

EVALUATION OF STEROL CONTENTS IN *VIVO* AND *IN VITRO* IN *BALANITES AEGYPTIACA* DEL.

Shashi Bidawat, T.N.Nag and B.B.S. Kapoor

Plant Tissue Culture Laboratory,

P.G. Department of Botany, Dungar College, Bikaner 334001, India

ABSTRACT

Evaluation of Sterol contents of roots, leaves, fruits and 8 weeks callus from the selected medicinal plant *Balanites aegyptiaca* growing in arid zone of Rajasthan was carried out. Among all the samples the β -Sitosterol and Stigmasterol were isolated and identified. Maximum sterol contents were observed in 8 week old callus of *Balanites aegyptiaca* (14.22 mg/g.d.w.), whereas minimum in fruits of *Balanites aegyptiaca* (10.00 mg/g.d.w.).

KEY WORDS: Sterol contents, *Balanites aegyptiaca*, *In vivo*, *In vitro*

INTRODUCTION

Arid region of Rajasthan is rich in medicinal plant species. This region exhibits a great variety of geological, physiographical, climatic, edaphic and biotic conditions and represents diversity of medicinal tree species, which occur on a wide range of habitat. These medicinal plant species are good source of phytochemicals of pharmaceutical interest such as flavonoids, sterols, alkaloids, phenolic compounds, sulphides, isothiocyanates, anthocynins, terpenoids etc. These are the active principles which act as antioxidants, anticarcinogenic, antimicrobials and immunity stimulants. A number of plant species have been screened by many workers for evaluation of steroidal contents. [1-11].

The present investigation describes the isolation and identification of Sterol contents from roots, leaves, fruits and 8 weeks old callus of selected medicinal plant of family Zygophyllaceae i.e *Balanites aegyptiaca*, it is locally called as Hingota. It is used as folk herbal medicine in the treatment of various ailments i.e. jaundice, intestinal worm infection, wounds, malaria, syphilis, epilepsy, dysentery, constipation, diarrhoea, hemorrhoid, stomach aches, asthma, and fever. It is also used as fodder for livestock.

MATERIALS AND METHODS

Fully matured and healthy roots, leaves and fruits of the selected plant species were collected from field area of Bikaner district.

The surface sterilized seeds were aseptically placed on hormone free MS medium for germination in the dark at $28\pm 2^{\circ}\text{C}$. Cotyledons and radicals from these aseptically grown 15 – 20 days old seedlings were taken as explants.

These explants were then established and maintained by frequent subculturing after 4 weeks on MS Medium supplemented with various concentrations and combinations of kinetin and 2, 4-D for callus induction and kinetin and BAP for induction of multiple shoots. Cultures were maintained in growth chamber with regulated temperature ($26\pm 2^{\circ}\text{C}$), relative humidity ($55\pm 5\%$), 3000 lux light intensity. Data was recorded after 2, 4, 6, 8 and 10 weeks.

The dried and powdered plant parts and 8 week old callus of selected medicinal plant. The surface sterilized seeds were aseptically placed on hormone free MS medium for germination in the dark at $28\pm 2^{\circ}\text{C}$. Cotyledons and radicals from these aseptically grown 15 – 20 days old seedlings were taken as explants.

These explants were then established and maintained by frequent subculturing after 4 weeks on MS Medium supplemented with various concentrations and combinations of kinetin and 2, 4-D for callus induction and kinetin and BAP for induction of multiple shoots. Cultures were maintained in growth chamber with regulated temperature ($26\pm 2^{\circ}\text{C}$), relative humidity ($55\pm 5\%$), 3000 lux light intensity. Data was recorded after 2, 4, 6, 8 and 10 weeks and growth indices were calculated.

Fresh and healthy roots, leaves and fruits of the selected plant collected from Bikaner district and 8 weeks old callus were dried and used for extraction of sterols.

Each of the dried samples was hydrolysed with 30% hydrochloric acid (2 gm/20 ml) for 4 hours on a water bath. The hydrolysed test samples were filtered and washed with distilled water till the filtrate attained pH 7. Test samples so obtained were dried at 60⁰C for 8 hours and Soxhlet extracted in benzene (200 ml) for 24 hours separately. Each of the benzene extracts of the various test samples were dried in vacuo and taken up in chloroform for further analysis by Thin Layer Chromatography method.

RESULTS AND DISCUSSION

β - Sitosterol and Stigmasterol were isolated and identified. Their quantitative estimation is given in the following Table 1.

Table 1: Sterol contents (mg. /g.d.w) from plant parts and callus of *Balanites aegyptiaca*

Name of Sterol	<i>Balanites aegyptiaca</i>			<i>Balanites aegyptiaca</i>
	Roots	Leaves	Fruits	Callus (8 weeks old)
®- sitosterol	6.14	5.86	5.13	8.14
Stigmasterol	5.08	4.83	4.87	6.08
Total Sterol Contents	11.22	10.69	10.00	14.22

The present investigation shows that among all the samples tested the total sterol contents were found to be maximum in 8 weeks old callus of *Balanites aegyptiaca* (14.22 mg/g.d.w.), whereas minimum in fruits of *Balanites aegyptiaca* (10.00 mg/g.d.w.).

The maximum β - sitosterol (8.14 mg/gdw) was found in 8 weeks old callus of *Balanites aegyptiaca*, while minimum (5.13 mg/gdw) in fruits of *Balanites aegyptiaca*.

The maximum amount of Stigmasterol (6.08 mg/gdw) was found in 8 weeks old callus of *Balanites aegyptiaca*, while minimum (4.83mg/gdw) in leaves of *Balanites aegyptiaca*.

.CONCLUSION

The medicinal plant species, under study area are potential source of secondary products. These retain potentialities to synthesize the sterol contents which play active role in metabolism. Due to presence of these secondary products in the selected medicinal plant species *Balanites aegyptiaca* growing in arid region of Rajasthan can be used in drug and pharmaceutical industries.

ACKNOWLEDGEMENT

The authors wish to acknowledge the UGC, New Delhi for providing the financial assistance for the research work.

REFERENCES

1. Akhisa T and Kokke W, (1991), "Naturally occurring sterols and related compounds in plants". In Patterson, G. W.; Nes, W. D. *Physiology and Biochemistry of Sterols*. Champaign, IL: American Oil Chemists' Society. 172–228.
2. Al-Yahya M, (1986), Phytochemical studies of the plants used in traditional medicine of Saudi Arabia. *Fitoterapia*. 57(3):179-182.
3. Kapoor BBS and Raksha Mishra Raksha, (2013), Sterol Contents from Some Cappridaceous Medicinal Plants of North-West Rajasthan. *International Journal of Medical and Pharmaceutical Sciences Research and Review*. 1 (2) : 1- 6.
4. Nag TN Mathur CS and Goyal S, (1979), Phytochemical studies of *Tribulus alatus*, *T.terrestris* and *Agave wightii* for Primary and Secondary Products. *Comp. physiol. Ecol.* 4: 157-160.
5. Sauerwein M Yoshimatsu K and Shimomura, (1992), Further approaches in the production of secondary metabolites by plant tissue cultures. *Plant Tissue Culture Lett.* 9: 1-9.
6. **Savikin Fodulovic Katarina Grubisic Dragoljub Culafic Ljubinka Menkovic Nobojsa Ristic Mihailo, (1998), Diosgenin and phytosterols content in five callus lines of Dioscorea balcanica. *Plant Science (shannon)*. 135(1): 63-67.**
7. Singh D and NagTN, (1981), Steroidal components of seeds of *Peganum harmala* growing in Rajasthan. *Comp. physiol. Ecol.* 6(3):163-164.
8. Valsta L M Lemström A Ovaskainen M L Lampi A M Toivo J Korhonen T Piironen V, (2007), "Estimation of plant sterol and cholesterol intake in Finland: Quality of new values and their effect on intake". *British Journal of Nutrition*. 92 (4): 671–8.
9. Vieno P Jari T ,Riitta P and Anna M , (.2003), Plant sterols in vegetables, fruits and berries. *J. Sci Food Agric.* 83: 330-337.
10. Zirvi K A and But A, (1971), Chemical Investigation of Germinated *Peganum harmala* Seeds. *Pakistan Journal of Scientific and Industrial Research*. 14.
11. Kapoor BBS and Veena Purohit, (2013), Sterol Contents from Some Fabaceous Plant Species growing in Rajasthan Desert. *Indian Journal of Pharmaceutical and Biological Research*. Vol. 1 (4): 13-15.