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Immobilization of *Andrographis paniculata* leaf cells for enhanced production of andrographolide

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Abstract

Traditional techniques of callus and suspension culture do not fully explore the range possibilities of cultured cells. Immobilization is the advanced novel biotechnological approach, through which it is possible to achieve a physiological state conducive for metabolite production that may not have been attended otherwise. andrographolide is the major alkaloid found in Andrographis paniculata, has been used for the treatment of various diseases and disorders paerticulerly related with liver. Leaf cell homogenate was immobilized in calcium alginate and carragennan and the effect of immobilization on cell growth, cell viability and cells biosynthetic activity was observed.

Key words: Andrographis paniculata, plant cell immobilization, secondary metabolite, andrographolide, HPLC.

INTRODUCTION

With emerging trends of exploiting plant cell culture for the production of high value phytopharmaceuticals, immobilization of plant cell has a very important role. Immobilization can overcome many limiting factors of suspension culture with distinct advantage of easier separation from product and also being amenable for biotransformation of low value compounds to higher value product. Plant cell immobilization is a valuable addition to the general techniques used in the plant tissue culture [1]. Immobilization induces or increase secondary metabolite production [2] and may even stimulate its excretion. Enclosure in a support exerts certain stress on the plant cell leading to restricted growth, the conditions normally considered favourable and as a prerequisite for enhanced metabolite production [3]. Here we describe studies on immobilized *Andrographis paniculata* leaf cell line homogenate in calcium alginate and carrageenan gel, these agents were selected as carrier for immobilization because it doesn't

affects cell viability, and cells are not subjected to expose to the strong chemical forces. Also a distinct advantage is the reproducibility of result and easier study of cell physiology.

A. paniculata is an herbaceous plant in the family Acanthaceae, native to India and Sri Lanka. It is used as a wonder drug in traditional Siddha and Ayurvedic system of medicine as well as in tribal medicine in India for multiple clinical applications [4]. It is a component of over 50% of the multi-ingredient herbal formulations available in India for the treatment of liver ailments [5]. The plant has been reported to possess antipyretic, analgesic, antibacterial, antiinflammatory and immuno suppressive properties due to its bitter content. Andrographolide ($C_{20}H_{30}O_5$; MW 350.44), a bicyclic diterpene lactone is the major constituent extracted from the plant [6]. After immobilization, cell growth, cell viability and the ability of immobilized cells to synthesize andrographolide from de-novo with sucrose as a sole carbon source has been investigated.

MATERIAL AND METHODS

The standard sample of andrographolide was obtained from Laila Impex, Vijayawada, A.P., India, and calcium alginate was purchased from CDH, Mumbai. All other chemicals were of analytical grade. Methanol (Qualigence Fine Chemicals, Mumbai) and water for HPLC (CDH, Mumbai) used for HPLC of andrographolide.

The immobilization of *A. paniculata* leaf cell homogenate was carried out under sterile conditions. Fresh leaves were collected from every part of plant i.e. upper. Lower and middle part and surface sterilized with 0.1% $HgCl_2$ for 2 min. Surface sterilized leaf material was homogenized using sterilized pestle mortar. Homogenate leaf material was then passed though net (425 µm), to remove the large cell aggregate and fibrous material.

2 gm fresh weight cells were suspended in 2% sodium alginate (10 ml) and the suspension was added drop wise to 50 mM Calcium chloride through sterilized needle and syringe. The alginate beads of diameter 2-3 mm were formed and were left in Cacl2 silution for 30 min for the stabilization of beads and then washed with sterilized water.

Cells were immobilize in carrageenan by suspended 2.0 gm fresh weight cells in 3% at 500 C and the suspension was added dropwise to 0.3 M KCL solution, The beads are formed and left in solution for 1 hr then washed with sterilized water.

Calcium alginate and carrageenan entrapped *A. paniculata* cells were cultivated in MS media [6] supplemented with 1 ppm 2-4 D and 0.5 ppm Kinetin. Cultured cells both in freely suspended and immobilized state in Erlenmeyer flasks 250 ml. capacity were placed in Remi shaker cum incubator at 40 rpm. Maintaining temperature of 25± 2^0 C, and under direct light of 40 W for 16 hrs light and 8 hrs. dark phase.

To quantify the cell number increment, 10 ml of cell suspension culture was pipette out after proper shaking for cell growth studies. Randomly selected 10 beads are placed in 10 ml. of 0.05M potassium phosphate buffer (pH 6.5), after ensuring complete dissolution of beads, the cells were counted under microscope with heamocytometer [2] and examined for cell growth studies. Cell viability was checked by using 23.9 mM Triphenyl tetrazolium chloride (TTC) [8].

Estimation of biosynthetic activity of andrographolide:
The culture media was harvested at the regular interval of 4 days to estimate the biosynthesis of andrographolide from immobilized cells. The sample was prepared analyzed by HPLC method [9] for 20 days because the beads lost their integrity and starts to disintegrate after 22 days using Shimadzu HPLC having Resolve C18 spherical 5 u (3.9 mm X 15 cm) column and SPD-M 10 Avp detector at 223 nm.

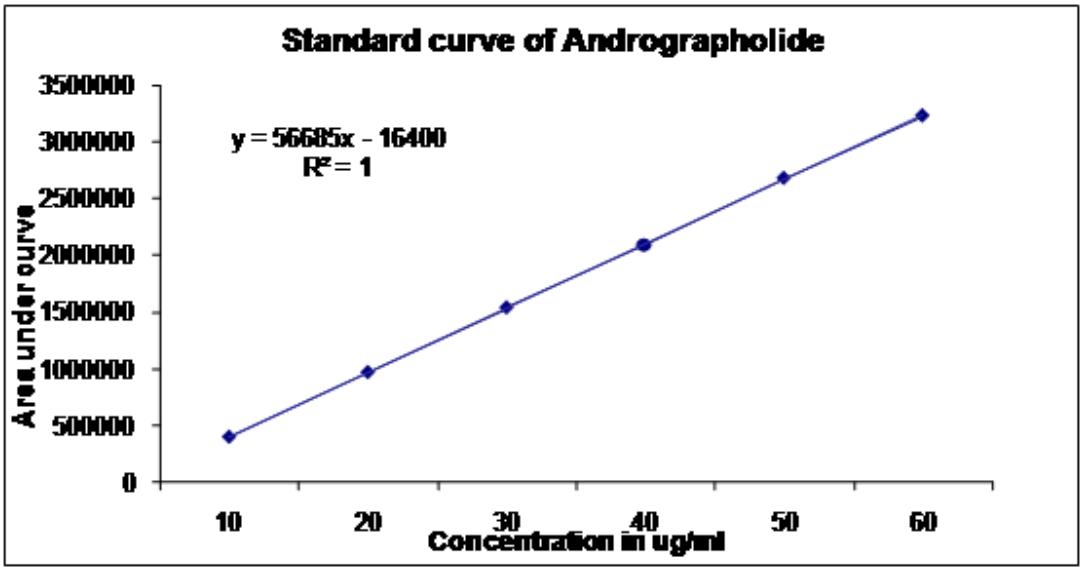

Fig. 1: Standard HPLC estimation curve for Andrographolide

Table 1. Cell viability % at different time intervals

Type of cultured cell in MS Medium	Cell viability % on day				
	04	08	12	16	20
Freely suspended cells	92	86	86	81	72
Calcium alginate immobilized cells	90	82	76	69	62
Carrageenan immobilized cells	85	74	68	61	53

Table 2. Andrographolide productions in plant organ, cell suspension culture and immobilized cells

Type of cultured cell in MS Medium	Andrographolide production in µg/ml/gram fresh weight on day				
	04	08	12	16	20
Intact plant organ	0.42				
Freely suspended cells	0.42 ±0.14	0.56 ± 0.16	0.61 ± 0.22	0.68 ±0.17	0.73 ±0.25
Calcium alginate immobilized cells	0.45±0.08	0.59 ± 0.13	0.64 ±0.18	0.71 ±0.25	0.78 ±0.33
Carrageenan immobilized cells	0.41 ±0.11	0.53 ± 0.17	0.58 ±0.21	0.63 ±0.24	0.68 ±0.28

Values are mean ± SD

RESULTS AND DISCUSSION

The present studies shows that the immobilization of leaf cells in calcium alginate and carrageenan markedly reduced the increment in cell number and biomass. There is not much significant difference in cell viability; however immobilized cells exhibit comparatively low viability table 1 but not in case of carrageenan as it shown lower cell viability, but calcium alginate entrapped cells shows higher biosynthetic capacity as compare to freely suspended and carrageenan entrapped cells.

CONCLUSION

It appears from the results that the formation of secondary metabolite can be increase by restricted growth of plant cells as immobilization restricted the growth. It is also concluded that the immobilization method and chemical can affect the cell viability and cellular metabolism as the carrageenan entrapped cells shows lower cell viability and andrographolide synthesis. Thus calcium alginate is found the better option for higher andrographolide synthesis. Immobilization of whole cell system can not disrupt any step of biosynthetic pathway, however there are some shortcomings are also observed that to increase the integrity of immobilized cell system and leaching of stored product from alginate and carrageenan matrix to the culture media and these can be investigate as further studies.

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