Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(5): 2411-2415
Received: 28-07-2018
Accepted: 30-08-2018

N Anitha Chandran
Department of Post–Graduate Studies and Research in Botany, S D College, Alappuzha, Kerala, India

PR Unnikrishna Pillai
Department of Post–Graduate Studies and Research in Botany, S D College, Alappuzha, Kerala, India

Correspondence
PR Unnikrishna Pillai
Department of Post–Graduate Studies and Research in Botany, S D College, Alappuzha, Kerala, India

In vitro studies on the effect of Sodium azide treatment on secondary metabolites production in *Andrographis paniculata* (Burm. f.) Nees

N Anitha Chandran and PR Unnikrishna Pillai

Abstract
Effects of 0.01% SA (Sodium azide) for 3 hours on secondary metabolites production in *Andrographis paniculata* was carried out in this study. Calli produced from leaf explants inoculated on Murashige and Skoog's (MS) medium supplemented with 2 mg/l NAA, 1 mg/l Kinetin and 50 mg/l Phenylalanine. Treatment of 0.01% SA for 3 hours positively influence in secondary metabolites production when compared to the control. The study also showed that 0.01% SA for 3 hours treated samples showed enhanced production in the fresh weight, dry weight of calli and callus induction frequency compared to the control.

Keywords: Andrographis paniculata, andrographolide, sodium azide, HPLC, neo andrographolide

1. Introduction
Andrographis paniculata (Burm. F.) Nees of the family Acanthaceae is one of the most popular medicinal plants widely distributed in India, China, Sri Lanka, Taiwan and other southeast Asian countries. It is commonly used for the treatment of diarrhea, common cold, fever, respiratory tract infections (Negi *et al.* 2008, Sareer *et al.* 2014, Wang *et al.* 2014) [1-3] and has various therapeutic potentials including antimalarial Mishra *et al.* 2011 [4], antioxidant Lin *et al.* 2009 [5], antibacterial (Burm *et al.* 2010 [6] and anticancer activity Subramanian *et al.*2012 [7]. This herb has many vernacular names - Kalmegh in Bengali, Kiriyath in Malayalam, Nilavembu in Telugu etc. and is commonly known as king of bitters, because of its bitter taste. This plant contains pharmaceutically important compounds such as diterpenoids, flavonoids, and polyphenols (Chao & Lin 2010 [8]. In a clinical study, andrographolide was reported to inhibit human immunodeficiency virus (HIV) induced cell cycle dysregulation and also increases CD4+ lymphocytes in HIV-I infected patients (Calabrese *et al.* 2000) [9]. Andrographolide, neoandrographolide and 14-deoxy-11,12-didehydro andrographolide have been studied for their antiallergic, anti-inflammatory and cardiovascular effects Yoopan *et al.* 2007 [10]. Mutation breeding correlates with many advantages in plant improvement by improving a specific character without altering the original genetic makeup of the cultivar and supplementing to conventional methods in a favourable advantage Gottaschalk *et al.*1986 [11]. Sodium Azide (NaN3) is identified to be highly mutagenic in numerous plants and animals. Azide, commonly used as sodium or potassium azide (NaN3, or KN3) is a potent base substitution mutagen. The aim of the present investigation is the effect of Sodium azide treatment on the callus production and secondary metabolite production in *Andrographis paniculata*

2. Materials and Methods
2.1 Plant material
Healthy growing young branches with 4 to 5 nodes were collected from the botanical garden of S.D College, Alappuzha District, Kerala State, India. Collected healthy shoots were brought to the laboratory by wrapping with a wet muslin cloth. A voucher specimen has been deposited in Kerala University Botanical Herbarium, with the registration number KUBH 6031.
One week old seedlings were used for the study. The cotton swab method adopted was that of Biswas and Bhattacharya (1971) [12]. A cotton swab dipped in 0.01% Sodium azide solution for 3 hours was applied to the apical vegetative bud and Sodium azide was added to the cotton swab frequently by a dropper. Seedlings treated with distilled water kept as control. The third leaf from apex was taken from healthy seedlings swabbed with 70% alcohol soaked cotton and then washed in running tap water for 20 minutes followed by washing with 2 drops of labolene for 5 minutes.

After washing with distilled water brought the materials to laminar air flow. The explants were treated with 70% ethyl alcohol for 30 seconds for surface sterilization and rinsed in sterile double distilled water. Treatment of 0.1% concentration of mercuric chloride with different time duration were used and finally standardized the optimum concentration for sterilization. 0.1% mercuric chloride treatment for 6 minutes was found to be the optimum treatment time for surface sterilization. Different media for callus induction were tried in this study. Callus produced from these samples were subjected to HPLC analysis to detect the secondary metabolites.

2.2 Procedure of HPLC

Chromatographic conditions:

A. Mobilephase

1. Dissolve 0.14 gm of anhydrous potassium dihydrogen orthophosphate (KH2PO4) in 900 ml of HPLC grade water and add 0.5 ml of orthophosphoric acid. Make upto 1000 ml with water, filter through 0.45 membrane and Degas in a Sonicator for 3 minutes. (Solvent A)
2. Acetonitrile (Solvent B)

Standard preparation

20.0 mg andrographolide was weighed to a 100 ml volumetric flask. 50 ml of HPLC grade methanol was added. Sonicated for 5-10 minutes and warmed on a water bath at 60-70ºC for 5 minutes. Cooled to room temperature and volume was made up to 100 ml with methanol.

Sample preparation

1000 mg of given material were weighed in clean, dried 250 ml beaker, 50 ml of methanol was added into a 250 ml beaker and refluxed for 10 minutes, cool and sonicate for 6 minutes. Cool and transfer to 50 ml volumetric flask, repeat the above step for another 2 times and volume was made up to 50 ml with methanol.

2.3 Statistical analysis of the data

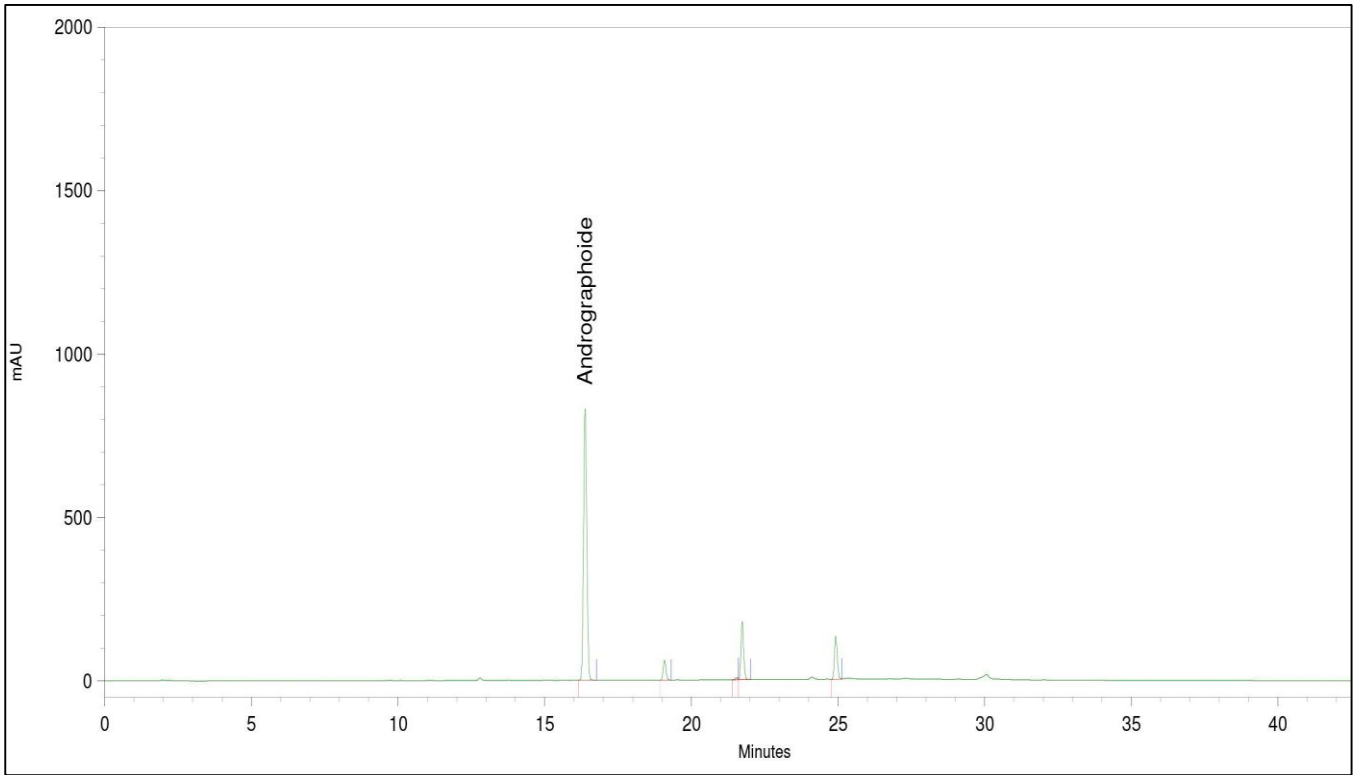
All the experiments were shown using a completely randomized block design (CRBD) method. Each treatment consist of three replications and each replication block was represented by 10 test tube per treatment. One way ANOVA was performed to determine the significance of treatments. The mean separation was done according to Duncan's Multiple Range Test ($P<0.05$).

3. Results and Discussion

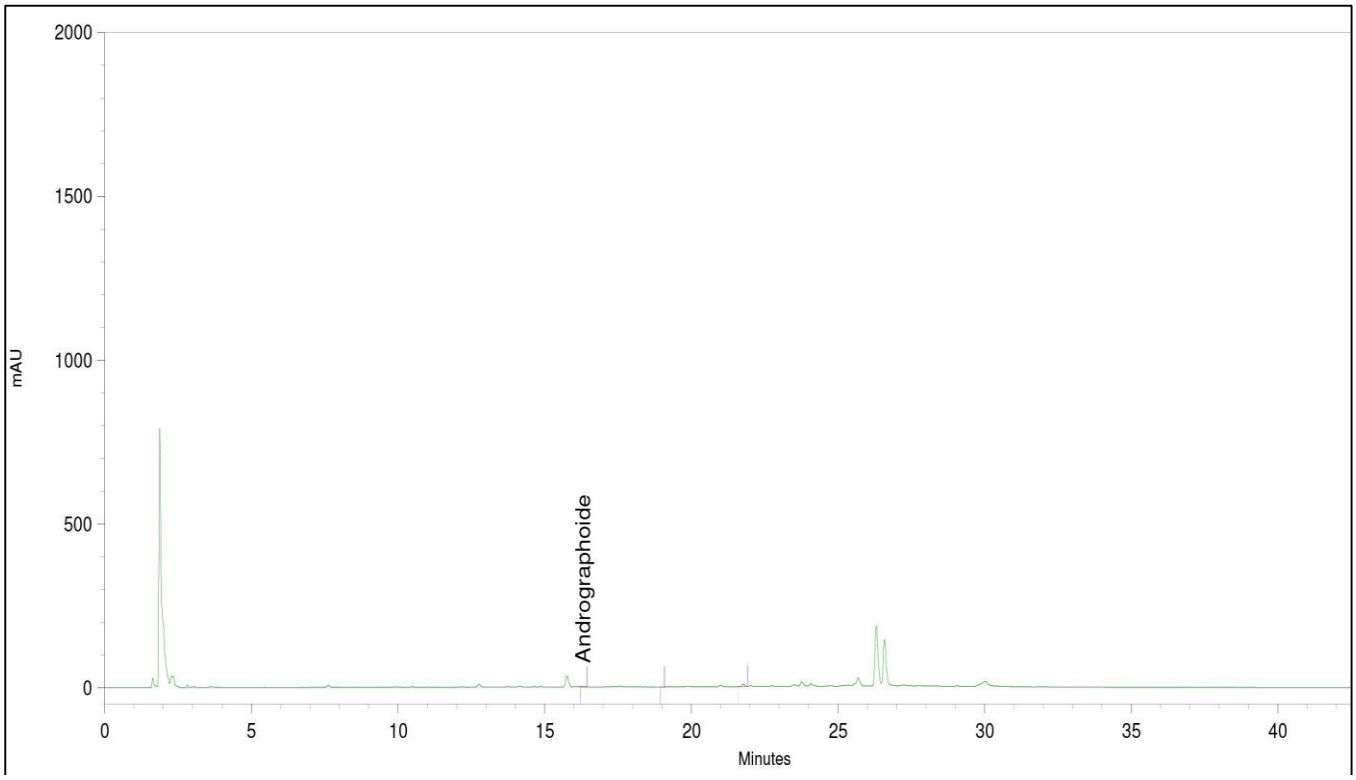
Explants taken from the control plants inoculated into full MS basal medium fortified with 2 mg/l NAA, 1 mg/l Kinetin and 50 mg/l phenylalanine. Control calli showed significant callus initiation and produced high amount of fresh weight (0.6113gm) and dry weight (0.04133 gm) in this medium. 0.01% of SA for 3 hours treated explants were inoculated on the medium containing 2 mg/l NAA, 1 mg/l Kinetin and 50 mg/l Phenylalanine produced high amount of fresh weight when compared to the control. The plant treated with 0.01% for 3 hours produced callus shows maximum response of callus induction (73.33±3.33), fresh weight (1.3537±0.041 gm) and dry weight of callus (0.1247±0.0097 gm). The calli produced from 0.01% SA treated explants showed higher amount of fresh weight, dry weight and percentage of the response of the callus induction than the control.

Fig 1: Control

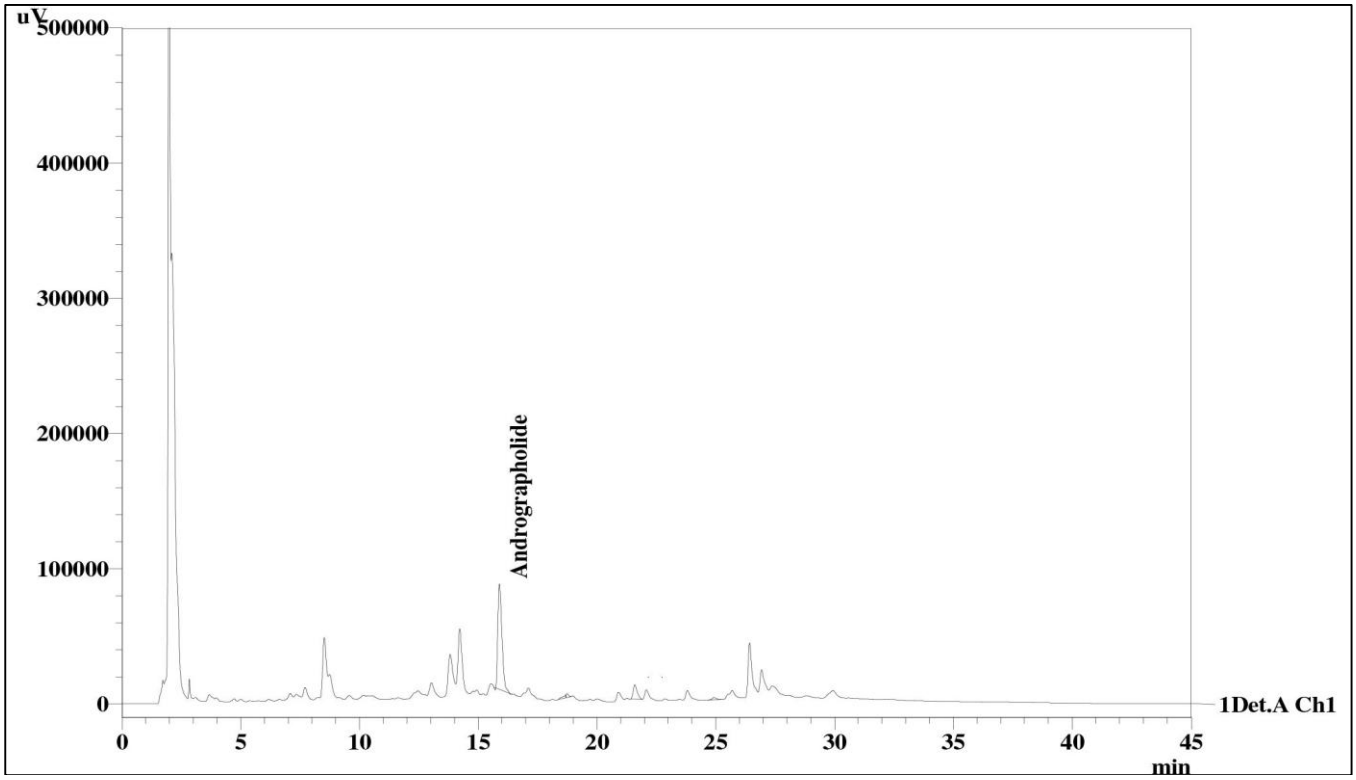
Fig 2: 0.01% SA for 3 hours


2000
1500
1000
500
0
mAU
Andrographoide
0
5
10
15
20
25
30
35
40
Minutes

A.


2000
1500
1000
500
0
mAU
Andrographoide
0
5
10
15
20
25
30
35
40
Minutes

B



C

HPLC chromatograms of andrographolide a. standard; b. Control; c. 0.01% SA for 3 hours;

By HPLC analysis, control calli and calli produced from explants treated with 0.01% Sodium Azide for 3 hours contain same amount of neo andrographolide (0.01% w/w) and 14-deoxy 11-12, didehydro andrographolide (0.01% w/w) Table 1. The same treated sample showed a maximum amount of andrographolide (0.06% w/w) and andrographonin (0.004% w/w).

Table 1: Amount of secondary metabolites present in control calli and treated calli.

Secondary metabolites	Control(%w/w)	Sample treated with 0.01% SA for 3 hours (%w/w)
Andrographoilde	0.01	0.07
Neo Andrographolide	0.01	0.01
14-Deoxy-11,12-dide hydro andrographoilde	0.01	0.01
Andrographonin	0.002	0.004

The amount of secondary metabolite was analyzed after 70 days.

Among the different media used, full MS medium produced good response. Maximum callus initiation was found in full MS medium fortified with 2 mg/l NAA, 1 mg/l Kinetin and 50 mg/l Phenylalanine. Kataky and Handique (2010b) [13] reported that MS medium was the most suitable medium compared to other culture media viz B5 and Nitsch's

Several reports revealed that Sodium azide (NaN3) was very effective in inducing mutation in tomato (Adamu *et al.* 2007, Adebola 2013) [14, 15]. El Kaaby *et al*., (2015) [16] reported that Sodium azide influenced the shoot and root length and also reduction in seed germination percentage in tomato cultivars. Sodum azide at various concentrations results either negative or positive responses at high or low concentrations respectively. The results of this study were in agreements with different reports (Al-Qurainy F and Khan S 2009, Asli *et al.* 2006) [17, 18].

4. Conclusion

The effect of sodium azide treatment on fresh weight, dry weight, callus induction frequency and secondary metabolite production on *in vitro* callus were recorded in this study. Lower concentration of Sodium azide was high effective in secondary metabolite production. MS medium supplemented with 2 mg/l NAA, 1 mg/l Kinetin and 50 mg/l phenyl alanine produced maximum amount of andrographolide. Calli produced from explants treated with 0.01% SA for 3 hours contain more amount of secondary metabolite.

5. Acknowledgement

This work was financially supported by University of Kerala, Thiruvananthapuram

6. References

1. Negi AS, Kumar JK, Luqman S, Shanker K, Gupta MM. Recent advances in plant hepato protectives a chemical and biological profile of some important leads Med Res Rev. 2008; 28:746-772
2. Sareer O, Ahmad S, Umar S. *Andrographis paniculata*: A critical appraisal of extraction, isolation and quantification of andrographolide and other active constituents. Nat Prod Res. 2014; 9:1-21
3. Wang J, Yang W, Wang G, Tang P, Saig Y. Determination of six components of *Andrographis paniculata* extract and one major metabolite of andrographolide in rat plasma by liquid chromatography–

tandem mass spectrometry, J Chromatogr B. 2014; 951-952, 78-88
4. Mishra K, Dash AP, Dey N. Andrographolide: a novel antimalarial diterpene lactone compound from *Andrographis paniculata* and its interaction with curcumin and artesunate, J Trop Med Article ID 579518. 2011, 1-6
5. Lin FL, Wu SJ, Lee SC, NG LT. Antioxidant, antioedema and analgesic activities of *Andrographis paniculata* extracts and their active constituent andrographolide Phyto Res. 2009; 23:958-964.
6. Burm F, Kumar OA, Naidu IM, Rao KG. *In vitro* antibacterial activity in the extracts of *Andrographis paniculata* Int J Pharm Tech Res. 2010; 2:1383-1385
7. Subramanian R, Asmawi MZ, Sadiku A. A bitter plant with a sweet future. A Comprehensive review of an oriental medicinal plant: *Andrographis paniculata*. Phytochem Rev. 2012; 11:39-75
8. Chao WW, Lin BF. Isolation and identification of bioactive compounds in *Andrographis paniculata* Chin Med. 2010; 17:1-15
9. Calabrese C, Berman SH, Babish JG. A phase I trial of andographolide in HIV positive patients and normal volunteers Phytother Res. 2000; 14:333-338
10. Yoopan N, Thisoda P, Rangkadilok Sahasitiwat S, Pholphana N, Cardiovascular effects of 14 deoxy 11, 12 didehydroandrographolide and *Andrographis paniculata* extracts Planta Med. 2007; 73:503-511
11. Gottaschalk W. Experimental mutagenesis in plant breeding. In: A.B. Prasad (Ed.). Mutagenesis: Basic and Applied; Print House, Lucknow, India, 1986, 81-96.
12. Biswas AK, Bhattacharya NK. Induced polyploidy in legumes. *Cyamopsis psoralioides*. dc *cytologia.* 1971; 36469-479
13. Kataky A, Handique PJ. Micropropagation and screening of antioxidant potential of *Andrographis paniculata* (Burm. f) Nees. J Hill Ag. 2010; 1:15–20
14. Adamu AK, Aliyu H. Morphological effects of sodium azide on Tomato *(Lycopersicon esculentum* Mill). Science World Journal. 2007; 2(4):9-12
15. Adebola MO. Mutagenic Effects of Sodium Azide (NaN3) on Morphological Characteristics of Tomato (Lycopersicon esculentum). Journal of Science & IT Management, 2013; 2(4).
16. El Kaaby, Ekhlas Abdulkareem Jasim, Al-Ajeel, Saadon Abdulhadi, AI-Anny, Jenan Abbas *et al*, Effect of the Chemical Mutagens Sodium Azide on Plant Regeneration of Two Tomato Cultivars under Salinity Stress Condition *in vitro*. Journal of Life Sciences. 2015; 9:27-31
17. Al-Qurainy F, Khan S. Mutagenic Effects of Sodium Azide and Its Application in Crop Improvement. World Applied Sci. World J. 2009; 6(12):15891601.
18. Asli DT, Khawar KM, Ciftçi CY, Özcan S, Effects of Mutagenic Sodium Azide (NaN3) on *In Vitro* Development of Four Pea (*Pisum sativum* L.) Cultivars. Int. J Agri. Biol. 2006; 8(3):349-353