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Identification, Isolation and Estimation of *Maytenus Emerginata* phytosterols *in vivo* and *in vitro*

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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 phytosterol content by Subramanian and Nagrajan 1969 method. Phytosterols have been identified and confirmed by TLC, M.P., UV maxima and IR studies. The maximum amount of β-sitosterol, stigmasterol and total sterol content has been estimated in leaves and minimum in fruits. Stem showed amount of individual sterols and total sterol higher than fruit but less than flowers and leaves .Unorganized tissue showed very little amount of sterols than leaves with highest amount of sterols. Amount of β-sitosterol was always greater than stigmasterol in vivo and in vitro.***

Keywords: *Maytenus emerginata*, phytosterols, medicinal plant, β-sitosterol, stigmasterol

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.

Steroids are tetra cyclic triterpenes, derived from cyclopentano-perhydroxy-phenanthrene ring and have structure similar to animal steroids. Most important class of steroid is cyclic terpenoids-phytosterols and sapogenins. Animal sterol cholesterol is the human precursor to all steroid hormones while β-sitosterol is the plant precursor to growth and reproductive hormones. The most common phytosterols are β-sitosterol, campesterol and stigmasterol which occurs in higher plants both *in vivo* and *in vitro* tissue culture. β-sitosterol has been found ***in vivo*** and ***in vitro*** tissue cultures of ***Morus alba*** by Kulkarni et al. (1970), ***Helianthus annuus*** by Sharma (1975), ***Datura metel*** by Khanna (1976), ***Tephrosia purpurea*** by Khanna et al. (1977c), ***Solanum aviculare*** by Gaur (1978) and ***Trigonella corniculata*** by Jain (1979). Lanosterol and β-sitosterol from plant parts of ***Citrullus colocynthis, Corchorus depressus, Fagonia cretica, Lycium barbarum*** by Harsh (1982), seeds of ***Peganum harmala*** by Singh and Nag (1981) and tissue cultures of ***Citrullus colocynthis, Peganum harmala*** by Harsh (1982), ***Lycium barbarum*** by Grover (1984) have also been reported

The phytosterols have been found effective in treating high cholesterol as the plant sterols compete for absorption sites with cholesterol; they thus reduce the amount of cholesterol absorbed. Phytosterols also contributes to the anti-inflammatory effect of cold-pressed flax seed and olive oils by **Visioli and Galli (2001).** Sitosterol and its related compound, sitosterolin decreases cholesterol absorption and helps to modulate immune function, inflammation and pain levels through its effects on controlling the production of inflammatory cytokines.

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 phytosterols.

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 phytosterols as ***in vivo***.

3. Analysis of Phytosterols

Different plant parts as well as tissue samples at maximum GI of selected plant species were air dried weighed, powdered, tissue samples were hydrolyzed with 30% (v/v) hydrochloric acid (2 gm/20ml) for 4 hours on water bath. The hydrolyzed test samples were washed separately with distilled water till the filtrate attained pH 7.0. Test samples so obtained were dried at 60^0C for eight hours and soxhlet

extracted in benzene (200 ml) for 24 hrs **(Nag *et al*., 1979)** separately. Benzene extracts of various test samples were dried separately *in vacuo* and taken up in chloroform for further analysis.

3.1 Qualitative and quantitative estimation

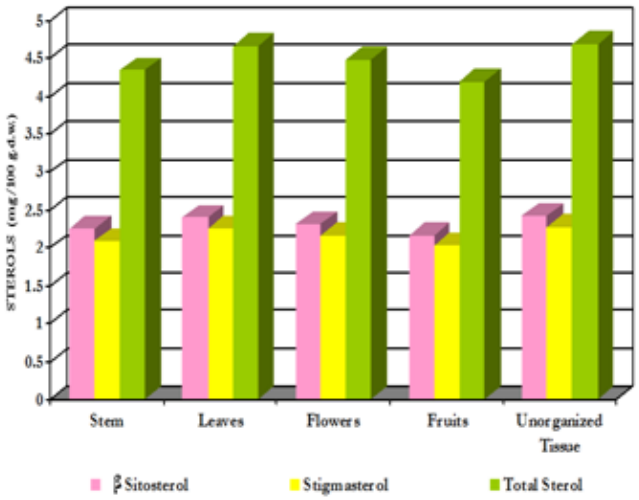
The isolates were identified by TLC silica gel G coated plates along with standard reference compounds - of sterols (β-sitosterol, campesterol, lanosterol and stigmasterol). Plates were developed in an organic solvent mixture of hexane and acetone (80:20 v/v), air dried, sprayed with 50% sulphuric acid and subsequently heated at 100^0C for 10 minutes. Two purple coloured spots coinciding with those of standard samples of β-sitosterol (Rf 0.60) and stigmasterol (Rf 0.64) were observed and collected along with silica gel from unsprayed plates. Each of the mixtures was then eluted with chloroform, elutes dried *in vacuo* and crystallized separately with acetone and methanol. Collected along with silica gel from unsprayed plates. Each of the mixtures was then eluted with chloroform, elutes dried *in vacuo* and crystallized separately with acetone and methanol. Quantitative estimation of the identified phytosterols was carried out colorimetrically following method of **Das and Banerjee** (1980).

4. Results and Discussion

Plant parts ***in vivo*** and unorganized tissue ***in vitro*** of ***M. emerginata*** analyzed for qualitative and quantitative estimation of phytosterols, confirmed presence of only two sterols β-sitosterol and stigmasterol. Maximum amount was estimated of β-sitosterol in all plant parts as well as unorganized tissue. Maximum amount of β-sitosterol, stigmasterol and total sterol content has been estimated in leaves 2.40 mg/100 g.d.w., 2.26 mg/100 g.d.w. and 4.66 mg/100g.d.w. respectively and minimum in fruits 2.17 mg/100 g.d.w, 2.03 mg/100 g.d.w. and 4.20 mg/100 g.d.w. respectively. Stem showed amount of individual sterols and total sterol (4.34 mg/100g.d.w.) higher than fruit (4.20 mg/100 g.d.w.) but less than flowers (4.48 mg/100g.d.w.) and leaves (4.66mg/100g.d.w.). Unorganized tissue showed very little amount of sterols (2.42 mg/100 g.d.w.,2.27 mg/100 g.d.w. and 4.69 mg/100 g.d.w. respectively) than leaves with highest amount of sterols. Amount of β-sitosterol was always greater than stigmasterol ***in vivo*** and ***in vitro***.

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

STEROLS	IN VIVO				IN VITRO
	Stem	Leaves	Flower	Fruit	Unorganized Tissue
β-Sitosterols	2.25 ± .04	2.40 ± .03	2.31 ± .05	2.17± .02	2.42 ± .01
Stigmasterol	2.09 ± .03	2.26 ±.02	2.17± .03	2.03± .04	2.27 ± .03
Total Sterols	4.34 ± .04	4.66± .03	4.48 ± .05	4.20 ± .04	4.69 ± .03



5. Conclusion

Present study has confirmed the presence of two sterols β-sitosterol and stigmasterol ***in vivo*** and ***in vitro*** in ***M. emarginata***. Higher concentrations of phytosterols in leaves of ***M. emarginata*** can be considered as medicinally valuable part of plant. Amount of phytosterols in unorganized tissue can be considered as better.

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