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In vitro propagation and secondary metabolite production in of *Beaumontia grandiflora*, Wall (Apocynaceae)

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Abstract

A simple protocol for *in vitro* propagation of *Beaumontia grandiflora*, Wall (Apocynaceae) has been established. *Beaumontia grandiflora* is a woody, latex bearing climber cultivated throughout the world for its beautiful flowers. It is used in medicinal preparations for modulating steroidogenic activity. *B. grandiflora* is known to possess steroidal alkaloids and flavonoids. Antioxidant as well as antifertility activity have been demonstrated by extracts of *B. grandiflora*. So far there are no reports on *in vitro* propagation of *Beaumontia grandiflora*. Therefore present work was undertaken. BA(6 Benzylaminopurine) in MS medium positively influenced *in vitro* shoot growth. 80% bud induction was obtained on MS medium supplemented 8 μ M BA. 10 μ M IBA (3-indole butyric acid) induced 70% rooting in *Beaumontia grandiflora*. Preliminary phytochemical investigations were carried out for *in vivo* plant material mainly for alkaloids. TLC of ethanolic extracts of stem bark showed presence of Camptothecin, an anticancer compound. Further confirmation will be carried out by using HPLC.

Keywords: *Beaumontia grandiflora*, Apocynaceae, *In vitro* propagation, alkaloids

Introduction

Beaumontia grandiflora, Wall (Apocynaceae) is a woody, latex bearing climber cultivated throughout the world for its beautiful flowers.- It is a constituent of different preparations used in Bhutan and used for antifertility or steroidogenic activities [1]. Pharmacognostic characterization of *Beaumontia grandiflora*. have been carried out by Aslam I(2018) [2]. So far there are no reports of *In vitro* propagation of this plant so the present study was undertaken.

Phytochemical analysis of this plant has been carried out long back, (Sharma *et al.*, 1981, Kanchanapom *et al.*, 2002) [3,4]. Sharma *et al.*, 1981, Khaled Abdelshafeek, 2010 demonstrated presence of palamitic acid, linoleic acid, ursolic acid, triacontane, hentriacontane, $\hat{\alpha}$ -sitosterol- $\hat{\alpha}$ -D-glucopyranoside [5]. Digitoxigenin cardinolides were extracted by Kanchanapom *et al.*, 2002. Li *et al.*, 2009 reported a new steroidal alkaloid beaumontamine from *B. grandiflora* [6]. So far there are no recent reports on phytochemical analysis of *Beaumontia grandiflora* so the present investigations were carried out.

Material and Methods

Collection of plant material: The plant material was collected from nursery, Pune, Maharashtra state and raised by cuttings and maintained, Department of Botany, S. P. College, Pune. The nodal sectors from the raised cuttings were grown on MS medium [1] supplemented with different concentrations of BAP and IAA. *In vitro* root induction was tried on MS medium supplemented with different concentrations of IBA. All the cultures were incubated under white fluorescent light at 2000-lux intensity for 16 hours at 24 \pm 2 $^{\circ}$ C. Shoot growth, root induction and growth was recorded after 4 weeks.

Phytochemical analysis: The powdered stem with bark was used for alkaloid extraction which carried out using standard methods described by Harborne [7]. TLC of ethanolic extracts was carried out using pre-coated silica gel plates (PF 254 Merck type 5715, 0.250 mm thick) using solvent system- cyclohexane: chloroform: diethylamine (6:3:1). TLC plates were observed under UV light (254 nm and 365 nm) and sprayed with reagents *viz.* Dragendorff's reagent, and ferric chloride-perchloric acid reagent for detection of alkaloids. Rf of different spots was calculated

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Results and Discussion

A positive effect of BA on shoot growth *in vitro* was observed on MS medium containing 8 μ M BA (Table 1). 80% bud induction was observed. The best results for root induction (70%) were obtained with MS medium containing 10 μ M IBA (Table 2). Thus the entire plantlets of *Beaumontia grandiflora* have been successfully raised *in vitro* and hardening experiments are being carried out in the lab. (plate 1) Similar response in *Chonemorpha grandiflora* has been reported earlier by the author (Kulkarni and Malpathak, 2006) [8].

The phytochemical analysis revealed a number of alkaloid spots observed under UV light (365 nm). (Plate 2). Pure Camptothecin in DMSO was loaded in lane 1. Lane 4 shows fluorescent spot with same Rf as that of pure Camptothecin. So primary investigations show that the bark extract of *Beaumontia grandiflora* contains Camptothecin. Presence of camptothecin in *Chonemorpha grandiflora* has been revealed earlier by the author (Kulkarni *et al.* 2010) [9]. Further investigations are necessary for further confirmation of presence of camptothecin by HPLC.

Table 1: Effect of growth regulators on shoot growth

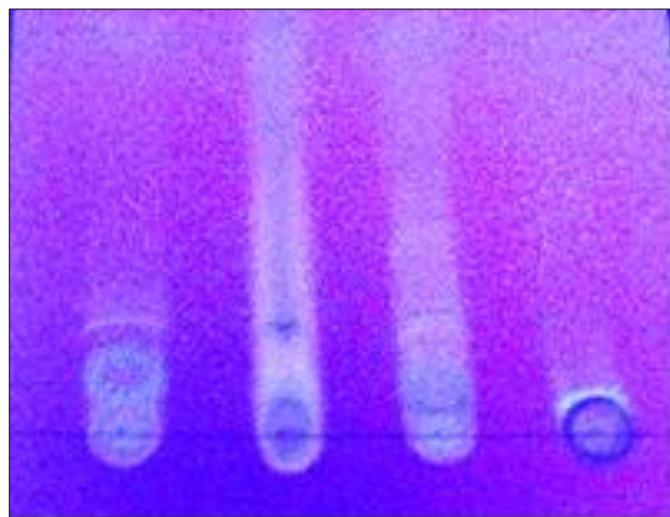
Medium Combination	*Shoot length in cm (4 weeks)	Bud break (% response(4 weeks)	*Days taken for bud break
MS Basal	1	40(no callus)	8
MS+5 μ M IAA	2	50 (heavy callus)	8
MS+4 μ M BAP	5	60(no callus)	4
MS+8 μ M BAP	7	80(no callus)	4
MS+12 μ M BAP	0.5	70(no callus)	4
MS+5 μ M IAA+4 μ M BAP	1	60(slight callus)	10
MS+5 μ M IAA+8 μ M BAP	1	50(slight callus)	15

*All results are mean of ten replicates

Table 2: Effect of IBA on root induction

	MS Basal	MS+2.5 μ M IBA	MS+5 μ M IBA	MS+10 μ M IBA	MS+15 μ M IBA
% Rooting	0	30	70	70	60
No. of roots	0	2	5	10	6
Days taken (root ind.)	---	13	7	6	9
Root length	---	0.6cm	1cm	3cm	2 cm

Plate 1



Lane 1 2 3 4

Lane 1-Pure camptothecin lane 4-*Beaumontia* ethanolic extract (stem bark)

Plate 2: Alkaloid profile of *Beaumontia grandiflora*

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Plate 1: *Beaumontia grandiflora*, at Amboli 2. Cutting raised 3. *In vitro* plantlet 4 - hardened plantlets *Beaumontia grandiflora*

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