PRODUCTION OF ANTICANCER DRUG PODOPHYLLOTOXIN BY PLANT CELL CULTIVATION OF *Podophyllum hexandrum*

By

SAURABH CHATTOPADHYAY

Department of Biochemical Engineering & Biotechnology

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Dedicated to

my beloved parents

to whom I am indebted for what I am

Certificate

This is to certify that the thesis entitled "**Production of anticancer drug podophyllotoxin by plant cell cultivation of *Podophyllum hexandrum***", being submitted by **Mr. Saurabh Chattopadhyay** to the Indian Institute of Technology – Delhi, for the award of the degree of "**Doctor of Philosophy**" is a record of the bonafide research carried out by him, which has been prepared under our supervision in conformity with rules and regulations of the "Indian Institute of Technology – Delhi". The research reports and results presented in the thesis have not been submitted for any degree or diploma in any other University or Institute.

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ABSTRACT

Plant cell culture is the most viable alternative to the whole plant cultivation for production of valuable pharmacologically active substances. *Podophyllum hexandrum*, an Indian medicinal plant, which grows in the Northern Himalayan region, produces an important medicinal compound podophyllotoxin. Podophyllotoxin is the raw material for the production of anticancer drugs, etoposide and teniposide. Currently podophyllotoxin is isolated by solvent extraction of the rhizomes of *P. hexandrum*, which has already been declared as 'an endangered plant species' from the Himalayan region. Therefore, mass propagation of *P. hexandrum* by means of tissue culture could provide an alternative route for the production of podophyllotoxin.

The seeds of *P. hexandrum* were germinated under *in vitro* conditions and the callus cultures were initiated from the root explants of the germinated seedlings. The suspension cultures were developed from the established callus tissue. Initiation of suspension cultures were associated with various problems *viz.* clumping of cells, browning of media and drop in pH. These were addressed by application of pectinase and polyvinylpyrrollidone in the culture medium. Glucose was found to be a better carbon source than sucrose for cell growth and podophyllotoxin production. *P. hexandrum* was cultivated in 3 l stirred tank bioreactor under low-shear conditions in the dark. The culture and environmental parameters were optimized by statistical tools for the improvement of podophyllotoxin production. Six parameters (indole-3-acetic acid, ammonium:nitrate ratio, phosphate, glucose, inoculum and initial pH) were identified to screen the most significant parameters by Plackett-Burman design. Among these, four

parameters (glucose, inoculum, indole-3-acetic acid and initial pH) were selected to determine their optimum levels by Response Surface Methodology using Central Composite Design. The statistically optimized culture conditions (inoculum: 8 g/l, glucose: 72 g/l, IAA: 1.25 mg/l and initial pH: 6.0) were used to cultivate the cells of *P. hexandrum* in 3 l stirred tank bioreactor using a low-shear setric impeller. Medium conductivity and on-line NADH fluorescence were correlated with cellular growth during the cultivation of *P. hexandrum* in shake flask and bioreactor, respectively. Different modes of cultivation (batch, fed-batch, continuous) of *P. hexandrum* were conducted for the production of podophyllotoxin. A mathematical model was developed by using the batch kinetics data and it was extrapolated to identify suitable feeding strategies for fed-batch and continuous cultivation with cell retention. A maximum of 48 g/l biomass and 43.2 mg/l podophyllotoxin were achieved by fed-batch cultivation of *P. hexandrum* in 60 d. The productivity was enhanced by 30% by fed-batch cultivation of *P. hexandrum* over the batch cultivation. An improvement in cell growth (53 g/l) and podophyllotoxin production (48.8 mg/l) was observed when *P. hexandrum* was cultivated in continuous mode with cell retention device. The *in vitro* produced podophyllotoxin was found to exhibit cytotoxic activity against human breast cancer cell line (MCF-7) and the IC_{50} of the compound was found to be 1 nM, when added at the beginning of the growth of the tumor cells.

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