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Study of Seasonable Variation in Vasicine Content and Influence of Plant Growth Regulator on Callus Culture of *Adhatoda vasica* Nees

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ABSTRACT

Adhatoda vasica is a well-known plant drug in Ayurvedic and Unani medicine. Adhatoda leaves have been used extensively in Ayurvedic Medicine primarily for respiratory disorders. Vasicine is the major alkaloid, responsible for pharmacological action. Vasicine was studies for seasonal variation and different concentrations of auxin and cytokinin was used to study their effect on callus formation. HPLC method was used to determine vasicine content.

Keywords: Vasaka, Vasicine, seasonal variation, callus culture, HPLC

1. INTRODUCTION

For centuries, human being are totally depends on the plants as source of food additives and as a source of medicines. Plant biotechnology may provide enormous possibilities and potentialities to solve these problems to a considerable extent. To overcome the problems in synthesizing the complex chemical structure, and limitations of natural resources, the alternative ways for the production of secondary metabolites are developed. Immobilization is now a well-established technique with the history of enzyme immobilization going back over 25 years and including many industrial applications. Through immobilization, the plant cells are protected from liquid shear forces. Moreover, immobilization facilitates the importance of cellular cross talk, which can establish inter-cellular communication by the action of signaling molecules. This should enhance the biosynthetic capacity of plant cells.

Various preparation of *Adhatoda vasica* leaves are used for curing bleeding, haemorrahge, skin diseases, wounds, headache and leprosy in Southeast Asia. The bruised fresh leaves are used for snake-bites in India and Sri Lanka. Usually, yellow leaves are exploited for cough and smoke from leaves is used for asthma.

2. MATERIALS AND METHODS

The leaves of *Adhatoda vasika* was collected from the Botanical garden of Department of Pharmaceutical Sciences, Hi-tech College of Pharmacy, Bhubneswar (Odi.). The plant material was dried under shed and then powdered using pestle mortar which was further subjected to extraction.

2.1 Study of seasonal variation on vasicine production in the intact plant [10]

The leaves of *Adhatoda vasica* are collected in different months and vasicine content was measured on moisture free basis. The leaves are collected from every part of plant (upper, middle and lower) were collected and thoroughly mixed to represent a homogeneous sample. Leaves (100 gm) were put in oven at 70^0C for 15 min. then leaves are subjected to extraction by acid alkali method.

2.2 Establishment of callus culture

For the preparation of callus culture studies two medium were selected as they are most commonly used for plant tissue culture studies and have almost all desired essential macro and micro nutrients which are essential for the growth of plant tissue, these medium are MS medium and B_5 medium. The plant growth regulators were added to the medium to promote the growth and also to study their effect on callus growth.

In the present study three plant growth regulators were selected in which one belongs to auxin class of plant growth regulator i.e. 2,4-Dichlorophenoxyacetic acid (2,4-D) and Indole 3 acetic acid (IAA) inducing cell elongation and cell division with all subsequent results for plant growth and development., another one is kinetin, a kind of cytokinin, that promotes cell division.

For establishment of callus cultures, Healthy and disease free plant from which the explant was obtained. For propagation, nodal stem explants with auxiliary buds were used and for raising callus, Auxiliary leaves explants from *Adhatoda vasica* were collected and washed under running tap water to remove dirt traces. Explants were immersed in a detergent for 5 min, washed in water once, and then surface sterilized with mercuric chloride (0.1%) for 4-5 minutes, and then explants were washed thrice with double distilled water.

The surface sterilized leaves cut in to small pieces (1-2 square mm). The cut segments were then cultured individually on MS and B_5 medium containing different concentration of 2, 4-Dichlorophenoxyacetic Acid (2, 4-D), Kinetin (Kin) and Indole acetic acid (IAA).

These inoculated explants were observed for 2 weeks of culture. The present frequency of callus induction was determined by counting the number of explants producing callus as the percentage of the total number of explants. The callus induced was subculture at an interval of 21 days on the same medium. They were monitored, developed and maintained on the same medium. Callus was healthy over a period of time on this medium.

2.3 Extraction of alkaloidal fraction [10]

The powder was then reflux with 50 ml methanol for 2 hrs Filtered and the marc was subjected for extraction for another two cycles (1 hr each) with methanol. The filtrate was concentrated to 1ml, diluted with water to about 20 ml, The aqueous extract was acidified with 5 ml of 3% hydrochloric acid, Partitioned twice with 10 ml chloroform. Reject the chloroform fraction, and basify the remaining aqueous phase with 3% ammonia solution (5 ml), extract with chloroform (5 x 10 ml), concentrate the pooled chloroform fraction (1 ml), and dissolved in 100 ml methanol.

2.4 HPLC estimation of Vasicine [10]

The dilutions of known concentration of vasicine (Provided by Prof. M.D. Kharya, Department of Pharmaceutical Sciences, Dr. H.S. Gaur University, Sagar, M.P.) was prepared by dissolving them in methanol in such a way to get the concentration range between 10-60 µg/ml.

Subject known volume of standard and sample preparation to HPLC, and the respective peak area for vasicine was recorded and accordingly its concentration in culture medium was calculated in µg/ml by using the standard calibration curve of vasicine.

Instrument: Shimadzu
Mobile phase: Methanol: water (2:3)
Flow rate: 0.7 ml/min
Column: Resolve C18 Spherical 5µ (3.9 mm X 15 cm)
Detector: SPD-M10 Avp
Wavelength: 298 nm
Retention time: 3.8 min

3. RESULTS AND DISCUSSION

The effect of season was observed in context to vasicine content and after extraction the results were shown are summarized in table 1.

The callus was grown with various mediums under influence of different concentrations of plant growth regulators (PGR). The results are tabulated (Table 2).

The result shows that the vasicine was synthesized more in winter season as the vasicine content was found highest in the month of September. various plant growth regulators affect the formation of callus. In this study we take seven different composition of auxin (2,4-D and IAA) and cytokinin (kinetin) in different concentrations. The callus induction was not done with the composition of 2,4-D, IAA, kinetin at 1.5 ppm concentration. All other compositions show callus induction and the maximum induction was found in 2,4-D, IAA, kinetin at 2.0, 0.5 and 0.5 ppm respectively.

Table 1: Seasonal variation in vasicine content estimated by HPLC

S. No	Month of Collection	Moisture %	Alkaloid content (residue) %	Vasicine % of total a alkaloidal residue
1	January	64.00	1.01±0.02	58±0.3
2	February	67.25	0.78±0.04	48±0.4
3	March	68.75	0.75±0.02	43±0.3
4	April	63.15	0.64±0.03	87±0.4
5	May	60.48	0.63±0.07	89±0.4
6	June	55.00	0.62±0.04	92±0.5
7	July	53.75	0.60±0.03	90±0.7
8	August	71.37	1.21±0.08	87±0.6
9	September	65.00	1.21±0.03	87±0.5
10	October	71.84	1.24±0.09	84±0.6
11	November	75.85	1.36±0.04	80±0.3
12	December	68.00	0.79±0.04	78±0.3

Values represent the mean n = 3, ± SE

Table 2: callus induction with various plant growth regulators

S.N.	MS medium				B_5 medium			
	PGR	Conc. (ppm)	Callus induction %	Observation	PGR	Conc. (ppm)	Callus induction %	Observation
1	2,4-D	0.5	50	Callus formation	2,4-D	0.5	40	Callus formation
	Kin	0.5			Kin	0.5		
	IAA	0.5			IAA	0.5		
2	2,4-D	1.0	50	Callus formation	2,4-D	1.0	50	Callus formation
	Kin	0.5			Kin	0.5		
	IAA	0.5			IAA	0.5		
3	2,4-D	1.5	50	Callus formation	2,4-D	1.5	40	Callus formation
	Kin	1.0			Kin	1.0		
	IAA	0.5			IAA	0.5		
4	2,4-D	2.0	70	Callus formation	2,4-D	2.0	60	Callus formation
	Kin	1.0			Kin	1.0		
	IAA	0.5			IAA	0.5		
5	2,4-D	2.0	80	Callus formation	2,4-D	2.0	80	Callus formation
	Kin	0.5			Kin	0.5		
	IAA	0.5			IAA	0.5		
6	2,4-D	1.5	60	Callus formation	2,4-D	1.5	60	Callus formation
	Kin	1.0			Kin	1.0		
	IAA	1.0			IAA	1.0		
7	2,4-D	1.5	20	No callus (very less developed)	2,4-D	1.5	10	No callus (very less developed)
	Kin	1.5			Kin	1.5		
	IAA	1.5			IAA	1.5		

4. CONCLUSION

The present study concluded that the seasonal variations lead to affect metabolic activities of the plant cells and this may be due to altered concentrations of plant hormones, as alteration in the concentrations in plant growth regulators results in variations in callus induction and cell growth which is related with secondary metabolite production.

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