INVESTIGATION, PRODUCTION, ISOLATION AND IDENTIFICATION OF SAPOGENINS FROM PLANT PARTS AND UNORGANIZED TISSUE OF *M. EMERGINATA*

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ABSTRACT

The present study aim is to isolate and identify sapogenins from plant parts and unorganized tissue of *M. emerginata*.Plant parts of *M. emerginata* were collected and then cut into small pieces, dried, powdered and then used for the estimation of steroidal sapogenins. Multiplied unorganized tissue samples of *M. emerginata* (harvested at maximum GI) were also dried, powdered and used for extraction of steroidal sapogenins. Among the plant parts of *M. emerginata* highest amount of diosgenin, kryprogenin and total sapogenins was observed in fruits (0.38 mg/100 g.d.w. and 0.30 mg/100 g.d.w. and 0.68 mg /100 g.d.w. respectively) followed by leaves (0.32 mg/100 g.d.w., 0.25 mg/100g.d.w. and 0.57mg/100g.d.w. respectively), stem (0.25 mg/100 g.d.w., 0.17 mg/100 g.d.w and 0.42mg/100g.d.w. respectively) and minimum in flowers (0.19 mg/100 g.d.w., 0.12 mg/100 g.d.w. and 0.31mg/100 g.d.w. respectively). Amount of diosgenin as well as kryptogenin and total sapogenins was much higher in unorganized (0.42 mg/100 g.d.w., 0.33 mg/100 g.d.w. and 0.75mg/100g.d.w.respectively) tissue of *M. emerginata*, than plant parts. Presence of diosgenin content is always in higher amount than kryptogenin *in vivo* and *in vitro*. Present work evidenced that diosgenins and kryprogenin can be isolated from various parts of *M. emerginata* in significant amount and plant could be utilized for the production of steroidal sapogenins which are of considerable importance as the main precursor of pharmacologically active steroids including sex hormones, corticosteroids and oral contraceptives.

KEY WORDS: ***M. emerginata*,** Unorganized Tissue, Sapogenins, diosgenin, kryptogenin.

No: of Tables: 1 **No: of Figures: 1** **No:of References:17**

INTRODUCTION

Sapogenins that are stored in plants with sugars attached are called Saponins. Diosgenin is a saponin a glycone obtained from the roots of Dioscorea species. Sapogenins are triterpenoids glycosides or glycosides of steroid, with well-known detergent properties. They are glycosidically linked to sugar chain at the C-3 hydroxyl position. They lower the surface tension of aqueous solutions and cause the formation of stable foams. They also increase the permeability of cell membranes; hence they are poisonous to animals if ingested, due to their bitter taste they may deter predators. Sapogenins are widely used in the field of medicine as they are the main precursors of many medicinally useful steroidal hormones such as sex hormones. These compounds are extensively distributed in plant kingdom. Some sapogenins can mimic on regulate steroid hormones or hormone precursors. Steroidal sapogenins have been reported from a number of plant species viz. Dioscorea Agave and Yucca Dioscorea spp. which contains considerable amounts of the sapogenins diosgenin can converted into corticosteroids, dehydroepiandrosterone (DHEA), estrogen and progesterone in laboratory (Araghinikhan et al., 1996). Fenugreek (Trigonella foenum-graecum) is richer in diosgenin and other saponins; its therapeutic effect has been by (Sharma et al., 1996). The most celebrated saponins and ginsenosides from Korean ginseng (Panax ginseng). Ginseng has been promoted as an aphrodisiac studied by (Wang and Lee, 1998).

MATERIAL AND METHODS

Plant parts of M. emerginata used in investigation were collected from the fields and then cut into small pieces, dried, powdered and then used for the estimation of steroidal sapogenins. Multiplied unorganized tissue samples of M. emerginata (harvested at maximum GI) were also dried, powdered and used for extraction of steroidal sapogenins.

a) Extraction Procedure Samples were hydrolyzed with 30% (v/v) hydrochloric acid (2 gm/20 ml) for 4 hours on a water bath and then hydrolyzed test samples washed separately with distilled water and filtrate attained pH 7.0. Test samples obtained were dried at 60°C for eight hours and soxhlet extracted in benzene (200ml) for twenty four hours separately. Benzene extract of each test sample was dried separately in vacuo and taken up in chloroform for analysis of its steroidal sapogenins.

b) Chromatography Thin Layer Chromatography (TLC)

Crude extracts with reference to sapogenins (diosgenin, gitogenin, hecogenin, kryptogenin, smilagenin, tigogenin and yamogenin) were dissolved in chloroform and applied separately on silica get 'G' coated and activated glass plates. These plates were developed in an organic solvent mixture of hexane and acetone (8.2, v/v). Developed glass plates were dried and visualized under UV light revealing two fluorescent spots in each of the test samples which on spraying with 50% sulphuric acid and subsequent heating at 100°C for 10 minutes showed two spots (brown, Rf 0.43, Rf 0.22) coincided with

that of the standard reference compound, diosgenin and kryptogenin.

c) Preparative Thin Layer Chromatography (PTLC) Two spots (brown, Rf 0.43, Rf 0.22) coinciding with that of diosgenin and kryptogenin were separately eluted by preparative TLC (silica gel 'G' dry thickness 0.4-0.5 mm, solvent system-hexane and acetone 8:2), from unsprayed plates along with silica gel. Each mixture was eluted with chloroform dried in vacuo and crystallized with methanol-acetone .The isolated compound and the standard reference compounds of diosgenin and kryptogenin were subjected to their mp and IR study.

d) Quantitative Estimation Steroidal sapogenins were estimated following the spectrophotometric method of (Sanchez, 1972). Standard stock solutions of diosgenin and kryptogenin were separately prepared in chloroform, out of which various concentrations were made ranging from 10µg to 120µg (each) and applied separately on silica gel 'G' coated and activated glass plates along with a parallel run of blank. These glass plates were run in a solvent mixture of hexane: acetone (8:2), air dried and kept in a chamber saturated with iodine vapours. Resulting coloured spots were marked and the plates were kept in an oven at 100°C for 15 minutes so as to evaporate excess of iodine. Spots of diosgenin and kryptogenin along blank zone from the parallel run were scrapped along with the absorbent, eluted with 5 ml of methanol and then centrifuged. From each of the sample 4 ml of aliquot was taken and evaporated to dryness on a water bath. To each of the resulting residue 4 ml of 80% methanolic sulphuric acid was added and kept for 2 hours. Absorbance from each of the known sample was measured on a spectronic-20 colorimeter set at 405 nm against a blank (80% methanolic sulphuric acid) and a regression curve of various concentrations against optical densities was computed which followed Beer's law. Absorbance from each of the unknown sample was also taken in a similar manner and their concentration (%) was determined by comparing with those of their standard curves. Five such replicates of each of the samples were examined and mean values were taken.

RESULTS AND DISCUSSION

Presence of diosgenin and kryptogenin from different plant parts and eight weeks old unorganized tissue of M. emerginata were confirmed by Co-TLC (diosgenin, Rf 0.42 mp 207-208° C and kryptogenin, Rf 0.22). The presence of diosgenin and kryptogenin was further confirmed by super imposable IR spectrum of the isolated and reference compounds. Among the plant parts of M. emerginata highest amount of diosgenin, kryprogenin and total genins was observed in fruits (0.38 mg/100 g.d.w. and 0.30 mg/100 g.d.w. and 0.68 mg /100 g.d.w. respectively) followed by leaves (0.32 mg/100 g.d.w., 0.25 mg/100g.d.w. and 0.57mg/100g.d.w. respectively), stem (0.25 mg/100 g.d.w., 0.17 mg/100 g.d.w and 0.42mg/100g.d.w. respectively) and minimum in flowers (0.19 mg/100 g.d.w., 0.12 mg/100 g.d.w. and 0.31mg/100 g.d.w. respectively). Amount of diosgenin as well as kryptogenin and total genins was much higher in unorganized (0.42 mg/100 g.d.w., 0.33 mg/100 g.d.w. and 0.75mg/100g.d.w.respectively) tissue of M. emerginata, than plant parts.

Presence of diosgenin content is always in higher amount than kryptogenin in vivo and in vitro.

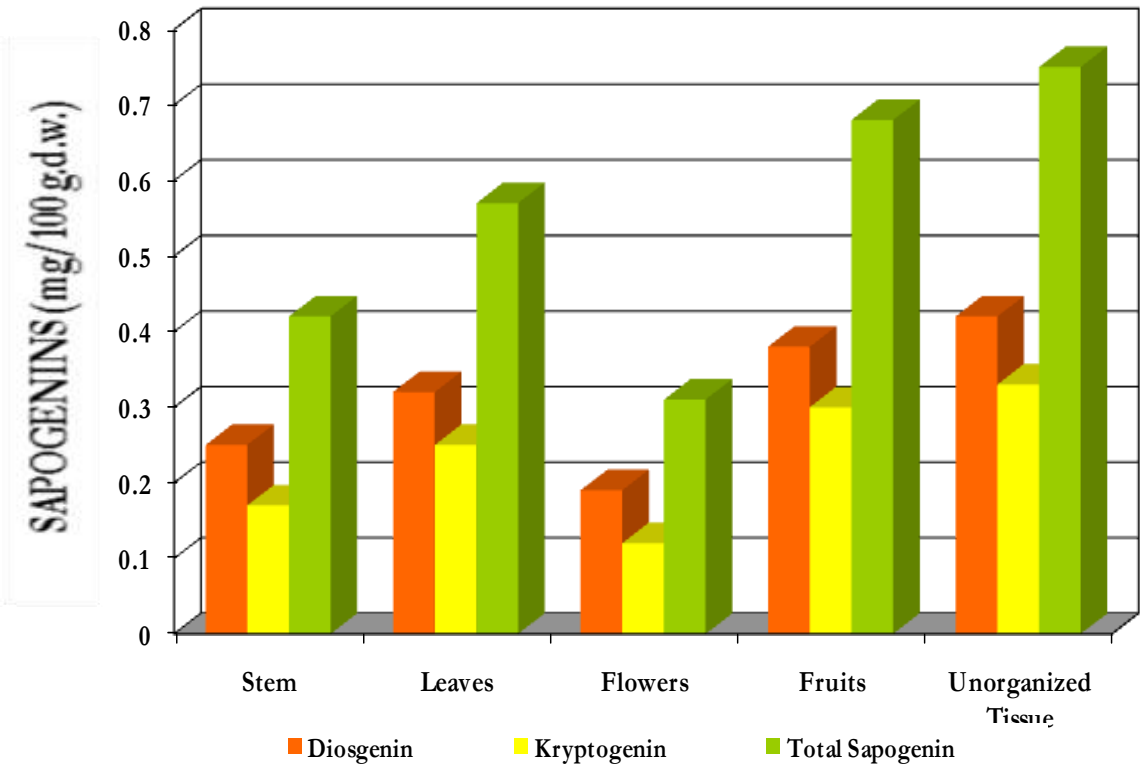
Steroidal sapogenins have been reported from a number of plant species. Dioscorea, Agave and Yucca have been reported among the promising source with regard to their valuable steroidal sapogenins. Diosgenin, a major raw material for the commercial production of steroidal contraceptives and sex hormones is useful in several medical therapies. Diosgenin, an important sapogenin and was reported in various species of Dioscorea (Staba, 1977, Singh, 1979, Khanna, 1980, Mandal and Chatterjee, 1985, Ravishankar and Grewal, 1986, Bishop and Yokata, 2001). Diosgenin, hecogenin, kryptogenin and tigogenin were detected from plant parts and cultures of Abutilon pannosum and Ocimum americanum. (Singh, 1989) .Kryptogenin and diosgenin were found in tissue culture of Fagonia cretica (Kapoor, 1991). Studies of the sapogenins from Albizzia lebbeck seed (Varshney and Badhwar, 1970) Occurrence of both sapogenins and alkaloid lycorine in Curculigo orchioiolis was observed by (Rao, 1978). Presence of some other sapogenins i.e. hecogenin, gitogenin and tigogenin in leaves of Agave wightii were showed by (Khanna 1979). Work on isolation and characterization of an intermediate steroid metabolite in diosgenin biosynthesis in suspension cultures of Dioscorea deltiodea cells was reported (Tal, 1984), Reviewed tissue cultures of a number of desert plant species for their steroidal content. (Nag, 1986). Khanna (1987a, b) has also reported a number of steroidal sapogenins from tissue cultures of various plants. Analysis of steroidal sapogenins from Amber Fenugreek (Trigonella foenum graecum by capillary gas chromatography and combined gas chromatography/Mass spectrometry) was carried by (Taylor, 1997). A new polyhydroxylated steroidal sapogenin and saponin were reported from the leave of Cestrum sendienerianum. (Haraguchi, 1999). Presence of diosgenin and effect of auxins on production of diosgenin in vitro in Balanites aegyptiaca was reported by (Bedawat, 2006). Diosgenin, a plant-derived sapogenin, enhances regulatory T-cell immunity in the intestine of mice with food allergy. (Huang, 2010). Genomic and co-expression analyses predict multiple genes involved in triterpene saponin biosynthesis in Medicago truncatula (Naoumkina, 2010). Biotechnological intervention of Agave sisalana: A unique fiber yieding plant with medicinal property. (Debnath, 2010).

Table- 1. Sapogenins (mg/100 g.d.w.) in *M. emarginata in vivo* and *in vitro*

SAPOGENIN	PLANT PARTS (*IN VIVO*)				*IN VITRO*
	Stem	Leaves	Flowers	Fruits	Unorganized tissue
Diosgenin	0.25±.02	0.32±.03	0.19±.04	0.38±.03	0.42±.04
Kryptogenin	0.17±.03	0.25±.04	0.12±.05	0.30±.04	0.33±.05
Total Sapogenins	0.42±.05	0.57±.07	0.31±.06	0.68±.07	0.75±.05

Values are mean of five replicates ± SD

Figure-1. Sapogenin content (mg/100 g.d.w.) in *M. emarginata in vivo* and *in vitro*



CONCLUSION

Present work evidenced that diosgenins and kryprogenin can be isolated from various parts of *M. emerginata* in significant amount and plant could be utilized for the productionsteroidal sapogenins which are of considerable importance as the main precursorarmacologically active steroids including sex hormones, corticosteraltraceptives.

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