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INVITRO STUDIES ON THE PRODUCTION OF SECONDARY METABOLITES FROM *HEMIDESMUS INDICUS*

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ABSTRACT

Hemidesmusindicus (L) is avaluable source of severaleffective principles of therapeutic value. It is an important medicinal herb used in the traditional system of Ayurvdicmedicine in India for drug development. The aim of our present study deals with the development of invitro propagation of callus from the explant of *Hemidesmus indicus*, production of biomass culture by the cell suspension culture. Leaf explants were cultured on MS medium supplemented with different concentrations of plant growth regulators such as BA (2 mg/l), 2, 4 D (1 and 2 mg/l), NAA + Kinetin (2 + 1 mg/l) and NAA + Kinetin (1 + 2mg/l) respectively. The established calli were transferred into a different carbohydrates suspension cell cultures was produced the secondary metabolites. Results showed the goodcallus establishment was observed by BA at 2 mg/l within 7 – 9 days of incubation than other supplements and the great biomass yield of secondary metabolites was produced by the treatment of G5, M3 and S1based on fresh and dry weight.

Key words: *Hemidesmus indicus*, explant, callus, tissue culture and secondary metabolites.

[MS – Murashige and Skoog, BA – 6 Benzyl Adenine, 2, 4 D – 2, 4 Dichlorophenoxy acetic acid, NAA – Naphthalene Acetic Acid].

Introduction

Hemidesmus indicus (L). R. Br. or Indian Sarsaparilla is belongs to the family Periploceae (formerly Asclepidaceae), is one of the medicinal plant used in Indian traditional medicine (Anonymous, 1997) and also as a drug in Indian Pharmacopoeia (Anonymous, 1996)

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and British Pharmacopoeia. This plant is mainly found in India throughout north, east, west and south regions. It is common in open shrub jungles, hedges, uncultivated soil etc. It has been used as folk medicine and as acomponent in Ayurveda and unani preparations against hypertension, diarrhea, skin and eye disease. Roots are useful in hemicranias, joint pains and syphilis (Heble, M.R., and Staba, E., 1980). It is a most valued herbs in Indian system of medicine (Sasidharan., 2004, Siddique et al., 2004).

The medicinal plants produce an enormous number of secondary metabolites. Secondary metabolites are compounds biosynthetically derived from primary metabolites. Secondary metabolites are primarily accumulated by plant cells in smaller quantities than primary metabolites. It may represent chemical adaptations to environmental stress (Brain K.R. 1976).Currently they are mainly important for the pharmacological activity. The several distinct chemicals derived from plants are important drugs used in one or more countries in the world.The increasing demand of the secondary metabolites for pharmaceutical industries, the natural plant sources are not sufficient. To overcome these problems the industry requires another methods of supply of plants throughout the year by plant tissue culture techniques.

Plant tissue culture is an effective and best alternative method of plantation as it offers anexact supply of natural independent of plant cell cultures are capable of producing a specific therapeutic compounds at similar or superior to that of integral plants (Gantel. M and Memelink. H., 2006). Plant cells grown in culture have potential to producephytochemicals are similar to parent plant. This technique is a boon in the studies of the biosynthesis of secondary metabolites and provides an efficient of producing economically important natural products (Short, K.C and Roberts A.V. 1989). Recent developments in tissue culture technology shows that transcription factors are proficient new molecular tools for plant metabolic engineering to enhance the production of therapeutic proteins including monoclonal antibodies, antigenic proteins that act as immunogenes, human serum albumin, interferon and human hemoglobinetc (Hiatt et al., 1989; Marden et al., 1997; Hahn et al., 1997).

Materials and Methods

Collection and preparation of sample

Healthy rapidly growing leaf segments of *Hemidesmus indicus* (L). R. Br.was collected from the tropical area of Kanyakumari Dist (South India) and the selected plant was identified

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by the Herbarium of TBGRI (Tropical Botanical Garden Research Institute and the flora of the presidency of Madras).The plant samples were washed with water and dried in shade for 20 days after that dried plant materials were crushed into fine powder and stored at room temperature for further study.

10 gm of dried leaf powder were soaked in 250 ml methanol contained 500 ml conical flask. This mixed contents were run in soxhlet apparatus for 24 hrs. After 24 hrs, the extract was collected from the apparatus. The extract obtained were evaporated to dryness using vacuum evaporator and stored in refrigerator in reagent bottles. Part of fresh plant material was used for the callus production.

Preparation of Media

Stock solution of MS (Murashige and Skoog, 1962) medium is supplemented with macro elements of 3% sucrose and 0.6% (w/v) agar (Himedia Laboratories, India). Adjust the pH of the medium to 5.7 by drop wise addition of 1N NaoH and 1N Hcl. The media were steam sterilized in an autoclaved at 121^0C and 1.06 kg/cm^3 pressure for 20 minutes,

Explants collection and Inoculation

Leaf segments of *Hemidesmus indicus* was collected from the tropical area. Leaf segments washed with running tap water. The explants were surface sterilized in 0.1 % (w/v) aqueous mercuric chloride solution for 12 minutes followed by four times washed with sterile distilled water then the sterilized leaves were cut into small pieces (0.5 – 0.7 cm) was inoculated into the sterilized medium. The cultures were incubated at 9 – 12 hrs with 3000 lux light intensity for 16 hrs. The temperature inside the culture room was maintained at 25 ± 2^0C and relative humidity at 60 – 80%.

Callus Establishment

Various plant growth regulators such as BA (2 mg/l), 2,4 D (1 mg/l), 2,4 D (2 mg/l), NAA + Kinetin (2 + 1 mg/l) and NAA + Kinetin (1 + 2 mg/l) were tried incorporation with MS media to study the effect of plant growth regulator for the establishment of callus from the explant.

Suspension Culture

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Dry and fresh weight of established calli were transferred into the suspension culture containing 100 ml of MS media is augmented with various precursors at different concentrations such as Glucose (20 – 60 g/l), Maltose (20 – 60 g/l) and Sucrose (20 – 60 g/l)were placed on a shaker (125 rpm) at 22 ± 1^0 C,under continuous light, were tried for the production of secondary metabolites.

Results and Discussion

Establishment of Callus

The plant *Hemidesmus indicus* was inoculated in basal MS medium (1962) augmented with various concentrations of BA, 2,4- D and combinations of NAA, Kinetin for callus induction (Figure: 1). Observations were taken based on the number of days taken for induction of callus and nature of the callus (Table 1). Benzyladenine at 2 mg/l was found to be best callus induction. The callus was formed within 7-9 days of incubation. The nature of the callus was yellowish in colour and friable. At 2, 4-D (1 mg/l) the callus induces within 9 – 11 days of incubation. The nature of the callus light green in colour. In 2, 4-D (2 mg/l) the callus was yellowish green and compact callus. According to Siddique et al 2004, MS medium containing

mg/l BAP and 2.0 mg/l Kin in 85% of organogenic callus was noticed in nodal segments of *Withania somnifera* whereas direct regeneration frominvitro leaf explants was showed by Kulkarni et al ., 1996. Murashige and Skoog Basal salt with Minimal Organics medium was supplemented with 3 mg/l of 2, 4 D and 2.5 mg/l NAA formed high callus induction rates in all seven varieties of sorghum. Plant tissue culture studies discussed with the combination of plant growth hormones Auxin and Cytokinin for callus induction (Liu, G et al., 2015, Pola, S et al ., 2008 and Shafi, A et al 2015) with low concentration of 2,4 D being commonly used in cereals (Chawla, H.S. 2009).

Table: 1 Effect of Plant Growth Regulators for callus Development

Plant Growth Regulators (mg/l)	Number of days taken for Callus Induction	Nature of Callus
BA (2.00)	7 – 9	Yellow
2.4 D (1.00)	9 – 11	Light Green
2.4 D (2.00)	8 – 10	Yellowish Green

NAA + Kinetin	20 – 25	Pale Green

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, S Iss 3, Dec 2022

(2.00 + 1.00)		
NAA + Kinetin (1.00 + 2.00)	15 - 17	Pale Yellow

Figure : 1 Establishment of Callus

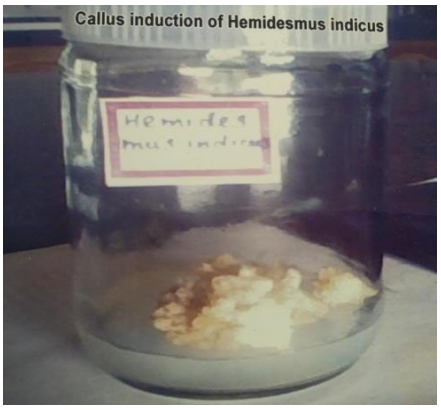

Figure : 2 Cell Suspension culture

Cell Suspension culture

50 mg of callus was transferred into a suspension culture with different carbohydrates to improve the yield of biomass and secondary metabolites (Figure: 2). The present study revealed that sucrose (20 g/l) in the medium induced maximum callus growth. S1 showed maximum fresh weight (824 mg) and dry weight (100 mg) of callus obtained in the same composition medium. In this study different level of glucose, maltose were used to produce

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high yield of biomass (Table: 2),at glucose 60 g/l (G5) was found to be good biomass production (Table: 2) (Fresh weight 804mg, dry weight 95 mg). Maltose at 30 g/l (M2) showed better biomass production (Fresh weight 455 mg, dry weight 52 mg) (Table: 2). The another study of FW and DW were noticed in suspension cultures of *Thevetia peruviana* and *Artemisia annua* (Arias et al., 2016; Wang et al., 2000). Cell suspension culture could be used for the extraction of secondary metabolites from culturing of plant cells through large scale system. The advantage of this technique is that it can finally offer a continuous, reliable source of natural products. Successful culture of plant cells in agitated liquid media was shown by Caplin and Steward (1948) and consequentlyby Muir et al., (1954). The suspension culture media color change is probably due to with cells maturing and aging, it had been proved that the suspension medium of *Coscinium fenestratum* Colebr. turned deep yellow because of the release of the alkaloids (Kashyap et al., 2016).

Table: 2 Effect of Carbohydrates for the Multiplication of Callus Suspension Culture

Sources	Treatment Number&Conc (g/l)	Fresh Callus Weight (mg)	Dry Callus Weight (mg)
Glucose	G1 (20)	620	80
	G2 (30)	619	78
	G3 (40)	615	73
	G4 (50)	630	82
	G5 (60)	804	95
Maltose	M1 (20)	160	25
	M2 (30)	455	52
	M3 (40)	260	36
	M4 (50)	375	41
	M5 (60)	53	10
Sucrose	S1 (20)	824	100
	S2 (30)	504	72
	S3 (40)	798	88
	S4 (50)	470	44
	S5 (60)	601	70

Conclusion

Tissue culture method is an important technique for its proliferation and production of phytochemicals for the conservation of large scale production of medicinally important plant. *Hemidesmus indicus* is a valuable source of medicinal plant for the development of new drugs. The main application of this plant contain active secondary metabolite compounds are used as

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chemotherapeutic and chemopreventive agents in molecular versatility. The present study focused that the recent pharmacological mode for the production of therapeutic compounds play a major role in human health care.

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