

Study of in-vitro Micro-propagation of Medicinally Important Plant *Andrographis paniculata* from its Different Parts

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Abstract: *Andrographis paniculata* is a plant of high medicinal importance. Explants from leaves, stem internodes and seeds had shown different patterns of callus stimulation under similar conditions of light duration, temperature and humidity with fixed concentrations of IAA and kinetin in MS media. During micro propagation, most efficient callus formation was observed in explants prepared from seeds and internodes. Under influence of IAA and kinetin, most viable callus culture was observed in seeds and internodes of stem. 18 of 25 nodal segments have developed callus of 4-6 mm with an efficiency of 72%. 21 of 25 seedlings have developed callus of 2-3mm with an efficiency of 84%. During callus development, few seedlings also exhibited some shoot development. 11 of 25 explants prepared from leaves have developed callus of 3-5mm with an efficiency of 44%.

Keywords: *Andrographis paniculata*, MS Medium, micro-propagation, in-vitro, callus

1. Introduction

Andrographis paniculata (Burm.f.) Nees, is an annual herb of Southeast Asian origin. This erect herbaceous plant is also known as "King of Bitters" in north eastern India, due to its extremely bitter taste. The plant grows up to 30-110 cm in height in shady places. Stem is square shaped, dark green and contains wings along angles of nodes. Leaves are lance shaped which lengths upto 8cm and flowers are white coloured in a raceme. Fruits are encapsulated in a 2 cm long pods and yellowish green in colour.

Indian Pharmacopoeia narrates that it is predominant constituent of at least 26 Ayurvedic formulations (Zhang, 2004; Mishra et al., 2007). Kalmegh is listed in 1992 Pharmacopoeia of People's Republic of China as a "cold property herb" used to rid the body from fever and dispel toxins (Deng, 1987). This plant was selected as one of the medicinal plants which were included in "National List of Essential Drugs A.D. 1999" in Thailand's Ministry of Public Health (Pholphana et al., 2004).

After a broad research on benefits of *Andrographis paniculata* in medical field, it has revealed that it has wide range of extremely beneficial pharmacological effects like anti-inflammatory (Sheeja et al., 2006), anti-diarrhoeal (Gupta et al., 1990), antiviral (Wiert et al., 2005), antimalarial (Misra P et al., 1992), hepatoprotective (Handa and Sharma, 1990; Trivedi and Rawal, 2000), cardiovascular (Rajagopal et al., 2004), anticancerous (Rajagopal et al., 2004), immune enhancement and anti-HIV activities (Calabrese et al., 2000), antioxidant (Trivedi and Rawal, 2000) and hypoglycemic (Borhanuddin et al., 1994). Leaves and stem are the main source of active ingredients found in *A. paniculata*. Besides these benefits, leaves of this plant are dry, bitter and cool and used as laxative and sometimes for treatment of breathing difficulties, burning sensation, cough, edema, thirst, skin diseases, syphilitic cachexia,

syphilitic ulcer, worms and acidity (Sivarajan and Balachandran, 2004).

Many investigations showed that the plant is abundant in various active chemical compounds. This valuable plant contains a rich source of diterpenoids and 2'-oxygenated flavonoids which includes andrographolide (Gupta et al., 1993; Neogy et al., 2008; Rastogi and Mehrotra, 1993; Visen et al., 1993) neoandrographolide (Balmain and Conally, 1973; Gupta et al., 1993), 14-deoxy-11,12-didehydroandrographolide (Rajagopal et al., 2004) 14-deoxyandrographolide, 19 β -D-glucoside (Rajagopal et al., 2004), homoandrographolide, andrographin, andrographosterin and stigmasterol (Chen and Liang, 1982; Pholphana et al., 2004). There are also some other compounds isolated from different parts of *Andrographis paniculata* are apigenin-7, carvacol, eugenol, myristic acid, hentricontane, oroxylon A and Wogonin (Rastogi and Mehrotra, 1993).

Among all the active compounds from *Andrographis paniculata*, andrographolide is most important chemical of medicinal importance. According to few researchers, andrographolide shows protective activity against paracetamol-induced toxicity on ex-vivo preparation of isolated rat hepatocytes and found to be more potent hepatoprotective than silymarin (Visen et al., 1993). Some investigations demonstrated that andrographolide, in an aqueous extract of *A. paniculata*, in combination with Vitamin E acts as putative protective agent against nicotine induced tissue injury in liver, kidney, heart, lungs and spleen by reducing lipid peroxidation, protein oxidation and increases the antioxidant enzyme status (Neogy et al., 2008)

This active ingredient is found to be potential cancer therapeutic agent in various investigations. This compound exerts direct anticancer activity on cancerous cells by arrest of cell cycle with help a cell cycle inhibitory of p27 and

depression in CDK4. Andrographolide also increases the cytotoxic activity of lymphocytes against cancer cells by enhancing the factor α -protective (tumour necrosis factor) and CD marker expression (Rajagopal et al., 2003).

Other diterpenes lactones like andrograpanin, a compound made from hydrolysis of andrographolide are used for treatment of infection and inflammation in China. Andrograpanin is a potential inhibitor for production of Nitric Oxide and pro-inflammatory cytokines (INF- α , IL-6 and IL-12p70). This activity includes the down regulation of p-38 nitrogen activated protein kinase (MAPK's) (Ge and Wang, 2008). Besides these benefits, its uptake should be avoided during pregnancy due to pregnancy terminating effects of andrographolide (Janarthanan, 1988). In Pharmaceutical Industry, there is high demand for andrographolide extracted from wild varieties of plants, but due to its limited availability, its commercial exploitation is hindered (Kanjilal et al., 2002). This limited commercial exploitation is because this plant is listed under vulnerable species in most of the Indian States (Sinha, 2013). Cultivation of this plant by commercial method is limited to vegetative means. In vitro micropropagation of medicinal and aromatic plants had proven efficient method to meet commercial demand of plant derived pharmaceuticals (Rout, 2002).

Expecting a preliminary report on micropropagation of *A. paniculata*, a wide research has been done in which different parts of plant like leaves, stem nodes and seeds were used to generate explants of different tissue culture media like MS medium (Murashige and Skoog, 1962), Gamborg's B5 medium (Gamborg et al., 1968) and Nitsch and Nitsch Basal Mixture (Nitsch and Nitsch, 1967) with changes in hormonal variations (Martin, 2003; Katakya and Handique, 2010- 11; Natrajan et al., 2006; Thatoi and Patra, 2011). Based on all research work on *A. paniculata*, there came various possibilities on efficiency of micropropagation for its conservation and commercialization.

2. Materials and Method

A collection of 10 plants of *Andrographis paniculata* were collected from a local Nursery, "Jayanti Kunj", Rewa, Madhya Pradesh, India in end of December 2014. Later, maintenance of plants till their flowering and fruiting was done at Botanical Garden at Department of Botany/Microbiology, Government T. R. S. College, Rewa, Madhya Pradesh, India. 15-20 days old seedpods were collected from these plants. Similarly, partially mature leaflets nodal segments of stem of length about 1-2cms were picked from plants.

Pods, leaves and nodal segments were undergone step by step surface sterilization process. Initially they were washed under running tap water for 20 mins to remove adherent particles like dust and soil, followed by washing with 0.1% (v/v) aqueous solution of TWEEN 20 (Qualigens) for 30 mins. The samples were then rinsed with 70% ethanol solution for 2-3 mins, followed by treatment of 1.2% Sodium Hypochlorite Solution (chlorine bleach) for 5-7 mins for complete removal of microbial contamination above the surface. Immediately, all the samples were rinsed

with sterile autoclaved distilled water 4 times to minimize the effect of chlorine bleach on viable plant tissues.

After complete sterilization process, pods were dried on sterilized blotting paper and carefully dissected with the help of tweezers to expose seeds for micropropagation and damaged ends of leaflets were nicked off with sterile. Similarly, outer covering of nodal buds and ends of nodal segments were removed with help of scalpel. These explants were then inoculated on MS medium (Himedia-PT099) containing Vitamins(B5), sucrose and calcium having hormonal concentration of 3mg/l of Indole-3-Acetic Acid(CDH-028558) and 0.3mg/l of Kinetin(Himedia-RM448) for callus induction. Each part was inoculated in a replica of 5 with 5 samples each. All the 75 samples were incubated at 30°C with 12hrs and 30mins of daylight (6.30am-7.00pm) daily for 4 weeks. After 4 weeks, size range in diameter of callus observed in most of the inoculated samples is as follows: Seed – 2-3mm (approx.), Leaves – 4-6mm (approx.), Nodal Segment – 3-5 mm (approx.)

3. Observation Table

Table 1: Observations from samples under PTC

Set No	Cytokinin (Kinetin mg/l)	Auxin (IAA mg/l)	Samples with Callus Induction (Max 5)	Samples Unaffected (Max 5)	% Viability of Callus
S1	0.3	3	4	1	84%
S2	0.3	3	5	0	
S3	0.3	3	5	0	
S4	0.3	3	4	1	
S5	0.3	3	3	2	
Total			21	4	
N1	0.3	3	3	2	72%
N2	0.3	3	4	1	
N3	0.3	3	4	1	
N4	0.3	3	4	1	
N5	0.3	3	3	2	
Total			18	7	
L1	0.3	3	3	2	44%
L2	0.3	3	2	3	
L3	0.3	3	3	2	
L4	0.3	3	2	3	
L5	0.3	3	1	4	
Total			11	14	

Where, S = Seed Samples, N = Internodal Segments, L = Leaflets and N=1,2,3,4,5 for number of replica.

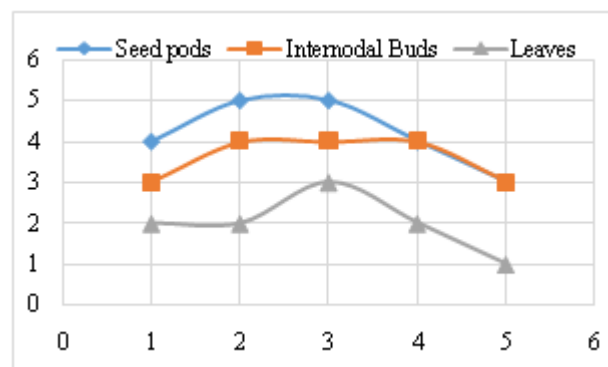


Figure 1: Graphical Representation of Callus Development in *Andrographis paniculata* in Different Somatic Parts

4. Result and Discussion

Callus Induction from all three parts were observed at a constant concentration of different growth regulators Kinetin and IAA. At a specific concentration of growth factors, the callus development was found to be highest in seeds and above 84% as compared to other parts of *Andrographis paniculata*. In nodal segments and leaflets, the effect of this range of growth regulators is observed to be less effective for callus development. The possibility of callus induction in the nodal buds was also above average i.e. 72% due to presence of active parenchymatous cells. On the other hand, due to least availability of undifferentiated cells in leaflets, the possibility of callus production has been below average i.e. around 44%.

It is also clear from the graphical observation that the totipotency of other parts of the plant is less as compared to seeds to develop callus. May be its due to cellular damage during sterilization process. On the other hand, the seed are completely enclosed inside the pods and kept them away from any severe contaminations. Somehow, at a certain point, the viability towards of seeds and internodes towards callus formation, is quite similar. We can consider both of them for a healthy callus formation.

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