Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (8):23-28
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Microscopic detection of flavonoid in suspension cultures of *Boerhavia diffusa*

S. Harini, Merina Paul Das and L. Jeyanthi Rebecca*

Department of Industrial Biotechnology, Bharath University, 173, Agaram Road, Selaiyur, Chennai-600 073

ABSTRACT

Boerhaavia diffusa is one of the well-known traditional medicinal plants. They are rich source of secondary metabolites like alkaloids, steroids and flavones. The chief function of flavonoids in plant includes UV filtration, symbiotic nitrogen fixation, and floral pigmentation. They also act as chemical messengers, cell cycle inhibitors and physiological regulators. The present study was focused on the detection and quantitative estimation of flavonoids in the in vitro plant cell suspension culture. The quantitative estimation showed that there was an increase in flavonoid production with increase in UV irradiation time, heavy metal concentration and number of days.

Keywords: *Boerhaavia diffusa*, flavonoids, microscopic examination, qualitative analysis, quantitative analysis, UV irradiation, heavy metal.

INTRODUCTION

Boerhaavia genus is a collection of 40 tropical and subtropical species. It is found as a weed during rainy seasons in Indian, Northern and Southern American continent and South Eastern Africa. The active principle contained in the herb is an alkaloid, known as 'Punarnavaine'. The whole plant *Boerhaavia diffusa* is a very useful source of the drug Punarnava, which is documented in Indian Pharmacopoeia as a diuretic [1]. The plant was named in honour of Hermann Boerhaave, a famous Dutch physician of the 18th century. People in tribal areas use it to hasten child birth [2]. It is used to cure Jaundice, Ascites, Asthma and in Scanty urine. The active constituent of the drug is the alkaloid Punarnavinne, the total alkaloid content of the roots being about 0.04% [3,4].

The genus *Boerhaavia* has several species and is distributed in the tropical, sub-tropical and temperate regions of the world. *Boerhaavia diffusa* is up to 1m long or more, having spreading branches (Fig 1). The stem is prostrate, woody or succulent, cylindrical, often purplish, hairy, and thickened at its nodes. The leaves are simple, thick, fleshy and hairy, arranged in un-equal pairs, green and glabrous above and usually white underneath. The shape of the leaves varies considerable ovate-oblong, round or subcordate at the base and smooth above. Flowers are white, pink or pinkish red in colour, minute, subcapitate, present 4-10 together in small bracteolate umbels, forming axillary and terminal panicles.

Fig. 1 The plant *Boerhaavia diffusa*

The root is mainly used to treat gonorrhoea, internal inflammations of all kinds, dyspepsia, oedema, jaundice, menstrual disorders, anaemia, liver, gall bladder and kidney disorders, enlargement of spleen, abdominal pain, abdominal tumours and cancers, then as a diuretic documented in Indian Pharmacopeia, digestive aid, laxative and a menstrual promoter. The root powder when mixed with mamira (*Thalictrum foliolosum*), is used to treat eye diseases. It cures corneal ulcers and night blindness and helps restore virility in men [5].

The juice of *Boerhaavia diffusa* leaves servers as a lotion in opthalmia. It is also administered orally as a blood purifier and to relieve muscular pain. The plant contains basic novel protein (30-34 KDa) capable of providing resistance immunity to several susceptible hosts against commonly occurring viruses. The roots of the plant are rich source of a basic protein or antiviral agent, which is used for inducing systemic resistance in many susceptible crops against commonly occurring viruses [4]. Study revealed that BD-SRIP induces the resistance against TMV infection [6]. Boerarinone G a compound found in *Boerhaavia diffusa* is considered as lead compound for the development of drugs potentially useful against those pathologies whose aetiology is related to ROS- mediated injuries [7].

Flavonoids are a group of plant metabolites thought to provide health benefits through cell signalling pathways and antioxidant effects. They can bind to nonheme iron, thereby decreasing its absorption in the intestine. Some flavonoids also inhibit cellular uptake of vitamin C and some experts advise avoiding flavonoid-rich foods or drinks when taking vitamin C. Among these compounds, flavonoids constitute one of the most ubiquitous groups of plant phenolics. Owing to their importance in food organoleptic properties and human health, a better understanding of their structures and biological activities indicates their potentials as therapeutic agents and also for predicting and controlling food quality. Due to the variety of pharmacological activities in the mammalian body, flavonoids are more correctly referred as “nutraceuticals” [8]. Initially flavonoid was detected in oranges and was classified as the vitamin P [9].

Moreover many methods have been used in localization studies that include light microscopy, fluorescence and electron microscopy [10,11], physical [12,13] or enzymatic [14] tissue preparation before chromatographic analysis or isolation of organelles for enzymatic studies [15] or protoplasts for preparation of vacuoles [16,17].The current investigation was carried out to study the effect of elicitors on the production of flavonoids from *B. diffusa* and to detect its presence microscopically.

MATERIALS AND METHODS

Plant Material

The plant samples are collected from Irula Tribal Women’s Welfare Society, Thandarai, Chengalpet. The collected plants were identified at the Centre for Floristic Research, Department of Plant Biology and Plant Biotechnology, Madras Christian College, Tambaram by Dr. D. Narasimhan.

Suspension cultures

The leaf samples taken freshly from the plant were surface sterilized using sodium hypochlorite and the epidermal layer was scrapped using a sterile blade. It was then cut into small pieces and inoculated in the Cocking, Peberdy and White medium or cell protoplast washing medium (CPW) [KH_2PO_4: 27.2 mg/l, KNO_3: 101 mg/l, $CaCl_2.2H_2O$: 1480 mg/l, $MgSO_4.7H_2O$: 246 mg/l, KI: 0.16 mg/l] supplemented with 13% mannitol. Amoxicillin 30mcg was added as an antibiotic.

Treatment with elicitors

The cells were treated with UV irradiation and heavy metal stress using mercuric sulphate. The cells were irradiated with 0, 5, 10, 15 and 20 min of UV radiation and the samples were taken for qualitative and quantitative analysis of flavonoids. Similarly for heavy metal stress, the cells were treated with 0, 100, 200 and 300 ppm of mercuric sulphate.

Qualitative Analysis of Flavonoid

The qualitative analysis of flavonoid was done using the ferric chloride test. An aliquot of 1ml of the cell suspension each was taken centrifuged and the pellets were taken. The dry weight of the cells was noted. Then the pellet was re-suspended in methanol, overnight for extraction of flavonoid. After 13-15 hours 10% of $FeCl_3$ was added to each. Appearance of greenish blue/ violet color confirmed the presence of flavonoids.

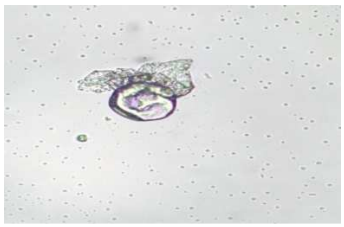
Quantitative Estimation of Total Flavonoid

The cell culture was centrifuged and the pellet was taken and suspended in methanol. The method used for the estimation of total flavonoid content is aluminium chloride calorimetric method [18]. Cell aliquots of 500µl were taken and mixed with 400µl of distilled water and 30µl of 10% aluminium chloride was added. At sixth minute 200µl of 1M sodium hydroxide was added and was made upto 2.5 mL with methanol. The absorbance was noted after 6 minutes in calorimeter at 510 nm. The amount of flavonoid was determined based on the amount of quercetin from the standard graph.

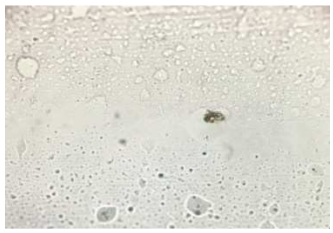
RESULTS AND DISCUSSION

The leaves of *B. diffusa* was surface sterilized and inoculated in the CPW medium and after 10-12 days of inoculation, the cell growth was observed as embryonic globules suspended in the liquid. The cell growth was seen as a globules suspended in the liquid. Then the cell presence is examined under microscope view stained with Evan's blue.

Qualitative Analysis



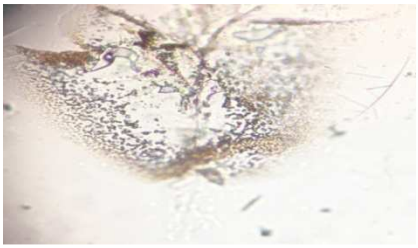
A. Control



B. 5 min UV irradiation

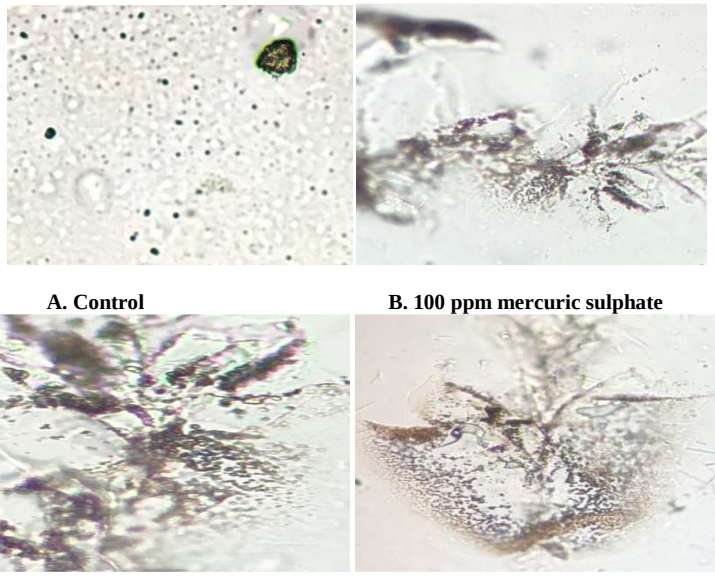
C. 10 min UV irradiation

D. 15 min UV irradiation



E. 20 min UV irradiation
Fig. 2 Microscopic view of UV irradiated cells in suspension after qualitative analysis

Fig 2 shows greenish or violet cells indicating the presence of flavonoids seen under the microscope of the UV treated cells (A-control, B-5min, C-10min, D-15 min and 20 minutes respectively).



A. Control **B. 100 ppm mercuric sulphate**

C. 200 ppm mercuric sulphate **D. 300 ppm mercuric sulphate**
Fig. 3 Microscopic view of heavy metal stress treated in suspension after qualitative analysis

Figure 3 shows the microscopic view of cells after heavy metal stress at different concentration after qualitative analysis (A-control, B-100 ppm, C-200 ppm and D-300 ppm, respectively). The qualitative samples are examined under trinocular microscope without any staining. The view of the samples at fourth and sixth day for metal stress and fifth and seventh day for UV stress were observed. Table 1 shows the effect of UV treatment on the suspension culture of *Boerhaavia diffusa* observed on the fifth and seventh day. There was a significant increase in flavonoid content with the increase in time.

TABLE 1 Effect of UV Treatment on flavonoid production in *Boerhaavia diffusa*

TIME(min)	AMOUNT OF FLAVONOID (mg)	
	Day 5	Day 7
0	0.99	1.05
5	1.47	1.53
10	1.59	1.59
15	1.71	1.68
20	1.74	1.74

There was a gradual increase in flavonoid production and there is not much difference in its production between Day 5 and Day 7.

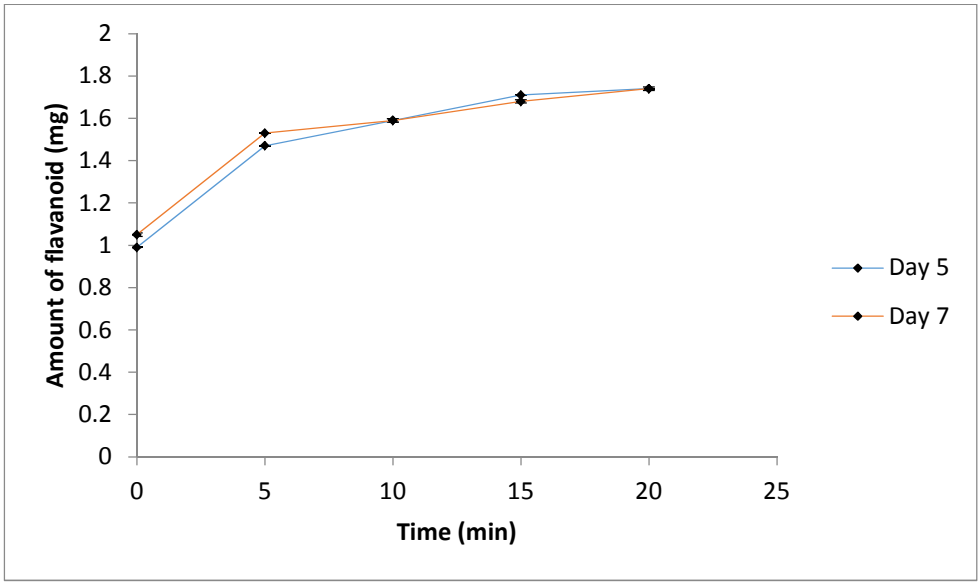


Fig. 4 Effect of UV Treatment on flavonoid production in *Boerhaavia diffusa*

Figure 4 shows the amount of flavonoid produced when the suspension cultures were subjected to UV irradiation. The amount of flavonoid increased with the increase in UV irradiation or by the increase in mercuric sulphate concentration when compared to the control.

TABLE 4: Effect of Metal Treatment on flavonoid production in *Boerhaavia diffusa*

CONCENTRATION (ppm)	AMOUNT OF FLAVONOID (mg)	
	Day 4	Day 6
0	0.96	1.05
100	1.2	1.2
200	1.29	1.35
300	1.41	1.5

Table 4 shows that the flavonoid amount had increased with the increase in the concentration of mercuric sulphate and there is significant difference between flavonoid amounts between the two days.

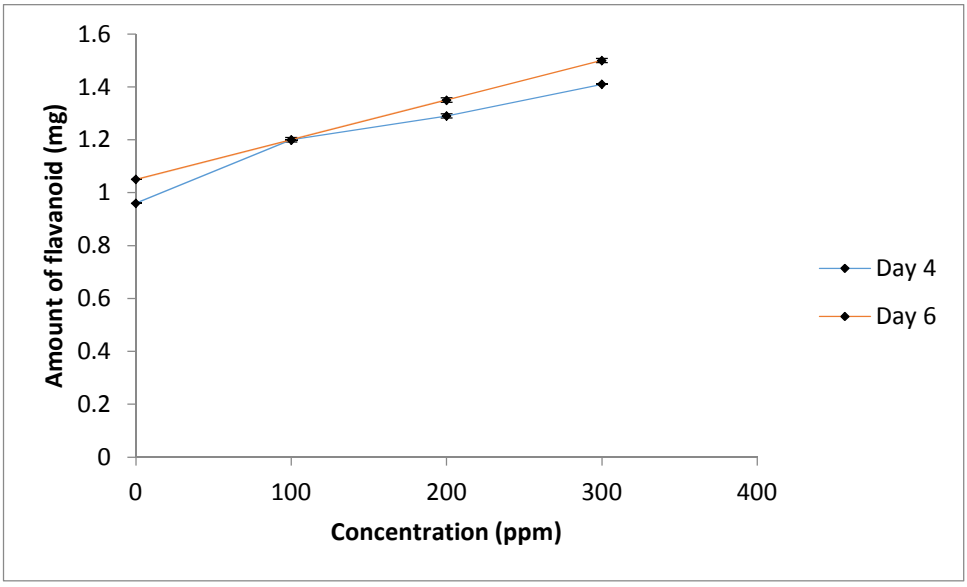


Fig. 5 Effect of heavy metal stress on flavonoid production in *Boerhaavia diffusa*

Figure 5 shows the amount of flavonoid produced when the suspension cultures were subjected to heavy stress as elicitor. The amount of flavonoid increased with the increase in mercuric sulphate concentration when compared to the control. UV radiation was a more effective elicitor than metal stress.

CONCLUSION

There was a gradual increase in flavonoid amount after stress although in some treatments there was no major change. This indicates UV and Metal stress induces the increase in amount of Flavonoid. It was found that UV radiation was a more effective elicitor than metal stress.

REFERENCES

[1] Akinrinmade, JF.; Oyeleye, OA.; *African Journal of Biotechnology*, **2010**, 9(31), 5028-5031.
[2] Arun Kumar,; Pingal Kumari,; Meenakshi Priyadarshini,; Kumari Anjali, ;Sukhla L.N,; *International journal of science research*, **2013**, 2(7) , 17-19.
[3] Awasthi, LP.; Verma, HN.; *Asian agri history*, **2006**, 10(1), 55-68.
[4] Sudarshana, MS.; Niranjan, MH.; Girisha, ST.; *Global journal of biotechnology and biochemistry*, **2008**,3(2), 83-86.
[5] Ramachandra, Y L.; Ashajyothi, C.; Padmalatha Rai, S.; *International journal of advances in pharmacy, biology and chemistry*, **2012**, 1(3), 420-433.
[6] Seema Lohani.; Arif Jain.; Verma, HN.; *Biotechnology*, **2007**, 6(3), 389-392.
[7] Aviello, G.; Canadanovic- Brunet, JM.; Milic, N.; Capasso, R.; Fattorusso, E.; Jaglialatela- Scafati, O.; Fasolino, I.; Izzo, AA.; Borrelli, F.; *PLoS one*, **2011**, 6(5)
[8] Tapas, A R.;' Sarkar, DM.; Kakde, RB:' *Journal of paramedical sciences*, **2013**, 4(3), 119-138
[9] Shashank Kumar.; Abhay K Pandey.; *The scientific world journal*, **2013**, 1-16
[10] Harris, PJ,; Hartley, RD.; *Nature*, **1976**, 259, 508–10.
[11] Charest,PM.; Brisson, L.; Ibrahim, RK.; *Protoplasma*, **1986**, 134, 95–101
[12] Hrazdina, G.; Marx, GA.; Hoch, HC.; *Plant Physiology*, **1982**, 70, 745–8.
[13] Knogge, W.; Weissenbo¨ck, G.; *Planta*, **1986**, 167, 196–205
[14] Schmelzer, E.; Jahnen, W.; Hahlbrock, K.; Proceedings of the National Academy of Sciences, USA, **1988**, 85, 2989–93.
[15] Hrazdina, G.; 1992. Compartmentation in aromatic metabolism. In: Stafford HA, Ibrahim RK, eds. Phenolic metabolism in plants. New York: Plenum Press, **1992**,1–23.
[16] Hopp, W.; Seitz, HU.; *Planta*, **1987**, 170, 74–85.
[17] Anhalt, S.; Weissenbo¨ck, G.; *Planta*, **1992**, 187, 83–8.
[18] Samidha Kamtekar.; Vrushali Keer.; Vijaya Patil.; *Journal of Applied Pharmaceutical Science*, **2014**, 4(09),061-065.