International Journal of Applied and Pure Science and Agriculture
ISSN Online - 2394-5532 Impact Factor : 4.446 by SJIF ISSN Print - 2394-823X

# A Comparitive Study Of The Effect Of SA And EMS On I*n Vitro* Production Of Andrographolide In *Andrographis paniculata* (Burm.F.) Nees

**Anitha Chandran N[1], Susila Kuruvilla[2] and P. R. Unnikrishna Pillai[3]**
[1,3]*Department of Post-Graduate Studies and Research in Botany, S D College, Alappuzha, Kerala-688003*
[2]*Department of Botany, St. Josephs College for Women, Alappuzha, Kerala- 688001*

**Abstract**
***Comparative effects of 0.01% of EMS and 0.01% SA (sodium azide) for 3 hours on secondary metabolite production in Andrographis paniculata were conducted in this study. Calli were derived 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 resulted in 7 fold increase in andrographolide production when compared to control and 0.01% EMS for 3 hours produced 6 fold increase in andrographolide production compared to the control. The study also showed that 0.01% SA for 3 hours treated samples positively influenced on the fresh weight, dry weight of calli, callus induction frequency and andrographolide production compared to the 0.01% EMS for 3 hours treated samples.***
***Keywords: Andrographis paniculata, andrographolide, sodium azide, EMS, HPLC***

## I. 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 common cold, diarrhea, fever, respiratory tract infections [1],[2],[3] and has numerous therapeutic potentials including antimalarial [4], antioxidant [5], antibacterial [6] and anticancer activity [7]. This herb has many common names - Kalmegh in Bengali, Kiriyath in Malayalam, Nilavembu in Telugu etc. and is commonly known as bhui-neem, because of its bitter taste as that of neem.

This plant contains pharmaceutically important compounds such as diterpenoids, flavonoids, and polyphenols [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 [9].

Andrographolide, neoandrographolide and 14-deoxy-11,12-didehydro andrographolide has been studied for their antiallergic, anti-inflammatory and cardiovascular effects [10]. Mutation breeding correlates with numerous 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 favorable advantage [11],[12].

There are 3 procedures to induce mutations, by either applying: 1) biological agents such as transposons and T-DNA, 2) physical agents such as fast neutron, UV and X-ray radiation, and 3) chemical agents such as Nmethyl-N-nitrosourea (MNU), 1,2:3,4-diepoxybutane (DEB) or ethylmethane sulfonate (EMS). Among these chemical mutagens, EMS has become one of the best effective, reliable, powerful and frequently used chemical mutagens in plants [13]. 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.

## II. MATERIALS AND METHODS

### 2.1 Plant material

Healthy growing young branches with 4 to 5 nodes were collected from 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)[14]. A cotton swab dipped in 0.01% Sodium azide solution and EMS solution for 3 hours was applied to the apical vegetative bud and Sodium azide solution and EMS were added to the cotton swab frequently by a dropper. Seedlings treated with distilled water kept as control. The third leaf 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. 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 were tried in this study. Callus produced from these samples were subjected to HPLC analysis to detect the andrographolide.

### 2.2 Procedure of HPLC

Chromatographic conditions:

**A. Mobilephase**

1. Dissolve 0.14 gm of anhydrous potassium dihydrogen orthophosphate ($KH_2PO_4$) 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.

## III. RESULTS AND DISCUSSION

Explants taken from control inoculated into full MS basal medium fortified with 2 mg/l NAA, 1 mg/l Kinetin and 50 mg/l phenylalanine. Control calli showed good callus initiation and produced high amount of fresh weight (0.6113gm) and dry weight (0.04133 gm) in this medium. Explant treated with 0.01% of SA and 0.01% EMS for 3 hours were inoculated on the medium supplemented with 2 mg/l NAA, 1 mg/l Kinetin and 50 mg/l Phenylalanine produced callus with high amount of fresh weight compared to control. The 0.01% SA for 3 hours showed, high callus fresh weight (1.3537±0.04) and dry weight (0.1247+0.00970) (Table 1). Percentage of callus response present in this concentration of Sodium azide was 73.33+3.33. The calli produced from 0.01% EMS treated explants showed a higher amount of fresh weight (1.4033+0.028), dry weight (0.08667+0.003333) and percentage of the response of the callus induction (73.333+3.3333) than that of control.

Andrographolide content present in the control calli was 0.1 mg/g. Comparative study of effect of 0.01% SA and EMS on andrographolide production revealed that, calli produced from

explants treated with 0.01% SA for 3 hours contained 0.7mg/g andrographolide. The andrographolide production was 0.6 mg/g in the calli derived from the explants treated with 0.01% EMS for 3 hours and the production of andrographolide was analyzed after 70 days.

**Table 1: Comparative effect of .01% of SA and EMS for 3 hours on callus induction**

| Samples | Concentration (%) | Time (Hr) | % of Response | Fresh weight | Dry weight |
|---|---|---|---|---|---|
| Control | | | 56.66±3.33 | 0.6113±0.13 | 0.041±0.069 |
| SA | 0.01 | 3 | 33.33±3.33 | 1.353±0.41 | 0.1247±0.09 |
| EMS | 0.01 | 3 | 73.33±3.33 | 1.4033±0.28 | 0.896±0.0033 |
| df(n-1)=2 | | | 8.333* | 200.406* | 36.819* |

Means within a column followed by same letters are not significantly ($p<0.05$) different as determined by Duncan's Multiple Range test. *significant ($p<0.05$) F value, NS- non significant

**Figure 1: Control**

**Figure 2: 0.01% SA for 3 hours**

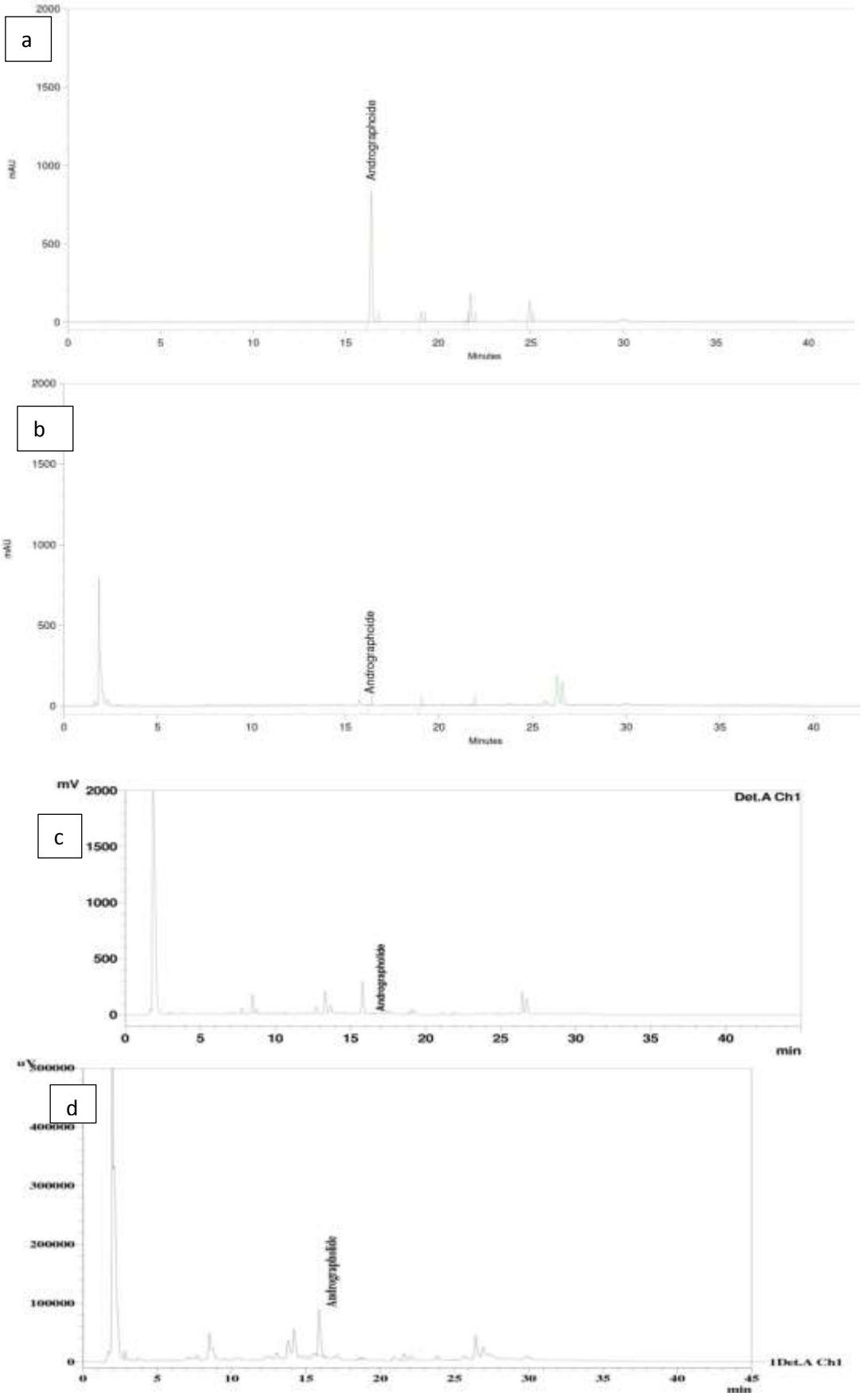
**Figure 3: 0.01% EMS for 3 hours**

In this study, among the different media used, full MS medium produced better results. 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) [15] reported that MS medium was the best suitable medium compared to other culture media viz B5 and Nitsch's media.

Maximum andrographolide (0.7mg/g) was produced in the samples treated with 0.01% SA for 3 hours and 7 fold increase in andrographolide production than the control and 1 fold increase in andrographolide production than the same concentration and treatment time of EMS. Several types of research proved that Sodium azide (NaN3) was very effective in inducing mutation in tomato [16],[17]. El Kaaby et al., (2015) [18] reported that Sodium azide influenced the shoot and root length and also observed reduction in seed germination percentage in tomato cultivars. In Dendrocalamus hamiltonii, the EMS treated seeds exhibited a significant increase in callus formation up to 0.1% concentration. Further more, there was marginal reduction noticed in the

percentage of callus induction. The callus induction in seed explants treated with a different dose of Sodium azide and colchicine hindered with the increase in the concentration of the mutagens [19].

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



## IV. CONCLUSION

The effect of sodium azide and EMS treatment on fresh weight, dry weight, callus induction frequency and andrographolide production on in vitro callus were recorded in this study. Lower concentration of Sodium azide was more effective in the andrographolide production than the lower concentration of EMS.

## BIBLIOGRAPHY

[1] Negi A S, Kumar J K, Luqman S, Shanker K & Gupta Mm (2008) Recent advances in plant hepatoprotectives a chemical and biological profile of some important leads Med Res Rev**28** 746-772
[2] Sareer O, Ahmad S & Umar S (2014) *Andrographis paniculata*: a critical appraisal of extraction,isolation and quantification of andrographolide and other active constituents. Nat ProdRes **9** 1–21
[3] Wang J, Yang W, Wang G, Tang P & Saig Y (2014) Determination of six components of *Andrographis paniculata* extract and one major metabolite of andrographolide in rat plasma byliquid chromatography–tandem mass spectrometry, J Chromatogr B **951–952** 78–88
[4] Mishra K, Dash A P & Dey N (2011) Andrographolide: a novel antimalarial diterpene lactone compound from *Andrographis paniculata* and its interaction with curcumin and artesunate, JTrop Med Article ID 579518 1–6
[5] Lin F L, Wu S J, Lee S C & Ng L T (2009) Antioxidant, antioedema and analgesic activities of *Andrographis paniculata* extracts and their active constituent andrographolide Phyto Res **23** 958–964
[6] Burm F, Kumar O A, Naidu L M & Rao K G (2010) In vitro antibacterial activity in the extracts of *Andrographis paniculata* Int J Pharm Tech Res **2** 1383-1385
[7] Subramanian R, Asmawi MZ & Sadiku A (2012) A bitter plant with a sweet future. AComprehensive review of an oriental medicinal plant: *Andrographis paniculata*. Phytochem Rev**11** 39–75
[8] Chao W W & Lin B F (2010) Isolation and identification of bioactive compounds in *Andrographis paniculata* Chin Med **17** 1-15
[9] Calabrese C, Berman S H and Babish J G (2000) A phase I trial of andographolide in HIV positive patients and normal volunteers Phytother Res **14** 333-338
[10] Yoopan N, Thisoda P, Rangkadilok Sahasitiwat S and Pholphana N (2007) Cardiovascular effects of 14 deoxy11,12 didehydroandrographolide and Andrographis paniculata extracts Planta Med **73** 503-511
[11] Gottaschalk W (1986) Experimental mutagenesis in plant breeding. In: A.B. Prasad (ed.), Mutagenesis: Basic and Applied, 81-96. Print House, Lucknow, India
[12] Toker C, Yadav S S and Solanki I S (2007) Mutation breeding. In: S.S. Yadav, D. McNeil and P.C. Stevenson (eds.), Lentil: An Ancient Crop for Modern Times, 209-224. Springer, Dordrecht
[13] Brockman H E, de Serres F J, Ong T M, DeMarini D M, Katz A J, Griffiths A J & Stafford R S (1984) Mutation tests in *Neurospora crassa.* A report of the U.S. Environmental protection Agency Gene-Tox Program. Mutat Res **133(2)** 87– 134
[14] Biswas A K & Bhattacharya, N K (1971) Induced polyploidy in legumes. Cyamopsis psoralioides. DC Cytologia **36** 469-479.
[15] Kataky A & Handique P J (2010b) Micropropagation and screening of antioxidant potential of *Andrographis paniculata* (Burm. f) Nees. Journal of Hill Agriculture. **1(1)** 15-20
[16] Adamu A K, and Aliyu H (2007) Morphological effects of sodium azide on Tomato *(Lycopersicon esculentum* Mill). Science World Journal **2** (4): 9-12
[17] Adebola M O (2013) "Mutagenic Effects of Sodium Azide (NaN3) on Morphological Characteristics of Tomato (Lycopersicon esculentum)." Journal of Science & IT Management 2 (4)
[18] El Kaaby, Ekhlas Abdulkareem jasim, Al-Ajeel, Saadon. Abdulhadi, AI-Anny, Jenan Abbas, Al-Aubaidy Ashwaq Abdulrazaq1 and Ammar Khalid (2015) Effect of the Chemical Mutagens Sodium Azide on Plant Regeneration of Two Tomato Cultivars under Salinity Stress Condition in vitro. Journal of Life Sciences **9** 27-31
[19] Abhishek Kumar, Meena Bakshi, Manpreet Kaur, Shatakshi Kapruwan, Manish Singh (2015) Effects Of Mutagen Treatment On The Seed Germination And Callus Induction In *Dendrocalamus hamiltonii.* Journal of Environmental and Applied BioresearchVol. **03**, No. 04, pp. 234-237