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Production, Isolation and Estimation of *Maytenus Emarginata* flavonoids *in vivo* and *in vitro*

Shweta Mathur[1], Asha Goswami[2]

Plant tissue culture and Biotechnology Lab, Department of Botany, Govt. Dungar College, Bikaner, Rajasthan, India

Abstract: ***Maytenus emerginata Willd. is an ever green medicinally useful tree that tolerates various types of stresses of the desert. In present study Maytenus emerginata plant parts stem, leaves, fruits and flowers and unorganized tissue established on MS medium supplemented with 1.5 mg/L BAP+1 mg/L NAA was analysed for flavonoid content by Subramanian and Nagrajan 1969 method. Flavonoids have been identified and confirmed by TLC, M.P., UV maxima and IR studies. The maximum amount of flavonoids in vivo luteolin, kaempferol, quercetin and total flavonoid content has been estimated in flowers and minimum in leaves. Individual as well as total flavonoid content was estimated little higher in unorganized tissue in vitro than flowers showing highest amount in vivo.***

Keywords: *Maytenus emerginata,* flavonoids, medicinal plant

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*** plant parts 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, pharmaceutical.

Flavonoids, one of the secondary metabolites, are water soluble phenolic glycosides imparting colour to flowers and fruits of higher plants. They have multiple biological effect including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic etc. Their contribution to physiological functions such as seed maturation and dormancy has already established Brenda, 1998.Presence of flavonoids has been reported from many plant species like ***Agave Americana*** Subramanian and Nair, 1970, ***Arachis hypogea*** Pratt and Miller, 1984,***Citrus*** Anis and Aminuddin,1981, Park *et al*., 1983, ***Citrullus colocynthis***, ***Corchorus depressus***, ***Fagonia cretica***, ***Lysium barbarum*** Harsh *et al*., 1983, ***Crotolaria retusa*** and ***C. striata*** Subramanian and Nagrajan,1969, ***Glycine max*** Hahlbrock,1972, ***Passiflora palmeri*** Ulubelen *et al*., 1984, ***Prosopis spicigera*** Bhardwaj *et al.*, 1979, ***Tribulus pentendrus*** and ***T. terrestris*** Saleh Nabiel *et al*.,1982.

The vital role of flavonoid is defences against pathogens and predators and contributes to physiological functions such as seed maturation and dormancy Winkel Shirley, 2001, 2002. The influence of flavonoids on lipid peroxidation shows that they could protect endogenous urate from oxidative degration and demonstrates an antioxidant synergy between urate and the flavonoids. They also show biological effects such as anti-anginal, antiallergic, anti-ulcer, anti-hepatotoxic, anti-viral, anti-inflammatory and anti-spasmolytic. Flavonoids: quercetin and rutin protect living cells against DNA strand breaks by way of their metal ion chelating mechanism. Quercetin play a significant role in the reduction of inflammatory naturally associated with the aging process. It is naturally occuring plant compounds with anti-ulcer activity.

2. Materials and Methods

Plant parts of ***M. emerginata*** like leaves, stem, flowers and fruits were collected from local area, separated, dried and powdered for analysis of flavonoids by **Subramanian and Nagarajan** 1969 method.

For in vitro studies various explants nodal segments, shoot apices and intact seeds and germinating seeds were used to initiate callusing. Plant parts were thoroughly washed with 50% solution of liquid detergent and running tap water, then surface sterilized with 0.1% w/v mercuric chloride for 5 minutes followed by two or three rinses of sterilized distilled water. Some seeds after sterilization were germinated in sterilized test tubes on paper bridges. These were used directly as explants.

Murashige and Skoog's medium 1962 supplemented with various concentrations and combinations of growth hormones were used for initiating callusing. Best results were achieved by germinating seeds on MS medium supplemented with 1.5 mg/L BAP+1 mg/L NAA. Calli were 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. Unorganized tissue Calli harvested at maximum GI 8 weeks was dried, powdered and analysed for flavonoids as ***in vivo***.

3. Analysis of Flavonoids

Different plant parts as well as tissue samples at maximum GI of selected plant species 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% H2SO4 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

The isolates were identified by TLC silica gel G coated plates along with standard reference compounds - Apigenin, Isorhamnetin, Scutellarein, Kaempferol, Luteolin, Quercetin, Myricetin, Scopoletin and Negretin. 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 CHCl3 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

Plant parts ***in vivo*** and unorganized tissue ***in vitro*** of ***M. emerginata*** analyzed for qualitative and quantitative estimation of flavonoids, confirmed presence of kaempferol, luteolin and quercetin. Maximum amount was estimated of luteolin in all plant parts while minimum of quercetin. Amount of kaempferol was slightly lower than luteolin but higher than quercetin in all plant parts.

Maximum amount of luteolin, kaempferol, quercetin and total flavonoid content (*in vivo*) has been estimated in flowers 0.55 mg/100 g.d.w., 0.49 mg/100 g.d.w., 0.46 mg/100 g.d.w. and 1.50 mg/100 g.d.w. respectively and minimum in leaves 0.37 mg/100 g.d.w., 0.31 mg/100 g.d.w., 0.26 mg/100 g.d.w. and 0.94 mg/100 g.d.w. respectively Table.1. Individual and total flavonoid content was estimated little higher in unorganized tissue ***in vitro*** 0.60 mg/100 g.d.w., 0.53 mg/100 g.d.w., 0.48 mg/100 g.d.w. and 1.61 mg/100 g.d.w. respectively than flowers 1.50 mg/100 g.d.w. showing highest amount ***in vivo***. Amount of luteolin is higher than kaempferol and quercetin in unorganized tissue of ***M. emerginata*** as in plant parts Table 1.

Table 1: Flavonoids Content mg/100g.d.w. in *M.emarginata in vivo* and *in vitro*

Flavonoids	Rf	Colour with spray reagent	Mp°C	UV maxima nm	In Vivo				In Vitro
					Leaves	Stem	Flower	Fruit	Unorganized tissue
Luteolin	0.78	Yellow to green	328-331	255-350	0.37 ± .05	0.42 ± .04	0.55 ± .04	0.50 ± .05	0.60 ± .02
Kaempferol	0.93	Deep yellow to brownish	271-273	268-369	0.31 ± .04	0.37 ± .05	0.49 ± .02	0.41 ± .03	0.53 ± .03
Quercetin	0.82	Yellow to bluish	309-311	258-373	0.26 ± .02	0.33 ± .04	0.46 ± .04	0.37 ± .04	0.48 ± .04
Total content	-	-	-	-	0.94 ± .05	1.12± .05	1.50 ± .04	1.28 ± .04	1.61 ± .04

Values are mean of five replicates ± SD

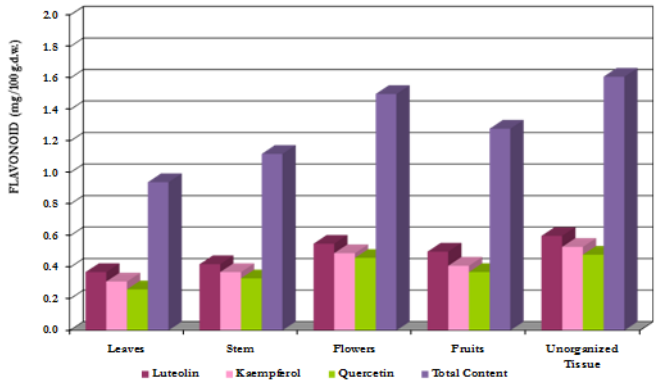

Figure1: Flavonoids Content mg/100 g.d.w. of *M.emarginata in vivo* and *in vitro*

5. Conclusion

Present study has confirmed the presence of three flavonoids- luteolin, kaempferol and quercetin ***in vivo*** and ***in vitro*** in ***M. emarginata***. Higher concentrations of flavonoids in flower of ***M. emarginata*** show the medicinal value of plants as antioxidant, antiallergic and antimicrobial. Sufficient amount of flavonoids in unorganized tissue can be considered as better achievement for large scale production of flavonoids being medicinally valuable.

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Author Profile

Shweta Mathur received her post graduation in Biotechnology from Allahabad Agriculture University in 2006. She did her Ph.D in Botany from Maharaja Ganga Singh University, (Bikaner). She worked as Senior Research Fellow in ICMR project for 2 years from 2007-2009.

Asha Goswami is Principal in Govt. College, Rajasthan. She received her post graduation in Botany, Ph.D in Botany and F.B.S degree. She has 35 years of teaching experience to U.G, P.G students. She had 20 M.Phil students and 10 Ph.D students under her guidance. Her papers are published in National and International Journals. Her research experience is of 20 years.