s). Roxithromycin could be successfully taste masked with Indion 204. The process for preparing drug resinates was optimized with respect to parameters like drug to resin ratio, volume of the medium and taste of the complex. 1:5 ratio of roxithromycin and Indion 204 gave a completely taste masked complex which could be easily incorporated into mouth-dissolve tablets. Thus, disappearance of the Indion 204 peak as well as the drug peak in the DSC thermograms confirmed complexation between roxithromycin and Indion 204.

Hardness of formulations F4, F5 and F6 was found to be within the range 3-4 kg/sq-cm. The drug content of the formulations was within the range 98-110% of the labeled claim. F4 and F5 exhibited *in vitro* dispersion time of 45 s. F6 was found to be palatable with *in vitro* dispersion time of 30 s. Dissolution studies of F6 showed more than 80% release of the drug within 30 min. *In vitro* dispersion time as well as *in vivo* disintegration time of F6 was found to be 30 s indicating effective disintegration. All the volunteers found the tablets to be non-bitter.

#### REFERENCES

- Siegel, S., Roy, H.R., James, A.Z. and Edward, J.H., J. Pharm. Sci., 1962, 51, 1068.
- Mehendale, S.V. and Malshe, V.C., Eastern Pharmacist, 1991, 41.
- Manek, S.P. and Kamat, V.S., Indian J. Pharm. Sci., 1981, 13, 209.
- Nanda, A., Kandaparu, R. and Garg, S., Indian J. Pharm. Sci., 2002, 64, 15.
- Agarwal, R., Mittal, R. and Singh, A., Drug Develop. Ind. Pharm., 2000, 26, 773.
- Reynolds, J.E.F., In; Martindale The Extra Pharmacopoeia, The Pharmaceutical Press, London, 1993, 201.
- 7. Markham, A. and Faulds, D., Drugs, 1994, 48, 297.
- Morella, A.M., Pitman, I.H., and Heinicke, G.W., US Patent No. 6197348, 2001.
- Yajima, T., Ishii, K., Itai, S., Nemoto, M., Suetake, K., and Tsukui, N., US Patent No.5972373, 1997
- 10. Indurwade, N.H., Rajyaguru, T.H., and Nakhat, P.D., Indian Drugs, 2000, 39, 405.
- Walter, S.P., Norman, E.J., and Sally, J.T., US Patent No. 5298261, 1994.
- 12. Habib, W., Khankari, R. and Hontj, J., Crit. Rev. Ther. Drug Carr. Syst., 2000, 17, 61.

# Differentiation in Hypocotyl Cultures of *Solanum platanifolium* Sims and Solasodine Production

R. K. JAGGI\*, A. L. KAJLA, JAGDISH SINGH AND BALBIR SINGH
University Institute of Pharmaceutical Sciences
Panjab University, Chandigarh-160 014

Accepted 13 August 2004 Revised 12 April 2004 Received 22 April 2003

Hypocotyl cultures were developed from explants obtained from *in vitro* germinated seedlings of *Solanum platanifolium* Sims (Family-Solanaceae) on modified Murashige and Skoog's agar-solidified medium supplemented with 6-benzylaminopurine (2 ppm) and  $\alpha$ -napthaleneacetic acid (1 ppm). Shoots originated from callus were transferred to modified Murashige and Skoog's medium and modified White's medium supplemented with different growth adjuvants. 6-benzylaminopurine (4 ppm) produced optimum growth of somatic shoots on MS medium. Chemical analysis of different tissues grown showed that organogenesis enhanced solasodine production.

Solanum platanifolium Sims is a good source of solasodine (1.93% dry weight)<sup>1</sup>, a steroidal alkaloid that is easily converted into 16-dehydropregnenolone acetate, helpful in the synthesis of steroidal drugs employed for the

treatment of sex harmone imbalances, oral contraceptives, asthma and inflammatory disorders. Plant tissue culture work on this plant is already in progress. Morphological differentiation in plant tissue cultures may affect the production of secondary metabolites e.g. alkaloid accumulation in callus of *S. dulcamara* was enhanced by the induction of organogenesis<sup>2</sup>. The aim of the present investigation

\*For correspondence E-mail: drrajjaggi@hotmail.com

TABLE 1: CALLUS FORMATION FROM HYPOCOTYL EXPLANT OF SOLANUM PLATANIFOLIUM

ON HORIZONTAL SURFACE				ON SLANTED SURFACE			
12 h/day light		Continuous light		12 h/day light		Continuous light	
Callus initiation (d)	Callus formation (%)	Callus initiation (d)	Callus formation (%)	Callus initiation (d)	Callus formation (%)	Callus initiation (d)	Callus formation (%)
11 - 22	80	14 – 32	100	11 – 19	100	9 – 14	90
12 - 30	95	12 – 30	95	12 – 19	100	8 – 18	95
10 - 14	95	10 – 21	100	10 – 14	100	10 – 14	100
13 - 27	100	10 – 28	95	11 – 17	95	11 – 16	100
12 - 18	95	12 - 25	95	11 – 17	95	11 – 20	100

Solanum platanifolium is grown on medium containing MS+BAP(2ppm)+NAA(1ppm), n=20 Number of cultures.

was to take up a detailed study on organogenesis in cultures of *S. platanifolium*.

Ripened and mature berries of *Solanum platanifolium* were collected from the Medicinal Plants Garden of the University Institute of Pharmaceutical sciences, Panjab University, Chandigarh. The standard solasodine was obtained by isolating the same from the ripened and matured berries of *S. platanifolium*.

The berries from S. platanifolium plants were washed with running tap water, cleaned with Teepol solution, again washed with tap water and finally rinsed with distilled water. These berries were sterilized with 0.1% solution of HgCl. for 8-10 min, washed 3-4 times with sterile distilled water. The fruits were further sterilized by dipping in alcohol and passing over the flame. Seeds were separated from the sterilized fruits and after removal of adhering mesocarp by shaking with warm sterile distilled water were planted on solidified seed germination medium under strictly aseptic conditions at a temperature of  $25\pm2^{\circ}$  . The hypocotyl stalk segments excised from seedlings grown in vitro were cultivated on modified Murashige and Skoog's (MS)3 agar-solidified medium (ferric citrate was used in place of ferrous sulphate and manganese sulphate monohydrate in place of manganese tetrahydrate and edamine was not used). The medium was supplemented with 2% sucrose and growth regulators 6-benzylaminopurine (BAP, 2 ppm) and (c-napthaleneacetic acid (NAA, 1 ppm). The pH of the medium was adjusted to 5.7-5.8. The inorganic macronutrients (100 ml) of MS medium diluted with distilled water (to 1.0 l) solidified with 1.5 % agar constituted the Seed germination

medium. The cultures were maintained at 25±2°. Some cultures were illuminated for 12 h/day and others with continuous light provided by fluorescent tubes (20 watts each). Shoots originated from callus were transferred separately to modified MS³ and White's⁴ media (ferric citrate and MnSO₄.H₂O were used instead of Fe₂SO₄ and MnSO₄. 4H₂O, respectively. In White's medium cysteine hydrochloride was used and not cysteine). The media were supplemented with growth regulators: BAP (2 ppm)+NAA (1 ppm), BAP (4 ppm), indole-3-propionic acid (IPA, 2 ppm) and indole-3-acetic acid (IAA, 2 ppm).

Different calli produced were dried in oven at 60°, powdered, extracted and tested for the presence of solasodine both qualitatively and quantitatively. For the analysis of solasodine, extraction of each powdered tissue sample was carried out by maceration with 2% acetic acid in 90% ethanol for 12 h followed by refluxing for 4 h. After filtration the mixture was concentrated under vacuum, the concentrate was refluxed with 90% ethanol containing 10% HCl for 2 h. The hydrolysate after cooling was made alkaline (pH 9-10) with ammonia solution it was again refluxed for 2 h. The residue on the filter paper obtained after filtration was washed with alcohol and alcohol was removed. Distilled water (30 ml) was added and after partitioning with CHCl<sub>3</sub> the combined CHCl<sub>3</sub> layers were concentrated under vacuum.

The CHCl<sub>3</sub> residues obtained as above were subjected to thin layer chromatography (TLC) along with standard sample of solasodine on silica gel G (Merck) coated plates (0.25 mm thick), activated at 110°. The solvent systems used



Fig. 1: Seedlings of *S. platanifolium*.

One-month-old seedlings of *S. platanifolium* on seed germination medium.

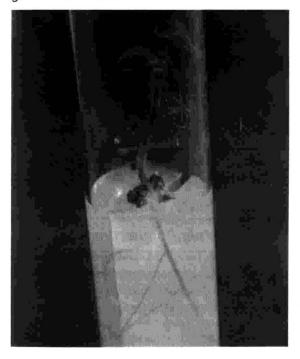


Fig. 3: Somatic shoot culture of *S. platanifolium* on MS+IPA (2 ppm).

Two and a half-month-old somatic shoot culture of *S. platanifolium* on MS+IPA (2 ppm) illuminated with 12 h/day light showing elongation of shoot and development of new root.

were (i) chloroform:methanol (9:1) and (ii) hexane:ethylacetate (7:3). The presence of solasodine was confirmed after visualizing the chromatograms by spraying with antimony trichloride in chloroform and activated at 110° for 10 min. For quantitative estimation of solasodine in dif-



Fig. 2: Somatic shoot culture of *S. platanifolium* on MS+BAP (4 ppm).

Two and a half-month-old somatic shoot culture of *S. platanifolium* on MS+BAP (4 ppm) illuminated with continuous light showing the elongation of two shoots.



Fig. 4: Somatic shoot culture of *S. platanifolium* on MS+IAA (2 ppm).

Three-week-old somatic shoot culture of *S. platanifolium* hypocotyl developed on MS+IAA (2 ppm) illuminated with 12 h/ daylight showing adventitious roots.

ferent calli, colorimetric method of Birners was used. Standard curve was obtained using standard solution (40  $\mu$ g/ml) of solasodine. Each tissue sample was extracted in alcohol and the later was removed under vacuum. The residue was hydrolyzed with 1N HCl. The acid was neutralized

with 1N sodium hydroxide. Resultant solution was treated with acetic acid, diluted with distilled water to obtain 1 ml of diluted solution equivalent to 1 mg of dry material. Two milliliters aliquots were made up to 5 ml with 20% acetic acid, then added acetate buffer (pH 4.7) and 1 ml of methyl orange solution (0.05%). After shaking for 10-30 s, solasodine was extracted from colored solution with chloroform. Chloroform layer was dried with anhydrous sodium sulphate and absorbances read on a spectrophotometer at 420 nm. The concentration of solasodine was determined on dry weight bases with the use of standard curve for solasodine.

Seed germination was about 92% (fig. 1). The initiation of callus and callus formation were better on slanted surface of the medium and cultures exposed to continuous light (Table 1) when compared with those developed on horizontal surface. The hypocotyl callus showed the formation of small shoots. Slanted surface of medium had better effect on the formation of shoots than the horizontal surface. BAP (4 ppm) was found to be the best growth regulator tested for the production and development of more somatic shoots (fig. 2), but IPA (2 ppm) caused the growth of the roots (fig. 3). After 2-3 days of subculturing of well developed shoots from hypocotyl callus on MS+IAA (2 ppm) shoots producing new leaves arise and the adventitious roots arisen from the callus as well as from the shoot base (fig. 4). White's medium did not show good results for the growth of somatic shoots.

The chloroform solution obtained after extraction of the powdered calli confirmed the presence of solasodine. On quantitative evaluation of callus tissues, the content of solasodine was found to be 0.52 mg/g and 0.66 mg/g in undifferentiated and differentiated callus, respectively. If shows that differentiation has caused increase in solasodine production. Differentiated tissue from hypocotyl callus of *Seleagnifolium* also produced higher yield of solasodine than non-differentiated tissue. Solasodine concentration was enhanced by the induction of organogenesis in leaf callus of *S. laciniatum*?

### **ACKNOWLEDGEMENTS**

Financial assistance received from UGC is gratefully acknowledged.

### REFERENCES

- Bhatnagar, J.K. and Puri, R.K., Lloydia, 1974, 37, 318.
- Empe, A. and Eilert, L.I., Plant Cell Rep., 1986, 5, 31.
- Murashige, T. and Skoogs, F., Physiol. Plantarum, 1962, 15, 473.
- White, P.R., In; The Cultivation of Animal and Plant Cells, 2nd Edn., Ronald Press, New York, 1963, 59.
- Birner, J., J. Pharm. Sci., 1969, 58, 258.
- Nigra, H.M., Alvarez, M.A. and Giulietti, A.M., Plant Cell Rep., 1989, 8, 230.
- 7. Chander, S. and Dadds. J., Plant Cell Rep., 1983, 2, 69.

## Gastric Antiulcer Activity of the Leaves of Caesalpinia Pulcherrima

ANIL KUMAR\*1 AND V. NIRMALA

Department of Pharmacology, C. L. Baid Metha College of Pharmacy, Thoraipakkam, Chennai-600096

'TIFAC CORE HD, Department of Phytopharmacy and Phytomedicine, J. S. S. College of Pharmacy, Ootacamund-643001.

Accepted 18 August2004

Revised 16 April 2004

Received 19 December 2003

Petroleum ether extract of *Caesalpinia pulcherrima* was examined in HCl/ethanol and aspirin and pylorus ligation models in the rat. Pretreatment of the extract prevented the formation of gastric lesions in HCl/ethanol model. In aspirin and pylorus ligation model, the extract was able to significantly reduce the ulcer score and increase in mucus content, but had no effect on gastric juice volume or acid content. Thus the results indicate that the extracts' antiulcerogenic effect is attributable to augmentation of gastric defense mechanisms.

Caesalpinia pulcherrima (L.) Swartz belonging to

\*For correspondence E-mail: aktifac@yahoo.com Caesalpiniaceae family is an ornamental shrub popularly known as peacock flower or *Mayuram* in Tamil Nadu, India. The leaves of *Caesalpinia pulcherrima* are used in