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Phytochemical Profiling and Micropropagtion of Catharanthus Roseus

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Abstract: Catharanthus roseus is also called periwinkle or Vinca rosea, is an important medicinal plant of the apocyanceae family. It is known as Indian originated herb which grows wild in the south Asia. It has indigenous medicine in various parts of the world. In vitro propagation of C.roseus cultures were established and maintained in Vitro on MS medium supplemented with BAP, IBA, 2, 4-D for shooting and better shooting was observed on MS medium supplemented with IBA 0.5 mg/l. The shoot induction was observed after 15 days of the explant. This showed that In vitro shoot induction of plant can be performed with promising results.

HPLC analysis of the methanolic leaves extract of the plant showed the presence of phenolic and flavoinds compounds, which are present in Catharanthus roseus.

This is Qualitative analyses to determine the presence of bioactive compounds. For determination of gallic aicd, Tannic acid, Resorcinol, Kaempherol in a simple and rapid HPLC method was developed. Phosphate buffer (pH = 5.8) and solvent Acetonitrile are used in the ratio of 55: 45 as mobile phase. gallic aicd, Tannic acid, Resorcinol, Kaempherol were determined by HPLC by using Type C-18 column and absorbance were measured at 254 nm. Retention time of standards of catharanthus roseus the Retention times of gallic aicd, Tannic acid, Resorcinol, Kaempherol are 3.435, 5844, 7517, 1.612.

Keywords: Catharanthus roseus, HPLC, shoot induction, MS medium

I. INTRODUCTION

Catharanthus roseus is an important medicinal plant of the apocyanceae family. It is known as Indian originated herb which grows wild in the Indian subcontinent in southern Asia. The plant *Vinca rosea* (periwinkle) is an Apocynaceae, herb or subshrub which has been shown to be a source of many alkaloids.

It has indigenous medicine in various parts of the world. It helps in relieving muscle pain, depression of central nervous system. A variety of different alkaloids is present in *Catharanthus roseus* more than 130 different compounds. Medicinal plants are great importance to the health of individuals and communities. The medicinal value of these plants in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive substances include alkaloids, tannins, flavonoids, steroids, carbohydrates and phenolics compounds. Phytochemicals are naturally occurring, biologically active chemically compounds in plants.

II. MATERIALS AND METHOD

1) *Sample Collection:* The explants were collected from Loyola college campus. The inter node of the plant *Catharanthus roseus* was washed thoroughly with running tap water. Subsequently, washing was done in Tween 20 (3-5) drops in 200ml water followed by a series of treatments: 0.1% HgCl2 for 1min and final washing was done with autoclaved double distilled water 4-5 times, under laminar airflow chamber. The sterile nodal segment was inoculated in Murashige and Skoog's (MS) Medium with different concentrations of phytohormones; BAP, Indole - 3- butyric acid (IBA).The pH of the medium was adjusted to 5.8 and 10 ml of the medium incorporated with phytohormones was dispensed into culture tubes. The culture tubes were sterilized and allowed gradually to cool down. The inoculation was done under laminar air flow chamber and cultures were maintained in culture room under controlled conditions.
2) *Extraction of Plant Material For Hplc Analysis:* The plants leaves were washed with tap water to remove soil and dust particles. Then the leaves were shade and dried and powdered with mechanical blender and stored in for air tight container. The *Catharanthus roseus* leaf extracts were prepared by dissolving 20 g of the leaf powder in 100 ml of methanol respectively. This mixture was kept in the shaker for 24 hours, filtered and was allowed to evaporate.

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III. CHROMATOGRAPHIC CONDITIONS:

The presence of Phytochemicals in the extracts were qualitatively analysed by High Performance Liquid Chromatography. The methanol extracts Catharanthus roseus were analysed. The specifications for HPLC for the analyses of the samples are:

Equipment : HPLC, Water make, Pump 515
Column : HPLC, C-18 (5µl)
Injection volume : 20(µl)
Temperature : 28˚C
Mobile Phase : Solvent A - Phosphate Buffer (pH – 5.8)
Solvent B – Acetonitrile
Flow rate : 1ml per/m
Wavelength : 254 nm
Detector : UV- VIS detector

IV. RESULTS AND DISCUSSION

A. In Vitro Studies Of Catharanthus Roseus

Explants were collected and surface sterilized, then inoculated in the media with the following hormones at concentration of 0.5mg/l to 2mg/l. Three different concentrations (0.5 mg/L IBA, 1 mg/ L 2,4-D and 0.5,1.0 mg/ L BAP) Successfully induced.(Table.1) The successful Plant shoots were transferred to rooting medium supplemented with1.0 mg/L Indole-3-acetic acid (IAA).

Table 1 Effect Of Iba, Bap And 2, 4-D On Shoot Formation

HORMONES	CONCENTRATION mg/l	Number of explants responded with shoot buds
IBA	0.25	1
	0.50	4
	1.0	0
	1.5	1
BAP	0.25	3
	0.50	1
	0.75	0
	1.5	1
2,4-D	1.0	5
	2.0	1

B. Ms Medium For Rooting Of In Vitro Developed Shoots

In vitro developed shoots were excised from plantlets which attained a height of 2-3 cm and inoculated on MS medium supplemented with 1.0 mg/l IAA.

C. HPLC Analysis

High Performance liquid chromatography was done for the methanol extract of the plant. This is qualitative analyses done to determine the presence of various bioactive compounds. The chromatogram shows various peaks at different retention period time.

Table 2.HPLC ANALYSIS OF METHANOL EXTARCT OF *C.roseus*

Peak	Retention time	Area (mAU)	Area%	Compound
1	1.612	4998.51025	2.00060	Gallic Acid
2	3.435	647.75781	1.54369	Tannic Acid
3	5.844	282.92484	3.53451	Kaempherol
4	7.517	895.87146	1.11623	Resorcinol

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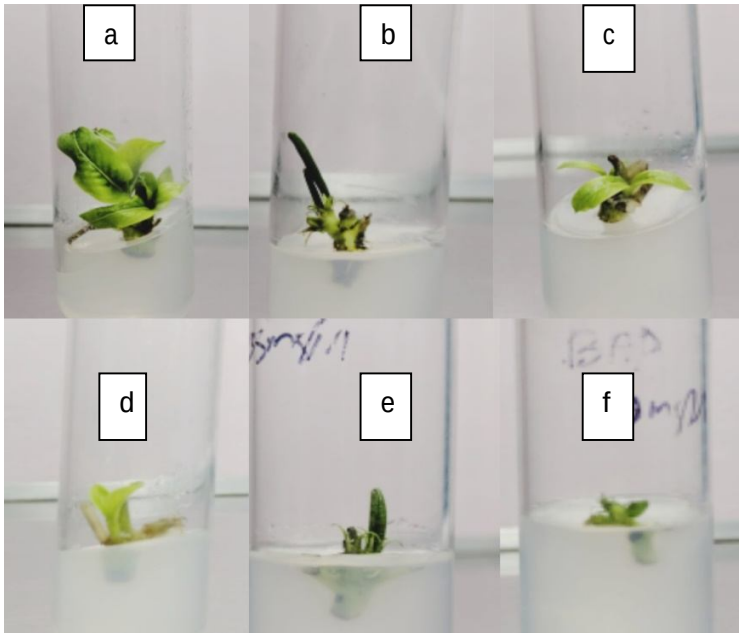

Figure.1 a) shoot induction on 0.50 mg/l IBA: b) shoot induction on 1.0 mg/l 2,4-D; c) shoot induction on 0.50 mg/l BAP; d) shoot induction on 1.5 mg/l BAP; e) Shoot induction on 1.0 mg/l BAP; e) Shoot induction on 1mg/l 2,4-D

Figure.2 Elongated shoot bud cultured on MS medium supplemented with 1.0 mg/l IAA for rooting

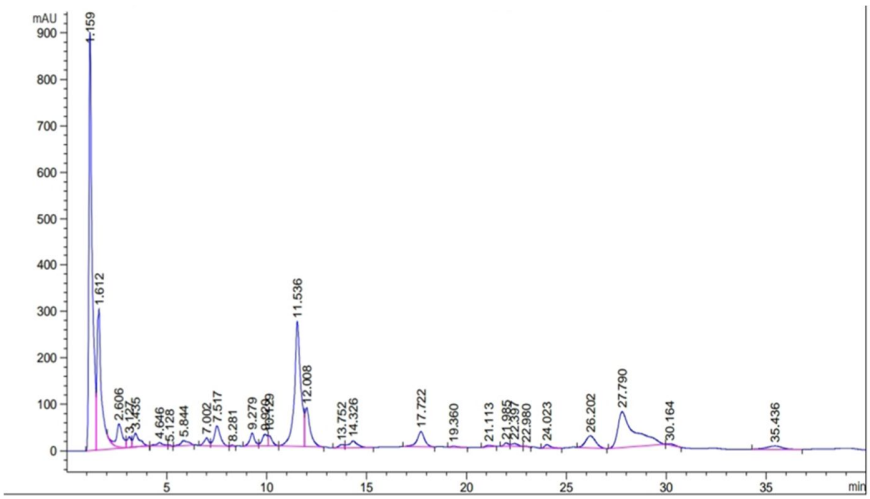

Figure.3- HPLC chromatogram of Extract of *Catharanthus roseus*

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V. CONCLUSIONS

In vitro propagation of Catharanthus roseus cultures were established and maintained in vitro on MS medium supplemented with BAP, IBA, 2,4-D for shooting and better shooting was observed on MS medium supplemented with IBA.0.5 mg/l. The induction of shoots was observed after 15 days of inoculation of the explant of Catharanthus roseus first with appearance of leaves, further shoots developed. This showed that In vitro shoot induction of plant can be performed with promising results. The successfully induced plant regenerations were transferred to rooting medium supplemented with 1.0 mg/l (IAA).

HPLC analysis of the ethanolic leaves extract of the plant showed the presence of phenolic and flavoinds compounds, which are present in Catharanthus roseus. This is a qualitative analyses to determine the presence of bioactive compounds. For determination of gallic aicd, Tannic acid, Resorcinol, Kaempherol in a simple and rapid HPLC method was developed. Phosphate buffer (pH = 5.8) and solvent Acetonitrile are used in the ratio of 55: 45 as mobile phase. gallic aicd, Tannic acid, Resorcinol, Kaempherol were determined by HPLC by using Type C-18 column and absorbance were measured at 254 nm. Retention time of standards of catharanthus roseus the Retention times of gallic aicd, Tannic acid, Resorcinol, Kaempherol are 3.435, 5844, 7517, 1.612. This HPLC method was found to be simple and convenient.

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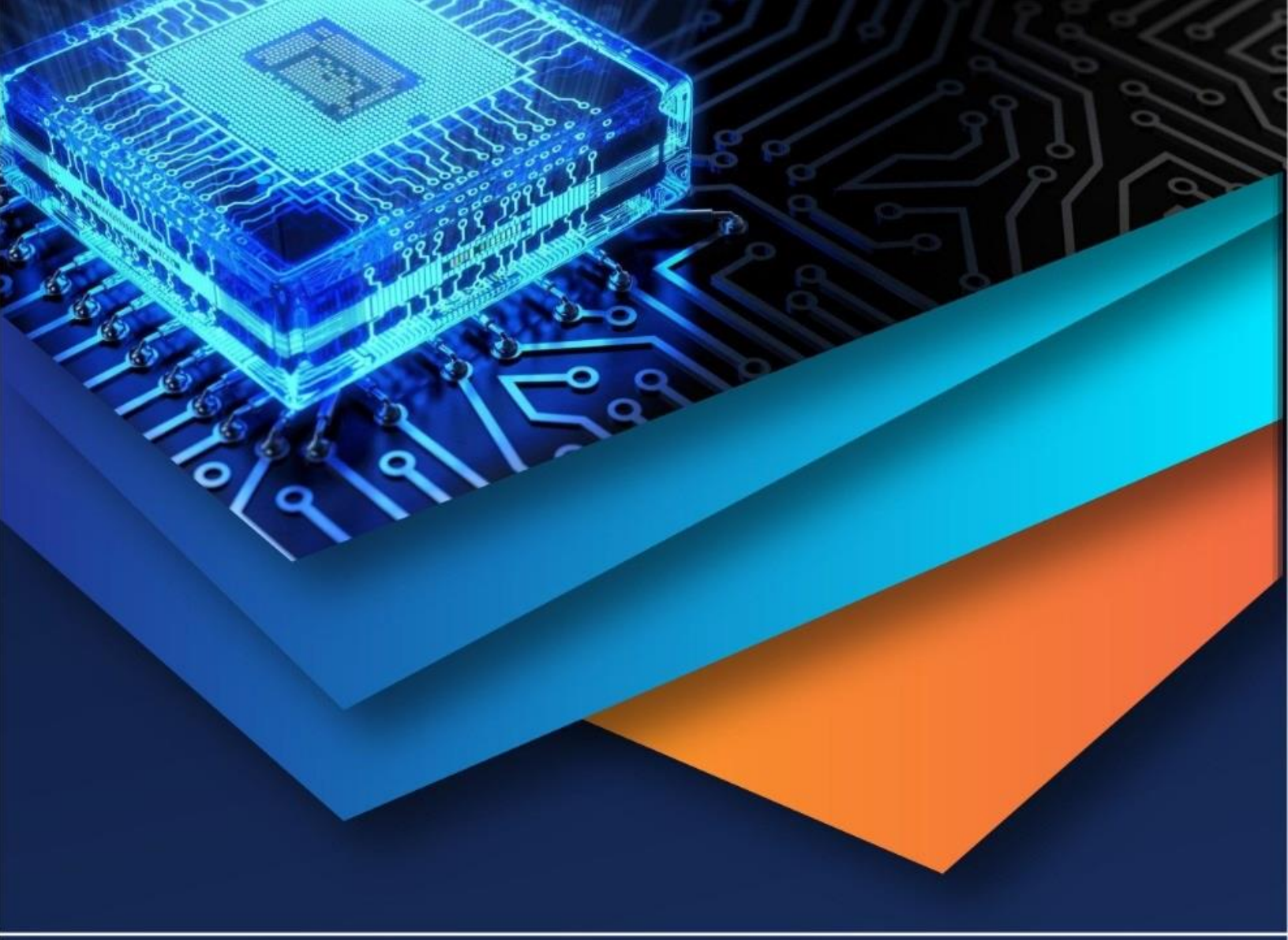

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