

Production of camptothecines from callus cultures of *Nothapodytes foetida* (Wight) Sleumer

R Sundravelan, B Desireddy and Veeresham Ciddi*

Faculty of Pharmaceutical Sciences, Kakatiya University
Warangal 506 009, India

Received 28 October 2002; revised received 21 May 2003;
accepted 14 July 2003

Murashige and Skoog (MS) medium supplemented with picloram (2 mg/l), N⁶-benzyladenine (BA) (1 mg/l) and gibberellic acid (GA₃) (1 mg/l) was found to be suitable for the establishment of callus cultures from leaves of *Nothapodytes foetida* (Wight) Sleumer. The callus upon extraction and analysis revealed the presence of a cytotoxic quinoline alkaloid, camptothecine (CPT) (2.893±2.38 mg %) and 9-methoxy camptothecine (MCPT) (0.4±0.4 mg %).

Keywords: *Nothapodytes foetida*, camptothecine, 9-methoxy camptothecine

IPC Code: Int. Cl.⁷ A 01 H 4/00; A 01 N 63/00

Nothapodytes foetida (Wight) Sleumer, a small tree of the family *Icacinaceae* is a potential source of camptothecine (CPT) and its analogs¹. It is reported that *N. foetida* has much higher contents of CPT and its analogs than all the other botanical sources of camptothecines². CPT, a cytotoxic quinoline alkaloid is used as an antitumour drug in the treatment of colon, head, breast and bladder cancers by stabilizing the DNA-protein complex by forming topoisomerase I-DNA adduct³. It also inhibits the replication of retroviruses such as human immunodeficiency virus (HIV)⁴. No commercial viable method of synthesis, low yields from intact plants, poor seed germination, high market price, coupled with various biological activities has encouraged to look for alternative methods for the production of CPT and its analogs from *N. foetida*. Plant tissue culture technique, emerged as a viable route for the production of paclitaxel (Taxol), provides a model for production of anticancer agents from woody plants⁵; it can also be applicable for producing CPT. There are some reports pertaining to the

tissue culture of *N. foetida* for the production of CPT and its analogs⁶⁻⁹. However, there is no report on the production of camptothecines from its leaf initiated callus cultures. In the present paper, the authors report production of camptothecines from the leaf initiated callus cultures.

The seeds and healthy plantlets of *N. foetida* were procured from the nursery of Forest Ranger's Office, Pykara, Nilgiris (TN) India and identified by Mr. D. Rajan, Taxonomist, Botanical Survey of India (Southern region), Coimbatore. The explants of *N. foetida* were washed thoroughly with tap water, followed by washing with 10% v/v solution of tween-20 for 3 min and then with distilled water. The explants were (w/v), picloram (2 mg/l), BA (1 mg/l) and GA₃ (1 mg/l) and then the tubes were kept in dark for 30 days at 25±2°C in B.O.D. incubator. The callus cultures were maintained by subculturing onto the same media at a regular interval of 4 weeks. The dried callus (0.5 g) was homogenized and kept on maceration with methanol (30 ml) for 24 hrs. The solvent was filtered and the same callus was extracted thrice. The combined methanolic extracts were evaporated to dryness, dissolved in 5 ml distilled water and partitioned with chloroform (30 ml). The extracts were pooled together and evaporated to dryness. The residue was dissolved in 1 ml methanol (HPLC grade) and subjected to TLC and HPLC analysis⁹.

Aluminium plates precoated with Silica gel-G (Merck) were used with a solvent system of methanol:water (6:4) and detection under UV light (254 nm). The HPLC analysis of the extracts was carried out on Shimadzu LC-10AT Model, by injecting 20 µl of each standard solution and extract with Hamilton syringe, using C-18 column (Tracer Analytica, Nucleosil- 100, 25 cm×0.4 cm diam, 5µ) with Shimadzu Photo diode array (SPD- M10A VP Model, Japan) detector. The mobile phase (methanol:water, 60:40) was pumped isocratically at a flow rate of 0.8 ml/min and the camptothecines were detected by their absorption at 256 nm.

MS (1962) media¹⁰ with sucrose (3% w/v) and different hormonal combinations were tried for the initiation of callus cultures from leaves of *N. foetida*. The MS medium supplemented with picloram (2 mg/l), BA (1 mg/l) and GA₃ (1 mg/l) was suitable

*Author for correspondence:

Tel: 91-8712-2446259; Fax: 91-8712-2438844

E-mail: ciddiveeresham@yahoo.co.in

for induction and maintenance of callus cultures. The initiation of callus began in the third week and complete conversion of explant into callus observed at the end of 8 weeks. The callus developed from vein endings was fragile, white in colour turning to light brown. The highest frequency of callus induction was observed in young leaves (95%) followed by immature embryos (40%). It was also found that young leaves were more suitable than mature ones for the initiation of callus cultures. It may be due to the fact that they are delicate and grow more actively than mature leaves.

TLC of the extracts derived from callus cultures upon co-chromatography with authentic samples had given the blue and violet spots. The R_f values of the extracts were found to be same as that of authentic samples (R_f values of CPT and MCPT were 0.35 and 0.45, respectively) indicating the presence of CPT and MCPT. The detection of camptothecines was observed in more than 12-week-old calli.

On HPLC analysis, the extracts derived from the callus cultures had given the same retention time (R_t) values as that of standard substances of CPT (9.8 min) and MCPT (14.5 min), indicating the presence of camptothecines. The amounts of CPT and MCPT of each sample were calculated, using respective standard graphs.

The callus and media produced 2.893 ± 2.38 mg% and 0.4 ± 0.4 mg% of CPT and 0.121 ± 0.0914 mg% and 0.13 ± 0.114 mg% MCPT, respectively. Roja and Heble⁶ reported that the differentiated plantlet cultures showed slightly higher amounts of MCPT (0.7 mg% d wt) than the undifferentiated callus cultures (0.1 mg% d wt). Veeresham and Shuler⁷ reported that the callus from excised embryos of *N. foetida* produced CPT (0.95 mg% d wt) and MCPT (traces). However, the present study indicates the higher yield of CPT and MCPT than the earlier reports.

In conclusion, the callus cultures developed from leaf explants of *N. foetida* were found to have biosynthetic potential to produce CPT and MCPT. The amount of CPT and MCPT produced in callus was

low, than the intact plant. However, the yield improvement strategies have to be developed before commercialization of this technique for the production of camptothecines from tissue cultures. So far, this is the first report on detection of camptothecines in leaf callus cultures of *N. foetida*.

Acknowledgement

Sundaravelan is thankful to Council of Scientific and Industrial Research, New Delhi for the award of Senior Research Fellowship. The authors are also thankful to AICTE for financial assistance.

References

- 1 Govindachari T R & Viswanathan N, Alkaloids of *Mappia foetida*, *Phytochemistry*, 11 (1972) 3529-3531.
- 2 Ku K Y & Tang T C, Several botanical sources of camptothecine, an antitumour alkaloid. *Zhong Cao Yao*, 11 (1980) 476-479.
- 3 Covey J M, Jaxel C & Kohen K W, Protein-linked DNA strand breaks induced in mammalian cells by camptothecin, an inhibitor of topoisomerase I, *Cancer Res*, 49 (1989) 5016-5022.
- 4 Pantazis P, Camptothecin—A promising anti retroviral drug, *J Biomed Sci*, 3 (1996) 14-19.
- 5 Cragg G M, Schepartz S A, Sufness M & Grever M R, The taxol supply crisis, New policies for handling the large-scale production of novel natural product anticancer and anti-HIV agents, *J Nat Prod*, 56 (1993) 1657.
- 6 Roja G & Heble M R, The quinoline alkaloids camptothecin and 9-methoxy camptothecin from tissue cultures and mature trees of *Nothapodytes foetida*, *Phytochemistry*, 36 (1994) 65-66.
- 7 Veeresham C & Shuler M L, Camptothecine from callus cultures of *Nothapodytes foetida*, *Biotechnol Lett*, 22 (2000) 129-132.
- 8 Satheesh Kumar K & Seeni S, *In vitro* multiplication of *Nothapodytes foetida* (Wight) Sleumer through seedling explant cultures, *Indian J Exp Biol*, 38 (2000) 273-277.
- 9 Thengane S R, Kulkarni D K, Shrikhande V A & Krishnamurthy K V, Effect of thidiazuron on adventitious shoot regeneration from seedling explants of *Nothapodytes foetida*, *In vitro Cell Dev Biol Plant*, 37 (2001) 200-210.
- 10 Murashige T & Skoog F, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures, *Physiol Plant*, 15 (1962) 473-497.