J. Indian bot. Soc. Vol. 92 (3&4) 2013 : 229-232

IN VITRO PRODUCTION OF PHYTOSTEROLS IN SOME MEDICINAL PLANTS

TAMANNA TALREJA, LALITA YADAV AND ASHA GOSWAMI

Plant tissue culture and Biotechnology Lab, Department of Botany
Govt. Dungar College, (Accredited with 'A' Grade by NAAC),
Bikaner (Rajasthan) India
E-mail: talrejatamanna@gmail.com

Medicinal plants are the most exclusive source of life saving drugs for the majority of the world's population. Phytosterols are a group of steroid alcohols, naturally occurring in plants. In the present study production of the phytosterols from medicinally useful plants *Cocculus pendulus, Moringa oleifera* and *Tinospora cordifolia* were studied *in vivo* and *in vitro*. Plant parts (separately) and unorganized tissues of the selected plants were separately analyzed for qualitative and quantitative estimation of sterols. ß-sitosterol, stigmasterol and lanosterol in *Cocculus pendulus* and *Tinospora cordifolia*, ß -sitosterol and stigmasterol in *Moringa oleifera* were identified and estimated in plant parts as well as in unorganized tissue. Amount of sterols was higher in unorganized tissues than plant parts.

Key words: *:* Phytosterols, *S*itosterol, Stigmasterol, Lanosterol, Growth index, Unorganized tissue

Steroids are economically useful class of secondary metabolites. These are waxy, soapy or greasy in texture, more soluble in oil than water. Among phytosterols, the most common ones are í- sitosterol, campesterol, lanosterol and stigmasterol. Researches have shown that phytosterols are beneficial in treating various diseases that ensure optimal functioning of the body's defense mechanisms (Daisy *et al.* 2008, Ebner *et al.* 2006**,** Heftmann1973**,** Subhisha and Subramoniam 2005). 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 and are used to treat enlarged prostates and prostatitis

C. pendulus is quite common in plains upto 200 metres. The juice of leaves mixed with water has the property of coagulating in to a green jelly like substance which is taken internally with sugar as tonic. The shoots and the leaves are used in rheumatic pains.

M.oleifera is considered as highly nutritious plant but along with nutritional values it has medicinal values also. Leaves are used in the treatment of fever, bronchitis, eye and ear infection, inflammation of mucous membrane, diarrhoea and gastric ulcer. Flower juice is useful in urinary infections, roots and bark for cardiac and circulatory problems as tonic, bark as appetizer and digestive.

T. cordifolia is most versatile rejuvenating herb that has medical potency to keep the body free from illness. It acts as hepatoprotectant, protecting liver from hepatotoxicity. Skin diseases are cured with extracts of its stem. Bark of *T. cordifolia* has antiallergic, antileprotic, antipyretic, antispasmodic and anti inflammatory properties.

*Cocculus pendulus***,** *Tinospora cordifolia* (family- Menispermaceae) and *Moringa oleifera* (family- Moringaceae) have been selected for ***in vivo*** and ***in vitro*** study of sterols (Anjaneyulu and Radhika 2000**,** Auilliam *et al.* 1977, Bishop and Yokota 2001**,** Singh and Nag 1981**,** Singhvi 1980). The main objective of this study is to encourage attempts to commercially produce plant products from plant tissue culture (Santosh *et al.* 2009, Staba and Kaul 1971, Stohs and Rosenberg 1975, Stohs *et al.* 1975, 1977).

MATERIALS AND METHODS

Fresh plant parts of selected plant species were collected from different local areas of Bikaner. Plant parts were separated, dried in shade and analyzed for phytosterols. Five replicates were taken for each plant part and mean value was

Received on October 11, 2013 Accepted on October 15, 2013

Table 1. Growth indices (GI) of static cultures of selected plant species

Name of plant	Growth indices at the age of					
	2 weeks	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks
C.pendulus	0.67±0.08	1.24±0.06	4.01±0.11	6.38±0.26	6.02±0.44	5.34±0.12
T.cordifolia	0.88±0.09	1.62±0.04	4.87±0.09	8.03±0.31	7.81±0.39	6.49±0.15
M.oleifera	0.92±0.06	1.86±0.08	5.01±0.13	8.34±0.35	8.02±0.27	6.68±0.18

Table 2. Sterol content (mg /100 g.d.w.) of selected plant species

STEROLS	Plant Species *Cocculus pendulus*					*Moringa oleifera*					*Tinospora cordifolia*				
	L	S	Fl	Fr	C	L	S	Fl	Fr	C	L	S	Fl	Fr	C
β-sitosterol	4.46	5.35	3.98	-	5.42	9.73	8.13	7.70	7.87	9.85	5.06	6.19	4.40	-	6.29
Stigmasterol	3.47	3.67	3.09	-	3.82	5.33	4.17	3.90	3.93	5.46	3.50	3.75	3.13	-	3.88
Lanosterol	4.25	5.01	4.06	-	5.11	-	-	-	-	-	4.89	5.37	4.03	-	5.48
Total sterol	12.18	14.03	11.13	-	14.35	15.16	12.30	11.60	11.80	15.31	13.45	15.31	11.56	-	15.65

calculated.

Unorganized cultures with profuse callusing were established using seeds as explant in *M.oleifera*, nodal segment in *C. pendulus* and floral buds in *T. cordifolia*, on Murashige and Skoogs medium supplemented with 2mg/L BAP +1mg/L 2,4-D in *C. pendulus*, 2mg/L BAP + 1.5mg/L IAA in *T. cordifolia* and 1.5 mg/L BAP+1.5mg/L 2,4-D in *M. oleifera*. These cultures were maintained for a period of six months by frequent subculturing at interval of 6 to 8 weeks at 26± 1°C, 55% relative humidity and diffused light conditions (3000 lux). The growth indices (GI) were calculated at different time intervals of 2,4,6,8 and 10 weeks.

Unorganized tissues were harvested at their maximum growth indices *i.e.* eighth week. Tissues were dried at 100°C for 15 minutes to inactivate enzymes followed by 60ºC till constant weight was achieved.

Five such replicates were analyzed for calculating mean value. Plant parts as well as tissue samples harvested at their maximum GI were analyzed separately for their phytosterols content following the procedure of Kaul and Staba (1968).

Extraction Procedure

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°C for eight hours and soxhlet extracted in benzene (200 ml) for twenty four hours separately. Benzene extracts of various test samples were dried separately *in vacuo* and taken up in chloroform for further analysis.

Qualitative Analysis

Each of the crude extract was applied separately on silica gel 'G' coated and activated thin (0.2-0.3 mm) glass plates along with the standard reference samples of sterols (ί-sitosterol, campesterol, lanosterol and stigmasterol etc.). The 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°C for 10 minutes. The three fluorescent spots (Rf 0.60, Rf 0.64 and Rf 0.92) corresponding with those of the standard reference samples of ß-sitosterol, stigmasterol and lanosterol in *Cocculus pendulus* and *Tinospora cordifolia* while two purple coloured spots coinciding with those of standard samples of ί-sitosterol (Rf 0.60) and stigmasterol (Rf 0.64) were observed in *Moringa oleifera*. These spots were separately

marked 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.

The crystallized isolates from all the samples tested were subjected to colorimetry (for quantitative estimation), melting point (melting point apparatus Toshniwal, India) and Infra-red spectral (Perkin-Elmer, 337, Grating, Infra-red spectrophotometer, using nujol or potassium bromide pellets) studies along with their respective standard reference sterols.

Estimation of sterols

Quantitative estimation of í-sitosterol and stigmasterol test samples was carried out using the method of Das and Banerjee (1980). Stock solution of í-sitosterol, stigmasterol and lanosterol in chloroform (500µg/ml) was separately prepared. From this solution six concentrations (0.1,0.2,0.3,0.4,0.5 and 0.6 ml) were prepared and spotted on silica gel 'G' coated and activated plates. The plates were developed in a solvent system of hexane: acetone (80:20). Such developed chromatograms were air dried and exposed to iodine vapors. Iodine positive spots were marked and heated to evaporate excess of iodine. The spots were scrapped along with silica gel and each was eluted with 5 ml of chloroform in test tubes. Each of the tubes was centrifuged, supernatants were collected, evaporated to dryness and proceeded further. To the dried samples, 3 ml of glacial acetic acid was added and shaken on a vortex mixer at room temperature for 1 minute and then immersed in crushed ice. 2 ml of freshly prepared chromogenic reagent (0.5 ml of 5% anhydrous ferric chloride in glacial acetic acid and 100 ml of concentrated sulphuric acid; Klyne, 1965) was added drop wise at 0°C to the frozen samples and mixed thoroughly. All the reaction mixtures were then incubated at 40°C for 30 minutes and their optical density was determined using a spectronic-20 colorimeter (Bausch and Lomb) set at 540 nm against a blank solution (3 ml glacial acetic acid and 2 ml of chromogenic reagent). For every concentration, five such replicates were run and average optical density was plotted against their respective concentrations to complete a regression curve which followed the Beer's law.

The crude extracts obtained from various plant parts and culture samples were separately dissolved in chloroform and spotted along with reference β-sitosterol and stigmasterol on silica gel 'G' coated and activated glass plates and developed in a solvent system of hexane: acetone (80:20). Three spots in *Cocculus pendulus* and *Tinospora cordifolia* and two spots in *Moringa oleifera* coinciding with those of the authentic samples of standards and were marked in all the respective samples. Each of these spots were eluted and extracted as described earlier. Elutes were dried, taken up in 5 ml of chloroform and processed further as mentioned above. The concentration of respective sterols present in various tissue samples of selected *plants* were calculated (in mg/100g.d.w.) by comparing the optical density of the experimental sample with the regression curves of β-sitosterol, stigmasterol and lanosterol separately. Five such replicates were examined in each case and their mean values were determined.

RESULTS AND DISCUSSIONS

Plant parts of all selected plant species analyzed showed presence of phytosterols although *M. oleifera* showed only two sterols β-sitosterol and stigmasterol while *C. pendulus* and *T. cordifolia* three sterols-β-sitosterol, stigmasterol and lanosterol. Amount of β-sitosterol was maximum in all plants than stigmasterol in *M. oleifera and* stigmasterol and lanosterol in *C. pendulus* and *T. cordifolia.* Stigmasterol in *C. pendulus* and *T. cordifolia* was in least amount among β-sitosterol and lanosterol**.**

As far as different plant parts were compared for sterol content, maximum amount calculated in leaves of *M. oleifera* and stem of *C***.** *pendulus* and *T. cordifolia* while minimum amount in flowers of all the three plant species. Fruits of *C. pendulus* and *T. cordifolia* could not analyze

due to unavailability.

Dried, powdered unorganized tissues (calli) of all plant species showed significantly high percentage of phytosterols even than maximum amount present in plant parts.

Comparing the three analyzed plant species *T. cordifolia* has greatest concentration of sterols and *M. oleifera* has slightly less amount of sterols than *T. cordifolia*. *C. pendulus* showed lowest concentration of sterols. (Table 2).

In this research paper, our data show that tissue cultures of selected plants are able to produce high levels of phytosterol. Present study showed that tissue cultures of the selected plant species showed significantly high amount of phytosterols even more than maximum amount present in plant parts**.**

Thus it can be concluded that the tissue cultures of selected plants retain the potential to synthesize phytosterols in fair amount**.**

REFERENCES

Anjaneyulu V & Radhika P 2000 The Triterpenoids and steroids from *Mangifera indica* (Review Article). *Ind. J. Chem*. **39B** 883.

Auilliam MA, Templeton ZF & Westmore JB1977 Analysis of steroid metabolites. *Steroids*. **29** 613.

Bishop GJ & Yokota T 2001 Plants steroid hormones, brassinosteroids; current highlights of molecular aspects on their synthesis, metabolism, transport, perception and response, *Plant and cell physiology* **42(2)**114-120.

Daisy P, Jasmine R, Ignacimuthu S & Murugan E 2008 A noval steroid from Elephantopus scaber an Ethnomedicinal plant with antidiabetic a activiry. *J. phymed*.**16 (2-3)** 252-257.

Das SK & Banerjee AB 1980 A rapid method for quantification of sterols after thin layer chromatography. *Ind. J. Exp. Biol*. **18** 969-971.

Ebner MJ, Corol DI, Havlikava H, Honour JW & Fry JP 2006 Identification of Neuroactive Steroids and Their Precursors and Metabolites in Adult Male Rat Brain, *Endocrinology* **147(1)**179-190.

Heftmann E 1973 (Ed.) In: Modern methods of steroids analysis. Academic Press,.New York.

Kaul B & Staba EJ 1968 *Dioscorea* tissue cultures I: Biosynthesis and isolation of diosgenin from *Dioscorea deltoidea* callus and suspension cells. *Lloydia* **31** 171-179.

Santosh Kumar Singh, Zakhana Rathod & OP Saxena 2009 *In vivo* and *in vitro* comparison of phytosterols of *Bacopa monnieri* and *J. Indian Bot Soc.* **88(3&4)** 82-85.

Singh D & Nag TN 1981 Steroidal components of seeds of *Peganum harmala* growing in Rajasthan. *Comp. Phys. Eco*. **6(3)**163-164.

Singhvi S 1980 Production of sapogenins, steroidal alkaloids and some other products from *in vivo* and *in vitro* tissue culture of some Solanaceous and Compositae plants. Ph. D. Thesis, Univ. of Rajasthan, Jaipur.

Staba EJ & Kaul B 1971 Production of diosgenin by plant tissue culture technique. U. S. Patent No. **3**. 628

Stohs SJ & Rosenberg H 1975 Steroids and steroid metabolism in plant tissue cultures. *Lloydia* **38** 181-194.

Stohs SJ, Tarng CS & Rosenberg H 1977 Influence of cyclic AMP on growth and steroid formation in *Corchorus olitorius* suspension cultures. *Lloydia* **40** 370-373.

Stohs SJ, Wegner CL & Rosenberg H 1975 Steroids and sapogenins in tissue cultures of *Dioscorea deltoidea*. *Planta Med.* **28** 101-105.

Subhisha S & Subramoniam A 2005 Antifungal activities of a steroid from *Pallavicinia lyellii* (a liverwort). *Ind. J. Pharma*. **37(5)**304-308.