Induction of Andrographolide, A Biologically Active Ingredient in Callus of *Andrographis Paniculata* (Burm.F) Wallich Ex. Nees

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Abstract Andrographolide is the main bioactive component of the medicinal plant, *Andrographis paniculata*. Callus and suspension cultures of *Andrographis paniculata* are known to produce only paniculides (sesquiterpene lactones) and not andrographolides (diterpene lactone) that are produced by the intact plant. In this study, Indole-3 acetic acid (IAA), Naphthalene-acetic-acid (NAA) and Gibberellic acid (GA) were used at different concentrations such as 25, 50, 75, 100 mg/l to find their effect on andrographolide content in leaves of *A. paniculata*. Treatment of detached leaves with growth regulators showed increase in andrographolide content and maximum enhancement was observed with NAA at 100 mg/l. Spectrophotometric and HPLC analysis proved andrographolide induction in callus by treatment with NAA. Present study thus indicates that andrographolide production can be induced in the callus of *A. paniculata* by treatment with plant growth hormones.

Keywords Growth Regulators, In Vitro Induction, NAA, Tissue Culture

1. Introduction

The “King of bitters” (*Andrographis paniculata*), family Acanthaceae, is a small endangered medicinal plant native to India and Sri Lanka. It is widely cultivated in southern Asia, where the roots and leaves are used in traditional medicine and pharmaceutical industries [1]. Andrographolide, the main constituent of *Andrographis paniculata* is known for its pharmacological activities and chemically it is a bicyclic diterpenoid lactone. Andrographolide is known to possess antihepatotoxic [2], antibiotic [3], anti-inflammatory [4], anti-snake venom [5], anticancerous [6] and anti –HIV [7] properties. Besides all it is generally used as immunostimulant [8] agent.

Andrographolide content varies with in plant parts and with the geographical distribution. Leaves of *A. paniculata* are reported to contain maximum andrographolide [9]. The conventional vegetative propagation of *A. paniculata* is too slow to meet the demand of pharmaceutical industries. Variability among the seed derived progenies and delayed rooting of seedlings restrains propagation through seeds [10]. Thus attempts were made by many laboratories to increase the quantity of andrographolide in *A. paniculata* plant parts using different inducers. Further to meet the overgrowing demand of andrographolide by pharmaceutical companies, attempts were also made to multiply *A. paniculata* through tissue culture. But callus developed on tissue culture media do not show detectable level of andrographolide. So the aim of the study was to treat the leaves of the plant with different growth regulator and to induce andrographolide production in the callus with the inducer that show best induction of andrographolide in leaves.

2. Materials and Methods

Leaves were collected from *Andrographis paniculata* before flowering as leaves collected after flowering show reduction in andrographolide content [11]. Leaves were treated with different inducers like Indole-3 acetic acid (IAA), Naphthalene-acetic-acid (NAA) and Gibberellic acid (GA) at different concentrations such as 25, 50, 75, 100 mg/l to enhance the andrographolide content. The growth regulators used for the experiments were dissolved in water to get the appropriate concentration. While dissolving IAA, few drops of alcohol were used before dilution with water. The leaves that were treated with water alone served as control.

The treated leaves along with control were collected at the end of three days and were evaluated for andrographolide content by spectrophotometric determination at 494 nm using Baljet reagent [12]. The amount of andrographolide was estimated from curve constructed using standard andrographolide.

High Pressure Liquid Chromatographic (HPLC)
determination of andrographolide was done following the procedure of Senthil Kumaran et al., [13]. A Shimadzu HPLC system (model LC 20AD) with UV-Vis spectrophotometric detector was used to quantify andrographolide in samples. A reversed phase Neusclosil C18 column (5 um, 250 × 4.6 mm, Macherey-Nagel, Duren, Germany) was used. The mobile phase was methanol: water (65:35 v/v). Aliquots of 20 ul of clarified sample extracts were injected into the HPLC system and eluted with the mobile phase at a flow rate of 1.0 ml/min. Elutes were monitored at 223 nm with the detector range setting fixed at 0.1. HPLC analysis of standard andrographolide showed single peak with retention time of 5.00 min. A representative calibration graph of andrographolide was constructed with different concentration of andrographolide. The peak area versus andrographolide concentration in the range of 2-10 ug/ml resulted in the regression equation, r =0.99.

For the induction of callus, leaf explants were collected from A. paniculata plant before flowering. The leaf tips of approximately 1cm size were cut and washed thoroughly with normal tap water and then surface sterilized under aseptic condition in the laminar air flow chamber. The explants were first surface sterilized in 70 % ethanol for 1 min following which the leaf bits were immersed in 1 % sodium hypochlorite solution (v/v) and were shaken for 3 min. Later, they were rinsed three times with sterile distilled water for 3 min per wash. These leaf bits were blotted dry before placed on to the Murashige & Skoog medium. The plant growth hormones 2, 4-D and NAA were tried at different concentrations to find the best combination for the induction of callus. Once the calli were established they were collected and treated with growth hormone for three days. These calli were then collected and evaluated for andrographolide content by spectrophotometric and HPLC methods as done for the leaf samples.

### 3. Results and Discussion

Plant growth regulators are known to induce andrographolide content in intact plants of A. paniculata [14-15]. From this study it was found that the andrographolide content of the detached leaves can also be enhanced by treatment with plant growth regulators. Of the different growth hormones tested, GA at 25 mg/L concentration showed maximum enhancement and was 6 fold higher when compared to control leaves. Leaves treated with IAA showed andrographolide content of 34.6 mg/gdw at 75 mg/l concentration when compared to andrographolide content of control leaves which was only 5.8 mg/gdw. Maximum enhancement in the andrographolide content was observed when leaves were treated with NAA compared to other two growth hormones. It was observed that the concentration of andrographolide increased with increase in concentration of NAA used for treatment. Maximum of 40 fold increase in andrographolide content was observed in A. paniculata leaves when treated with NAA at 100 mg/l concentration (Fig.1).

![Figure 1](image.png)

**Figure 1.** Effect of different plant growth regulators on andrographolide content of leaves of A. paniculata (Means ± SD, n=9). Data were statistically analyzed using one-way ANOVA followed by Turkey’s HSD test. Statistical significance was set at P<0.05. * indicates level of significance. Treatment of leaves with NAA showed extremely significant induction of andrographolide when compared to treatment with IAA and GA and showed P<0.0001.

The Murashige Skoog medium with 2,4-D and NAA each at 1mg/l was found to be the best medium for establishing callus.
The callus on this medium was whitish green in nature. As NAA at 100mg/l showed maximum induction of andrographolide in leaves, the same concentration of NAA was used to treat the callus of *A. paniculata*. HPLC analysis of calli treated with NAA showed a peak at 223 nm with the retention time of 4.99 indicating the induction of andrographolide in these samples (Fig.2b). Calli that served as control did not show the peak that represents andrographolide (Fig.2a). This study clearly indicates that andrographolide can be produced under *in vitro* conditions by treating the callus with NAA.

![Graph](image)

*(a) Control  (b) Treated with NAA (arrow indicates andrographolide peak)*

*Figure 2. HPLC analysis of andrographolide content in calli of *A.paniculata* treated with NAA*

Analogues prepared from natural andrographolide are emerging as powerful anticancer agents. A series of β-amino-γ-butyrolactone analogues synthesized from naturally occurring andrographolide revealed their potential for being developed as promising anti-cancer agents [16]. Further, a novel andrographolide-lipoic acid conjugate (AL-1) was shown to protect pancreatic b-cells from reactive oxygen species (ROS)-induced oxidative injury [17]. Present study suggests that andrographolide can be induced in callus and the quantity can be increased by altering the concentration of plant growth hormones used for treating the callus. As callus can be produced throughout the year and is not limited by the time of the year or weather, this approach can be used well in development of andrographolide derivatives against various ailments.

Plant growth hormones play a major role in tissue culture. The composition of nutrient medium along with specific ratio of auxin to cytokinin plays a major role not only in successful establishment of cell cultures but also in the production of secondary metabolites. Rapid high performance thin layer chromatography (HPTLC) screening of callus extracts revealed that the callus established in MS medium supplemented with 4.5 µM NAA and 0.46 µM BAP produced the highest yield of Proscillaridin A (4.51 mg/g DW), Scilliroside (3.3 mg/g DW), Scillaren A (2.35 mg/g DW) and desacetyllscilliroside (8.62 mg/g DW), which was higher than from the intact plants of *Charybdis congesta* [18].

Plant tissue culture systems can be used for continuous production of secondary metabolite compared to whole plants which shows more variation based on physiological and geographical conditions. The quantity of active principle produced can also be increased by changing the concentration of the inducer. Further this approach can considerably decrease the exploitation of medicinal plants where the entire plant is medicinal.

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Conflict of Interest Statement
We declare that we have no conflict of interest.

REFERENCES

[1] Kumar A., Dora J., Singh A. & Tripathi R. A review of king of bitter (Kalmegh). Int. J. Res. Pharmacy Chem. 2: 116-124. 2012.

[2] Maiti K., Mukherjee K., Murugan V., Saha B.P. & Mukherjee P.K. Enhancing bioavailability and hepatoprotective activity of andrographolide from Andrographis paniculata, a well-known medicinal food, through its herbosome. J. Sci. Food. Agric. 90: 43–51. 2010.

[3] Sule A., Ahmed Q.U., Samah O.A. & Omar M.N. Screening for anti bacterial activity of Andrographis paniculata used in Malaysia folkloric medicine: a possible alternative for the treatment of skin infection. Ethnobot Leaflets. 14: 445–456. 2010.

[4] Lee K.C., Chang H.H., Chung Y.H. & Lee T.Y. Andrographolide acts as an anti-inflammatory agent in LPS-stimulated RAW264.7 macrophages by inhibiting STAT3-mediated suppression of the NF -κB pathway. J. Ethnopharmacol. 135:678-684. 2011.

[5] Premendran S.J., Salwe K.J., Pathak S., Brahmane R. & Manimekalai K. Anti – cobra venom activity of plant Andrographis paniculata and its comparison with polyvalent anti-snake venom. J. Nat. Sci. Biol. Med. 2: 198–204. 2011.

[6] Lim J.C.W., Chan T.K., Ng D.S., Sagineedu S.R., Stanslas J. & Wong W.F. Andrographolide and its analogues: versatile bioactive molecules for combating inflammation and cancer. Clin. Exp. Pharmacol. Physiol. 39: 300-310. 2012.

[7] Tang C., Liu Y., Wang B., Gu G., Yang L., Zheng Y., Qian H. & Huang W. Synthesis and Biological Evaluation of Andrographolide Derivatives as Potent Anti-HIV Agents. Arch. Pharm. Pharm. Med. Chem. 345: 647–656. 2012.

[8] Radhika P., Annapurna A. & Nageswara Rao S. Immunostimulant, cerebroprotective & nootropic activities of Andrographis paniculata leaves extract in normal & type 2 diabetic rats. Indian J. Med. Res. 135: 636–641. 2012.

[9] Mishra S., Tiwari S.K., Kakkar A. & Pandey A.K. Chemoprofiling of Andrographis paniculata(Kalmegh) for its andrographolide content in Madhya Pradesh, India. Int. J. Pharm. Biosci. 1:1-5. 2010.

[10] Martin K.P. Plant regeneration of protocol of medicinally important Andrographis paniculata (Burm. F.) Wallich Ex Nees via somatic embryogenesis. In vitro Cell. Dev. Biol. 40: 204-209. 2004.

[11] Sharma M., Sharma A. & Tyagi S. Quantitative HPLC analysis of Andrographolide in Andrographis paniculata at two different stages of life cycle of plant. Acta Chim. Pharm. Indica. 2: 1-7. 2012.

[12] Shah K. & Trivedi P.S. Pundarikakshudu K. Spectrophotometric determination of andrographolides in Andrographis paniculata Nees and its formulation. Indian J. Pharm. Sci. 69: 457-458. 2007.

[13] Senthilkumar K., Thirugnanasambantham P., Viswanathan S. & Sree Rama Murthy M. An HPLC method for the estimation of andrographolide in rabbit serum. Indian J. Pharmacol. 35: 109-112. 2003.

[14] Gudhate P.P., Lokhande D.P. & Dhurnal K.N. Role of Plant Growth Regulators for Improving Andrographolide in Andrographis Paniculata. Pharmacogn. Mag. 5:249. 2009.

[15] Anuradha V.E, Jaleel C.A, Salem M.A, Gomathinayagam M. & Panneerselvam R. Plant growth regulators induced changes in antioxidant potential and andrographolide content in Andrographis paniculata Wall.ex Nees. Pest Biochem. Physiol. 98:312-316. 2010.

[16] Kasemsuk S., Sirion U., Suksen K., Piyachaturawat P., Suksamrarn A. & Saeeng R. 12-Amino-andrographolide analogues: synthesis and cytotoxic activity. Arch. Pharmacal Res . DOI 10.1007/s12272-013-0152-0. 2013.

[17] Yan G-R., Zhou H-H., Wang Y., Zhong Y., Tan Z-L., Wang Y. & He Q-Y. Protective Effects of Andrographolide Analogue AL -1 on ROS-Induced RIN -mb Cell Death by Inducing ROS Generation. PLoS ONE 8(6): e63656. doi:10.1371/journal.pone.0063656. 2013.

[18] Reddy A.S., Devi P.S. & Kiran S.R. In vitro cell culture of Charybdis congesta for enhanced production of secondary metabolites: Prosclillardin A, Scillaren A and Scilliroside. Afr.J. Biotechnol.12:1754-1759.2013.