Research Article

Effect of Heavy Metals and UV Irradiation on the Production of Flavonoids in *Indigofera tinctoria*

J. Emmaline Jane Keziah, S. Sharmila, L. Jeyanthi Rebecca*
Department of Industrial Biotechnology, Bharath University, 173, Agaram Road, Selaiyur, Chennai, Tamil Nadu, India.
***Corresponding author's E-mail:** hodibt@bharathuniv.ac.in

Accepted on: 02-06-2016; **Finalized on:** 31-07-2016.

ABSTRACT

Indigofera tinctoria is a branching shrub used in traditional medicines possessing medicinal activities like antibacterial, antioxidant, cytotoxicity effect, anti-inflammatory activity, anti-hepatoprotective activity, antiviral activity etc. Their therapeutic potential is due to the presence of phenolic compounds specifically, flavonoids. The present study was undertaken to detect the presence of flavonoids and to estimate its content in the suspension culture of *Indigofera tinctoria* under heavy metal stress and UV irradiation. The qualitative and quantitative assay was done. The results indicate that, as the days progressed, there was a significant increase in the production of flavonoids in the UV and metal stressed cells compared to the control.

Keywords: *Indigofera tinctora*, flavonoid, UV irradiation, heavy metal stress, elicitor, suspension culture.

INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases and offer a new source of biologically active chemical compound as antimicrobial agent.

Medicinal plants are the richest bio-resources of drugs of traditional medicinal systems, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceuticals, intermediate and chemical entitled for synthetic drugs[1].

It has been estimated that 14-28% of higher plant species are used medicinally and that 74% of pharmacologically active plant derived components were discovered after following up on ethno medicinal use of the plants[2].

Recently there has been a great interest in the therapeutic potential of medicinal plants due to their phenolic compounds, specifically flavonoids because of their various health benefits.

Fruits and vegetables are the main dietary sources of flavonoids for humans, along with tea and wine. Most recent researches have focused on the health aspects of flavonoids for humans.

Many flavonoids are shown to have antioxidative activity, free radical scavenging capacity, coronary heart disease prevention, hepatoprotective, anti-inflammatory, and anticancer activities, while some flavonoids exhibit potential antiviral activities. In plant systems, flavonoids also help in combating oxidative stress and act as growth regulators[3].

Flavonoids can scavenge harmful active oxygen species including O^{2-}, H_2O_2, and OH by donating electrons to guaiacol-type peroxidases (GuPXs) for the detoxification of H_2O_2 produced under stress conditions.

As a result of such enzymatic as well as non-enzymatic antioxidant reactions, phenoxyl radicals are formed as the primary oxidized products. In contrast to their antioxidant activity, phytophenolics also have the potential to act as prooxidants under certain conditions[4].

Consumption of the flavonol quercetin, is inversely associated with the incidence of cancer of the prostate, lung, stomach, and breast. In addition, moderate wine drinkers also seem to have a lower risk to develop cancer of the lung, endometrium, oesophagus, stomach, and colon[5]. Major molecular mechanisms of flavonoids are downregulation of mutant p53 protein, cell cycle arrest, tyrosine kinase inhibition, inhibition of heat shock proteins, estrogen receptor binding capacity, inhibition of expression of Ras proteins[6].

Cell cultures have a higher rate of metabolism than intact differentiated plants because the initiation of cell growth in culture leads to fast proliferation of cell mass and to a condensed biosynthetic cycle.

As a result, secondary metabolite production can take place within a short cultivation time (about 2-4 weeks) with an added advantage of tunability. These cell cultures can be employed in scaled-up operation, for isolation of desirable compounds in bulk. Moreover, plant cell cultures provide a valuable platform for the production of high-value secondary metabolites and other substances of commercial interest[7,8].

Indigofera tinctoria, being a valuable medicinal plant with various uses and pharmacological actions, with this background, the present study was undertaken with an aim of isolating secondary metabolites from the plant in suspension cultures. *Indigofera tinctoria* (Indigo plant) belongs to the Family Fabaceae (Fig.1). *Indigofera tinctoria* is a shrub, 1.2-1.8 m high. The roots, stems and leaves are bitter, thermogenic, laxative, trichogenous,

expectorant, anthelminthic, tonic, naturopathy, splenomegaly, echolalia, cardiopathy, chronic bronchitis, asthama, ulcers, skin diseases, diuretic and are useful for promoting growth of hair[9].

The juice expressed from the leaves is useful in the treatment of hydrophobia. An extract of the plant is good for epilepsy and neuropathy[10]. The plant is an antiseptic and an astringent.

Externally, leaves crushed are used as stimulant and to heal wounds and ulcers. Powdered indigo is mixed with castor oil and applied to the navel of children to promote the action of the bowels[11]. The plant was found to contain indirubin and indigtone where the juice extracted from the leaves is useful in the treatment of hydrophobia[12]. The phytochemical analysis and the antibacterial effect of *Indigofera tinctoria* has been studied[13].

Figure 1: The plant *Indigofera tinctoria*

The main objectives of this research are to elicit the production of flavonoids in the suspension culture of *Indigofera tinctoria* under heavy metal stress and UV irradiation and to estimate the amount of total flavonoid content present.

MATERIALS AND METHODS

Plant Material

Indigofera tinctoria saplings were collected from Chengalpet, Tamil Nadu. The saplings were collected and planted in pots for further experimentation. 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 are taken freshly from the plant. The leaves were surface sterilized using sodium hypochlorite and the epidermal layer was scrapped using a sterile blade. This was then cut into 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 a antibiotic.

Treatment with Elicitors

The cells were treated with two classes of elicitors one physical (UV irradiation) and the other chemical (heavy metal stress). 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 lead acetate.

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 (the ratio of methanol and cell culture was 3:2). Appearance of greenish blue/ violet color confirms the 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[14]. 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 present study was carried out to study the effect of elicitors like UV irradiation and heavy metal stress using lead acetate on the production of flavonoids in *Indigofera tinctoria*.

Cell Growth in CPW Medium

After 10-12 days of inoculation of sterilized explants in the CPW media, the cell growth was observed as embryoid globules suspended in the liquid (however, the media was clear when globules were formed) Fig. 2.

The cell growth was seen as globules suspended in the liquid (the media will be clear when globules are formed).

The globules are observed within 10-12 days. Then the cell presence is examined under microscope view stained with Evan's blue.

Effect of Elicitors on Flavonoid Production

After the cells were subjected to UV irradiation and heavy metal stress, when seen under microscope, the cells are closer together compared to the control, more number of cells were visible and the size of the cells seemed to be

larger as the treatment time increased. The cells formed were found to be closer to each other and clumpy with the increase in metal concentration when compared with the control.

Qualitative Test for Flavonoid

The presence of flavonoid was observed in greenish blue and/or violet colour. The qualitative samples after mixing with ferric chloride were examined under trinocular microscope without any staining (Fig. 2).

The view of the samples at fourth and sixth day for metal stress and fifth and seventh day for UV stress were observed. This is because there was no significant growth was seen till the respective days.

The presence of flavonoid was observed in greenish blue and/or violet colour under microscopic view. Figure 2 shows greenish or violet cells seen under the microscope of the UV treated cells (A-control, B-5min, C-10min, D-15 min and 20 minutes respectively) which confirms the presence of flavonoids.

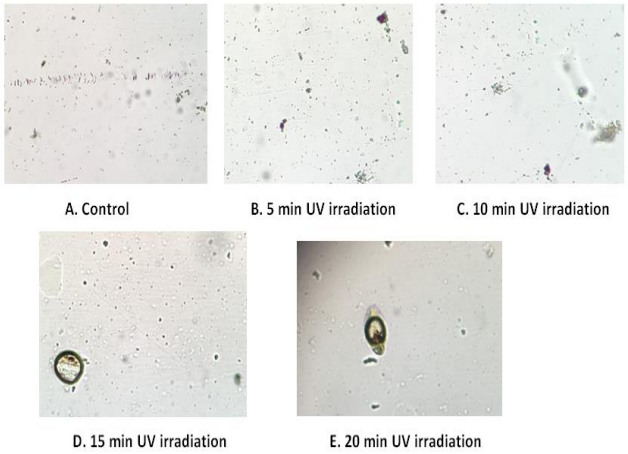

Figure 2: Microscopic view of UV irradiated cells in suspension after qualitative analysis

Quantitative Analysis for Estimation of Total Flavonoid

From the standard graph, the unknown concentration of total flavonoid present in the UV treated cells on fifth and seventh day and the metal stressed cells on fourth and sixth day was found out.

Table 1 and Fig. 3 shows the effect of UV treatment on flavonoid production in *Indigofera tinctoria* observed on the fifth day and seventh day.

Table 1: Effect of UV treatment on Flavonoid Production in *Indigofera tinctoria*

Time (min)	Amount of Flavonoid (mg)	
	Day 5	Day 7
control	0.96	1.05
5	1.35	1.62
10	1.68	1.68
15	1.71	1.8
20	1.86	1.83

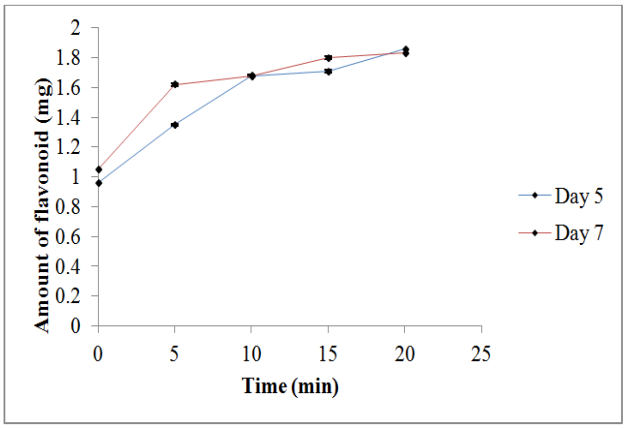

Figure 3: Effect of UV treatment on Flavonoid Production in *Indigofera tinctoria*

Table 2 and Figure 4 shows the effect of metal treatment on the suspension culture of *Indigofera tinctoria* observed on the fourth day and sixth day.

Table 2: Effect of heavy metal treatment on flavonoid production in *Indigofera tinctoria*

Concentration (ppm)	Amount of Flavonoids (mg)	
	Day 4	Day 6
Control	0.93	1.05
100	1.08	1.14
200	1.29	1.32
300	1.47	1.41

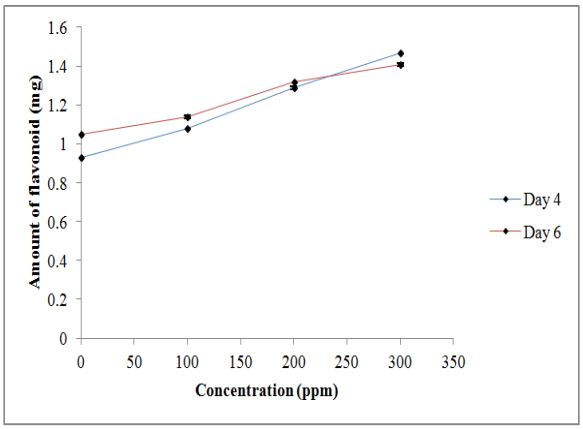

Figure 4: Effect of heavy metal treatment on flavonoid production in *Indigofera tinctoria*

Figure 3 and 4 shows the amount of flavonoid produced when the suspension cultures were subjected to UV irradiation and heavy stress as elicitors, respectively. The amount of flavonoid increased with the increase in UV irradiation or by the increase in lead acetate concentration when compared to the control.

CONCLUSION

The current study attempted to study the effect of stress on the secondary metabolite production in *in vitro* suspension culture of *Indigofera tinctoria*. The study demonstrated that the action of UV irradiation and heavy metal stress have a remarkable effect and greatly attributes to the significant increase in the production of flavonoids as the days progressed. The isolation of flavonoids from the *in vitro* suspension culture of *Indigofera tinctoria*, with various uses and pharmacological actions, could thus serve as potential source in herbal drugs. The obtained results could form a good basis for further investigations in the future enhancing the flavonoid production for therapeutic use.

REFERENCES

1. Hammer K.A, Carson CF, Riley TV, Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol., 86(6), 1999, 985.
2. Ncube NS, Afolayan AJ, Okoh AI, Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African Journal of Biotechnology, 7(12), 2008, 1797-1806.
3. Shashank Kumar, Abhay K. Pandey, Chemistry and Biological Activities of Flavonoids: An Overview. The Scientific World Journal, 2013, 16.
4. Sakihama Y, Cohen MF, Grace SC, Yamasaki H, Plant phenolic antioxidant and prooxidant activities, phenolics-induced oxidative damage mediated by metals in plants. Toxicology, 177, 2002, 67–80.
5. Koen B, Ruth V, Guido V, Johannes VS, Induction of cancer cell apoptosis by flavonoids is associated with their ability to inhibit fatty acid synthase activity, Journal of Biological Chemistry, 280(7), 2005, 5636–5645.
6. Davis RJ, Signal transduction by the JNK group of MAP kinases, Cell, 103, 2000, 239–252.
7. Allan E, Plant Cell Culture- In: Stafford A, Warren (eds.) Plant Cell and Tissue Culture, Open University Press, Milton, Keyens, 1991, 1-24.
8. Bhojwani S, Razdan M, Plant Tissue Culture: Theory and Practice, Elsevier Sci Pub Comp, Inc., The Netherlands, 1983, 43-53.
9. Saraswathi Motamarri N, Karthikeyan M, Rajasekar S, Gopal V, Indigofera tinctoria Linn - A Phytopharmacological Review, International Journal of Research in Pharmaceutical and Biomedical Sciences, 3(1), 2012, 164-169.
10. Warrier PK, Nambiar VPK, Ramankatty C. Indian Medicinal Plants, Published by Orient Longman Private Limited Chennai, 3, 2007, 210-213.
11. Nadkarni KM, Indian Meteria Medica, Published by Ramdas Bhatkal for Popular Prakashan Pvt. Ltd Mumbai, 2002, 680-681.
12. Muthulingam M, Mohandoss P, Indra N, Sethupathy S, Antihepatotoxic efficacy of *Indigofera tinctoria* (Linn.) on paracetamol induced liver damage in rats, IJPBR, 1(1), 2010, 13-18.
13. Ramanibai R, Madhavarani A, Phytochemical Screening and *in vitro* antibacterial activity of leaf extracts of *Indigofera tinctoria Linn,* Int. J of Advances in Pharmaceutical Res, 3, 2012, 872-877.
14. Samidha Kamtekar, Vrushali Keer, Vijaya Patil, Estimation of Phenolic content, Flavonoid content, Antioxidant and Alpha amylase Inhibitory Activity of Marketed Polyherbal Formulation. Journal of Applied Pharmaceutical Science, 4(9), 2014, 61-65.

Source of Support: Nil, **Conflict of Interest:** None.