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STEROIDAL SAPOGENINS OF *AGAVE WIGHTII* DR. AND PRAIN —A SYSTEMATIC STUDY *IN VIVO* AND *IN VITRO*

O.P. SHARMA
P.G. Department of Botany, M.S.J. College, Bharatpur-321001, India.

Various plant parts of *Agave wightii* (root, stem. flowering pole, flowers and bulbils) and their respective callus cultures were dried and extracted for their steroidal sapogenins. Gitogenin, hecogenin and tigogenin were identified by TLC, PLC, Colour reaction with 50% H_2SO_4, UV fluorescence, mp, mmp, and IR spectral studies. The quantitative estimation was done colorimetrically. Maximum steroidal sapogenin content *in vivo* (0.70%) and *in vitro* (1.33%) was observed in stem and bulbil-stem callus respectively. Though amount of individual sapogenins varied, tigogenin was found to be predominant in all cases. The seedling callus as well as callus from other parts yielded higher amount of sapogenins as compared with any of the plant part *in vivo*.

Keywords : *Agave wightii;* Sapogenins; *in vitro*.

Introduction

Agave, a genus of tribe Agaveae in family Agavaceae had been investigated for hecogenin and other steroidal sapogenins by a number of workers including, Marker *et al.* (1943), Blunden *et al* (1974, 1978), Srinivasulu and Mahapatra (1971) and Suba Rao and Sundar (1974). Leaves of some plant species of tribe Agaveae were also screened for their steroidal sapogenins by Blunden *et al.* (1978) *Agave wightii* a common species prevalent in Rajasthan has been worked out for its steroidal sapogenins *in vivo* by Khanna *et al.* (1979) and *in vitro* by Sharma and Khanna (1980). The present work was undertaken for systematic screening of *A. wightii* for its steroidal sapogenins *in vivo* and *in vitro*.

Material and Methods

Unorganised callii were separately raised from explants of various plant parts i e , root, stem and leaf of *A. wightii* on revised (Kaul and staba, 1968) Murashige and Skoog's (1962) medium (RT) supplemented with 1 ppm of 2,4-D and 1% agar. The tissues were maintained as static cultures by frequent subculturings of 6-8 weeks and each harvested at the culture age of 2,4,6 and 8 weeks. Each of the tissue samples were dried and growth index calculated

(Sharma and Khanna, 1980). The tissues at their culture age (six weeks in all cases) of maximum growth indices were used for the present study.

Various plant parts (root, stem, flowering-pole, flowers and bulbils, were collected afresh, dried separately, powdered and each defatted in xylene (Srinivasulu and Mahapatra, 1971).

Each of the defatted plant materials and powdered tissue samples were separately hydrolysed with 15% (V/V) ethanolic HCL for 4 hrs and extracted with ethyl acetate. Each of the ethyl acetate extracts was neutralized by distilled water washings and dried *in vacuo*. Each of the dried samples was reconstituted in chloroform and analysed for steroidal sapogenins by TLC (Silica gel G plates run in solvent mixture, Benzene-Ethyl acetate 3:2), isolated and purified by PLC. Each of the isolated substances was crystallized using methanol-acetone and subjected mp, mmp and IR spectral studies for further indentification and confirmation (Khanna *et al*., 1979). Quantitative estimation of various sapogenins was done colorimetrically (Sanchez *et al.*, 1972).

Results and Discussions

Gitogenin, hecogenin and tigogenin were identified from all the samples studied. Tigogenin was found to be a predominant sapogenin followed by hecogenin in all the instances (Table 1 and 2). Maximum amount of total sapogenins *in vivo* was observed in stem (0.70%) followed by 0.63% in root and 0.55% in leaf, with a minimum 0.45% in flowers (Table 1). Maximum sapogenin content *in vitro* 1.33% was observed in stem callus followed by 1.27% in root callus which was significantly higher than the amount obseved in seedling callus 0.70%. The yield of tigogenin and hecogenin *in vitro* showed a proportionate increase as compared to that of gitogenin which increased considerably (upto 0.30%, Table 2). The total as well individual amount of sapogenins was much higher *in vitro* cultures as compared with their respective plant parts *in vivo*. Although seedling callus yielded all the sapogenins, their total yield (0.70%) was less than the yield in other callii (Table 2).

Blunden *et al.*, (1974, 1978) when examined different plant parts of various species of *Agave* in general and *A. sisalana* in particular, observed that tigogenin was the predominant sapogenin alongwith other spogenins including hecogenin. The present work on *A. wightii in vivo* and *in vitro* confirms these findings. Further, the study supports observations of Khanna and Jain (1973) on *Trigonella foenum- graecum* where amount of steroidal sapogenins was observed to be higher *in vitro* as compared to that *in vivo*.

Table 1. Steroidal sapogenin contents (%) in different mature plant parts of *Agave wightii*.

Plant part	Gitogenin	Hecogenin	Tigogenin	Total sapogenin contents (%)
Root	0.05	0.17	0.41	0.63
Stem	0.05	0 13	0.52	0.70
Pole	0 05	0.05	0.41	0.51
Leaf	0.01	0.10	0 44	0.55
Flower	0.01	0.09	0.35	0.45
Bulbils	0 02	0.12	0.40	0.54

Table 2. Steroidal sapogenin contents (%) in six weeks old static cultures of *A. wightii* raised from different plant parts.

Source of callus (Plant parts)	Growth Index (GI)	Tigogenin	Hecogenin	Gitogenin	Total sapogedin content
Seedling	1.60	0.43	0.16	0.11	0.70
Leaf (young)	3.20	0.71	0.25	0.24	1.20
Stem	1.50	0.77	0.27	0.29	1.33
Root	2.50	0.70	0.27	0.30	1.27

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