

## Production of *Piper betle* L. Callus Tissues for Diosgenin

AMINUDDIN<sup>1</sup>, B.S. DIXIT<sup>2</sup> AND R. BANERJI<sup>2</sup>

<sup>1</sup>Virology Laboratory, <sup>2</sup>Phytochemistry Division  
National Botanical Research Institute, Lucknow-226001.

Accepted 6 June, 1998

Received 10 March 1998

**Piper betle** L. shoot tip and leaf-derived Callus tissues were established on Murashige and Skoog's (MS) medium fortified with  $\alpha$ -naphthalene acetic acid-3,6-benzylaminopurine 0.05 mg/l and solidified with 0.8% agar. The presence of diosgenin was revealed in leaf (0.003%) and shoot tip (0.013%) derived callus tissues, respectively. The study also confirmed that the potential of diosgenin can be extended to other tissues rather than only the roots.

**P**IPER *betle* L. (Piperaceae) comprises about 2000 species of them 50 are found in India. Betlevine is extensively cultivated in specially trellised gardens in hot-damp localities for its leaves which are used as a masticatory. Leaves and roots of *P. betle* are used for a number of medicinal properties by members of several tribes and by aborigines of India<sup>1-4</sup>. However, the essential oil contributes to the aroma and taste of betel leaves and is responsible for the characteristic flavour that reflects the quality of leaf of a particular cultivar<sup>5-7</sup>.

Roots of betlevine have been reported to contain diosgenin<sup>8</sup>. Work has also been carried out on regeneration of *P. betle* through somatic embryogenesis<sup>9,10</sup>. In continuation of our studies on *P. betle*, the potential of other tissues i.e. shoot tip and leaf-derived callus tissues have been explored for the presence of diosgenin.

Shoot tip and leaf pieces of *Piper betle* L. cv. *Deshi-Bangla* collected from Banthra Research Station of the Institute, were aseptically cultured on Murashige and Skoog's medium<sup>11</sup> fortified with  $\alpha$ -naphthalene acetic acid 3,6-benzylaminopurine 0.05 mg/l and jellied with 0.8% agar. About 2 year-old callus tissues were maintained by subculturing them on to fresh MS media. The respective tissues were harvested after 6-7 weeks and processed for the estimation of diosgenin by gas chromatography.

The respective tissues were dried at 60° and powdered. The tissues of leaf (5.77 g) and shoot tip (2.3 g), in triplicate, were hydrolysed with 2.5 N HCl for 3 h. The samples were

washed with water, dried and finally extracted with petroleum ether (60-80°) for 8 h in a soxhlet extractor. The solvent was distilled off under vacuum, redissolved in methylene chloride and transferred to a measuring flask (2 ml) and made up the volume. An aliquot (1  $\mu$ l) was then subjected to GLC analysis.

GLC analysis was carried out on a Varian gas chromatograph model Vista 6000 with a flame ionisation detector. A stainless steel column, 10' x 1/8" packed with chromosorb AWS (80-100 mesh) coated with SE-30 (3%) was employed. The column temperature was 110° initially for 5 min, then programmed at the rate of 2.5°/min upto 250°. The temperature for injector and detector blocks were, however, maintained at 250°. The flow rate of N<sub>2</sub> was 25 ml/min. The peaks were quantified by the data station attached with the gas chromatograph. The retention time for diosgenin was 34.15 min. A linear calibration curve was also obtained from the solutions containing 1, 2, 3 and 5 mg diosgenin in methylene chloride (1 ml). The diosgenin content in the respective tissues was calculated from the standard curve.

The results of analysis showed the presence of diosgenin in shoot tip and leaf callus tissues of *P. betle* 0.003% and 0.013% respectively.

### REFERENCES

1. Chopra, R.N., Chopra, I.C., Handa, K.L. and Kapoor, L.D., "Chopra's Indigenous Drugs of India", 1958, 371.

2. Brothakur, S.K., "Glimpses of Indian Ethnobotany", Oxford and IBH Publishing Co., 1984, 183.
3. Mudgal, V., Pal, D.C. and Jain, S.K., Natl Workshop on Betelvine, Agriculture Society of India, Calcutta, 1984, 17.
4. Kharkongar, P. and Joseph, J., "Glimpses of Indian Ethnobotany, Oxford and IBH Publishing Co., 1994, 133.
5. Lawrence, B.M., *Perfumer and Flavourist*, 1993, 18, 61.
6. Rawat, A.K.S., Banerji, R. and Balasubrahmanyam, V.R., *Feddes Repertorium*, 1989, 100, 331.
7. Rawat, A.K.S., Tripathi, R.D., Khan, A.J. and Balasubrahmanyam, V.R., *Biochem. Syst. Ecol.*, 1989, 17, 35.
8. Dixit B.S., Banerji, R., Aminuddin, Johri, J.K., Bhatt, G.R., and Sircar, K.P., *Indian J. Pharm. Sci.*, 1995, 57, 263.
9. Aminuddin, Johri, J.K., Mohd. Anis and Balasubrahmanyam. V.R., *Curr. Sci.*, 1993, 793.
10. Johri, J.K., Aminuddin and Pal, A., *Indian J. Exp. Biol.*, 1996, 34, 83.
11. Murashige, T. and Skoog, F., *Physiol. Plantarum*, 1962, 15, 473.

---

## Buccoadhesive Films of Triamcinolone Acetonide: Development and Evaluation of a Buccoadhesive-Erodible Carrier for Treatment of Oral Lesions

---

JAVED ALI, R.K. KHAR AND ALKA AHUJA

Department of Pharmaceutics, Faculty of Pharmacy, Hamdard University  
Hamdard Nagar, New Delhi - 110 062

Accepted 6 June 1998

Received 20 February 1998

**Buccoadhesive films of triamcinolone acetonide (TA) for local delivery of the drug to the oral cavity for the treatment of oral lesions were prepared by the solvent casting technique. Different bioadhesive polymers were evaluated for film formation. Propylene glycol was used as the plasticizer while the choice of solvents was based on the type of polymer chosen. The films were characterized on the basis of their physical characteristics, bioadhesive performance, release characteristics, surface pH, folding endurance and stretchability. The optimized film exhibited an *in vitro* adhesion time of 3.24 hours and an *in vitro* release of 89.98% in 3.5 hours. The buccoadhesive films were accepted well by healthy human volunteers and no irritation of buccal mucosa was reported.**

**T**OPICAL oral therapy with triamcinolone acetonide as kenalog in orabase is the most widely used preparation by dentists and physicians<sup>1</sup>. Oral bioadhesive mucosal sustained release devices have proved to be viable alternative to the conventional local oral medications<sup>2</sup> since they have the disadvantages of an initial burst of activity followed by rapid decrease in concentrations to below therapeutic levels and are difficult to retain in the mouth for longer periods of time<sup>3</sup>. The objective of the present study was to develop a sustained release device in the form of erodible-buccoadhesive polymeric film containing TA for treatment of oral lesions.

TA was obtained as gift sample from M/S Cyanamid India Ltd. Polymers were obtained as gift samples from M/S Ranbaxy Labs.Ltd. All other materials used were of analytical reagent grade. The films were prepared by the solvent casting technique<sup>4</sup>. A number of substrates were tried for the film formation but mercury surface gave best results. Initially, placebo films using various polymers, plasticizer and solvents in different combinations were prepared. Films which were complete, homogenous, flexible, non-sticky and smooth were then loaded with the drug and evaluated (Table1). The requirement of the drug was about 158.41 mg for the formation of a film of 6.23 cm diameter from which small patches of 14 mm diameter