific part of plant body. Secondary plant products are of major interest because of their biological activities ranging from antibacterial, antibiotic, insecticidal, hormonal, pharmacological and pharmaceutical.

β-phenylalanine amino acid is the precursor of flavonoids (Harborne, 1965 Barz, 1977). Biosynthetic scheme of flavonoids based mainly on C14 labelling experiments suggest that the C15 skeleton of the flavonoids is derived from phenylalanine through cinnamic acid, under the shikimik acid pathway. Effect of dl-β-phenylalanine on flavonoids has been studied in tissue cultures ***Tribulus terrestris*** (Saluja, 1981), ***Agave wightii*** (Sharma, 1982), ***Cheiranthus cheiri*** (Agarwal, 1982), ***Tribulus alatus*** (Jit, 1985), ***Lycium barbarum*** (Shekhawat, 1985), ***Zygophyllum simplex*** (Mathur, 1988), ***Seetzenia orientalis*** (Sethia, 1988), ***Abutilon pannosum, Ocimum americanum*** (Singh, 1988), ***Calligonum polygonoides, Lasiurus sindicus*** (Bhojak, 1991), ***Fagonia cretica*** (Kapoor, 1991), ***Lycium barbarum*** (Mukhi, 1995), ***Gossypium cultivars*** (Kaur, 1997, Goyal, 1997), ***Peganum harmala*** (Badia, 1999), ***Fragaria ananassa*** (Mori *et al.,* 2001), ***Vigna aconitifolia*** (Tyagi, 2002), ***Withania somnifera*** (Bains, 2002), ***Zizyphus mauritiana*** (Chauhan, 2003), ***Spirodela intermedia*** (Gitz *et al.,* 2004), ***Cassia angustifolia*** (Reddy, 2005), ***Balanites 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).

2. Material and Method

Murashige and Skoog's medium (1962) supplemented with 1.5 mg/L BAP and 1.0 mg/L NAA was used for establishment of unorganized tissue of ***M.emarginata***. Callus was maintained by frequent sub culturing at interval of 6 to 8 weeks at 26 ±1ºC, 60% relative humidity and diffused light conditions (3000 lux). Growth Indices (GI) of tissues were calculated at 2,4,6,8 and 10 weeks time intervals. Tissues were harvested at different age intervals and their growth indices (GI) were calculated.

Growth Index (GI)

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

These tissues established on MS medium (Standrized by1.5 mg/L BAP and 1.0 mg/ L NAA) were supplemented with various concentrations of dl β-phenylalanine (25, 50, 75 and 100mg/100ml) to observe the effect on GI and quantitative amount of flavonoids. GI was calculated at the age of 2,4,6,8, 10 and 12 weeks separately for each concentration of phenylalanine. Tissues were harvested at maximum GI separately, dried, powdered and analysed for flavonoids by **Subramanian and Nagarajan,** (1969) method.

3. Analysis of flavonoids

Tissue samples of M***. emarginata*** supplemented with various concentrations of phenylalanine were air dried, weighed, powdered, soxhlet extracted separately with 80% hot ethanol on a water bath for 24 hr to extract flavonoids and filtered. Filtrate was re-extracted with petroleum ether (Fr I), ethyl ether (Fr II) and ethyl acetate (Fr III) in succession following the method of **Subramanian and Nagarajan** (1969).Each step was repeated three times to ensure complete extraction in each case. Petroleum ether (Fr.I) was rejected due to its richness in fatty substances where as Ethyl ether (Fr II) was analyzed for free flavonoids while the Ethyl acetate fraction was hydrolyzed with 7% H_2SO_4 for 2 hr. The mixture was filtered, the filtrate extracted with ethyl acetate was

neutralized with distilled water and then dried in vacuum and analyzed for bound flavonoids.

3.1 Qualitative and quantitative estimation

Plates were developed in an air tight chromatography chamber containing about 200 ml of solvent mixture of n-butanol, acetic acid and water 4:1:5 (upper layer). Developed plates were air dried and visualized under UV light 254 nm which showed one fluorescent spot in ethyl ether fraction second and two spots in ethyl acetate fraction third.The plates were also placed in a chamber saturated with ammonia vapours to observe the colours of the spots. The developed plates were also sprayed the with 5% ethanolic ferric chloride solution for further confirmation. Each of the isolates was purified by preparative TLC in similar solvent system. Isolates were eluted with ethanol, crystallized by $CHCl_3$ and further confirmed by melting points, UV maxima on spectrophotometer and infra red spectral studies. Quantitative estimation of the identified flavonoids was carried out colorimetrically following method of **Kariyon *et al.*** 1953 and **Naghski*et al.*** 1975 in case of quercetin and of **Mabry *et al.*** 1970 in case of luteolin and kaempferol.

4. Results and Discussion

Growth indices (GI) of unorganized tissues of ***M. Emarginata*** established and multiplied on standardized MS medium (MS + 1.5 mg/L BAP + 1.0 mg/L NAA) and supplemented with different concentrations of β-phenylalanine (25, 50, 75 and 100 mg/100ml) separately, were calculated.

GI of callus was found to be increased from standardized MS medium to STMS medium fed with 25mg PA/100ml to 50mg PA/100ml (3.43 and 4.15 respectively) and maximum (4.42) with 75 mg/100ml phenylalanine fed medium at all growth intervals (2, 4, 6, 8, 10, and 12 weeks). Maximum GI was observed at the age of eight weeks old tissues fed with all concentrations of phenylalanine. **(Table.1)**

Table 1: Effect of β-phenylalanine on growth index of *M.emarginata* tissue cultures

MEDIUM	2 WEEKS	4 WEEKS	6 WEEKS	8 WEEKS	10 WEEKS	12 WEEKS
ST MS Medium	0.26± 0.24	0.49± 0.19	1.89± 0.20	3.24± 0.33	3.03± 0.17	2.11± 0.23
MS + 25 mg PA/100 ml	0.29± 0.21	0.54± 0.12	2.00± 0.11	3.43± 0.10	3.28± 0.11	2.03± 0.20
MS + 50 mg PA/100 ml	0.36± 0.18	0.73± 0.13	2.69± 0.15	4.15± 0.13	3.97± 0.18	3.03± 0.19
MS + 75 mg PA/100 ml	0.41± 0.15	0.92± 0.12	2.98± 0.13	4.42± 0.19	4.01± 0.20	3.51± 0.16
MS +100 mg PA/100 ml	0.21	0.11	0.13	0.14	0.14	0.13

Mean value of five replicates ± SD

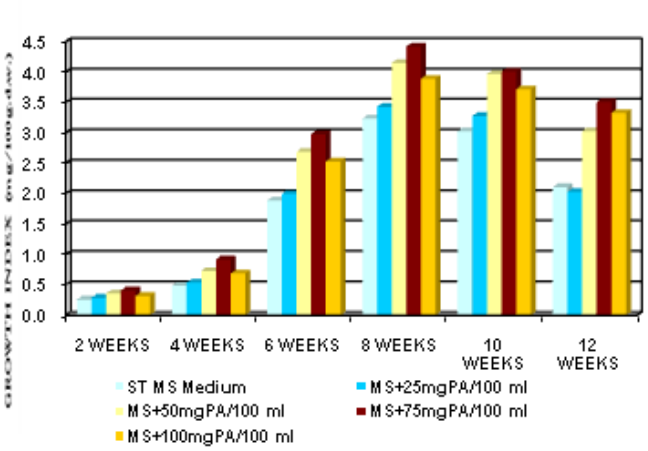

Figure 1: Effect of β-phenylalanine on growth index of *M.emarginata* tissue cultures.

Individual as well as total flavonoid content showed gradual increase in amount present in tissue grown on standardized medium (MS) to tissues fed with 25 mg PA/100ml medium, up to maximum in tissue fed with 75 mg PA/100ml. Maximum amount of leutiolin (0.70mg/100g.d.w.), kaempferol (0.68mg/100g.d.w.), quercetin (0.64mg/100g.d.w.) and total flavonoid (2.02 mg/100g.d.w.) was observed in the tissue grown on standardized MS medium fed with 75 mg PA/100ml. After that the amount started decreasing in tissues fed with 100 mg PA/100 ml (0.60 mg/100 g.d.w, 0.54 mg/100 g.d.w, 0.50 mg/100 g.d.w. and 1.64 mg/100 g.d.w. respectively) **(Table 2).**

Table 2: Effect of β-phenylalanine on flavonoid content (mg/100 g.d.w.) in tissue cultures of *M. Emarginata*

MEDIUM	Leuteolin	Kaempferol	Quercetin	Total Flavonoid
ST MS Medium	0.60±0.02	0.53±0.03	0.48±0.05	1.61±0.06
MS + 25 mg PA/100 ml	0.62±0.03	0.53±0.04	0.52±0.05	1.70±0.04
MS + 50 mg PA/100 ml	0.65±0.04	0.60±0.03	0.57±0.04	1.82±0.03
MS + 75 mg PA/100 ml	0.70±0.02	0.68±0.03	0.64±0.06	2.02±0.05
MS +100 mg PA/100 ml	0.60±0.05	0.54±0.04	0.50±.03	1.64±0.04

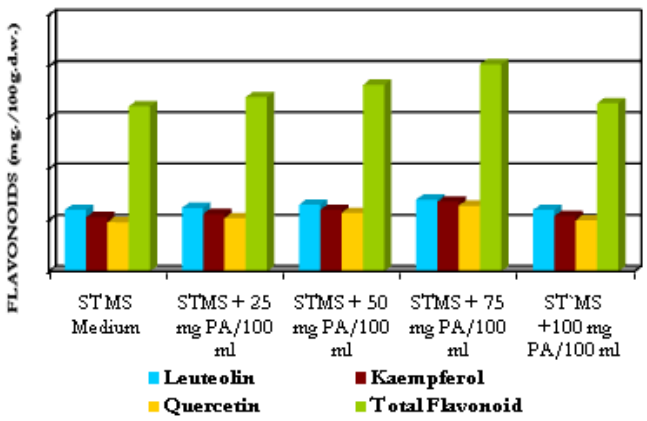

Figure 2: Effect of β-phenylalanine on flavonoid content (mg /100 g.d.w.) in tissue cultures of *M. Emarginata*

In all observations amount of leutiolin was slightly more than kaempferol and kaempferol than quercetin as in tissue grown on standardized MS medium as well as MS medium fed with all concentrations of phenylalanine.

5. Conclusion

The present study shows that plants and their tissue cultures retain the potential to synthesize flavonoids in fair amount. Dl-β-Phenylalanine being one of the precursors of flavonoids, when fed into the MS medium, increased the production of flavonoids appreciably, up to a certain limit. Hence, it can be concluded that specific concentration of phenylalanine can be fed into the medium, to obtain higher concentration of flavonoids, for large scale production being medicinally useful. All these findings strengthen the view of Barz (1977) that phenylalanine is a precursor of flavonoids and plays important role in flavonols biosynthesis.

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