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# Effect of Heavy Metal Stress on Secondary Metabolite Production in Murraya Koenigii

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***Abstract:*** ***The present study was aimed to study the phytochemical properties of Murraya koenigii plant. Previous literature has revealed a vast array of pharmacological activities of this plant. This study was dome to investigate upon the production of flavonoids by using heavy metals as elicitors in suspension cultures. There was an increase in flavonoid content with an increase in heavy metal concentration.***

***Keywords:*** ***Murraya koenigii, Antioxidants, Flavonoids, microscopic examination, qualitative analysis, quantitative analysis, UV irradiation, heavy metal***

## I. INTRODUCTION

Higher plants are a good source of natural products such as pharmaceuticals, agrochemicals, fragrance ingredients and pesticides [1]-[3]. The use of plant cell cultures for the production of natural compounds has been demonstrated [4]-[6]. Large amounts of secondary metabolites are to be produced for commercial exploitation. Efforts are being made to isolate these compounds using cell culture to meet the demands. This is achieved by optimizing the cultural conditions [7]. Hairy root culture is another method that has revolutionized the secondary metabolite production from plants [8]-[10]. Kieran et al. 1997, [11] had combined cell culture with genetic engineering to get increased yield. Transcription factors are also used as new molecular tools to produce valuable compounds [12].

Murraya koenigii, (family Rutaceae), is a native of India. It is an aromatic plant with a dense crown [13],[14]. Phenolic, antioxidant and flavonoids are produced by plants as a secondary metabolite and they exhibits many biological effects. They are estimated using Folin-Ciocalteau method [15]-[17]. The present study was done to study the stress on secondary metabolites produced by *Murraya koenigii* and to estimate the total polyphenol and flavonoid content.

## II. EXPERIMENTAL

The present work was undertaken to understand the stress on secondary metabolites in medicinal plants like *Murraya koenigii* and to understand their behaviour.

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### Plant Material

The Plant *Murraya koenigii* (curry leaf) is an easily available plant in India. For the experiment, the plant sample was collected from a nursery in Tambaram, Chennai (Fig 1).

**Fig 1. The plant *Murraya koenigii***

### Sample Preparation

The leaf samples taken freshly from the plant were first washed with tap water to remove the dust particles. After that, it was soaked in Tween 20 (detergent) for 15 min to remove grease from the plant. Then it was washed thoroughly to remove the foams. At last the sample was washed with distilled water for 2-3 times.

### Suspension Culture

The cleaned leaf samples were treated with mercuric chloride (0.01M) for 5 min. After that, the leaves were cut into small pieces using sterile blade. The explants were sterilized with absolute alcohol for 10 sec. After sterilization, the sample was inoculated in liquid MS media Tetracyclin/Ampicillin/Refampicin was added as an antibiotic.

### Treatment with Heavy Metal

After the inoculation for heavy metal stress, the culture was treated with 50, 100, 250, & 500 ppm of aluminium chloride.

### Quantitative Analysis of Total Polyphenol Content (TPC)

The Total phenolic content of the extracts were measured spectrophotometrically using Folin-Ciocalteau method as described by Singleton et al. 1999 [18] at 725 nm.

### Quantitative Analysis of Flavonoid Content

The amount of flavonoids was determined using aluminium chloride method with little modifications at 415 nm. The cell culture was centrifuged and the pellet was taken and suspended in methanol and it was used as sample.

## III. RESULTS AND DISCUSSION

The cell culture method for study secondary metabolite production was found to be highly useful for commercial production of pharmaceuticals. The leaves of *Murraya koenigii* was surface sterilized and inoculated in the MS medium and after 10-12 days of inoculation, the cell growth was observed as white precipitates suspended in the liquid. Then the cell presence was examined under microscope view stained with Evan's blue.

After addition of heavy metal, it was seen that the rate of growth of cell was faster (data not shown). The mean absorbance with concentration of Gallic acid is shown in Table 1. Fig 2 shows the effect of heavy metals on TPC. The amount of gallic acid increased with the increase in aluminium chloride concentration when compared to the control.

**Table 1 Absorbance of Standard Gallic Acid**

| Concentration of $AlCl_3$ (ppm) | Absorbance, λmax=725 nm | |
|---|---|---|
| | Day 4 | Day 6 |
| 10 | 0.329 | 0.420 |
| 20 | 0.50 | 0.738 |
| 30 | 0.746 | 1.073 |
| 40 | 1.275 | 1.322 |
| 50 | 1.358 | 1.536 |

**Table 2 Total Phenolic Content *Murraya koenigii* plant extract**

| Sample | Concentration(µg/ml) | Total flavanoids content (mg/g) |
|---|---|---|
| *Murraya koenigii* | 1000 | 4.83 |

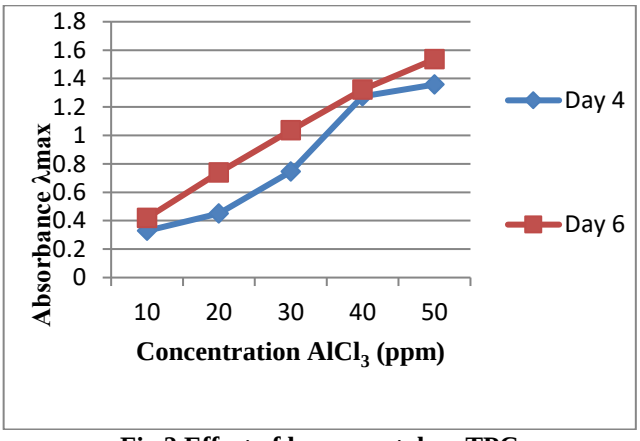


**Fig.2 Effect of heavy metal on TPC**

The amount of total phenol was determined using Folin-Ciocalteau reagent. The total phenol present in the extracts is 4.83 mg/g.

**Table 3 Absorbance of Flavanoid content in *Murraya koenigii* plant extract**

| Concentration of $AlCl_3$ (ppm) | Absorbance, λmax=415 nm | |
|---|---|---|
| | Day 4 | Day 6 |
| 10 | 0.159 | 0.317 |
| 20 | 0.212 | 0.475 |
| 30 | 1.328 | 1.587 |
| 40 | 1.464 | 1.718 |
| 50 | 1.593 | 1.826 |

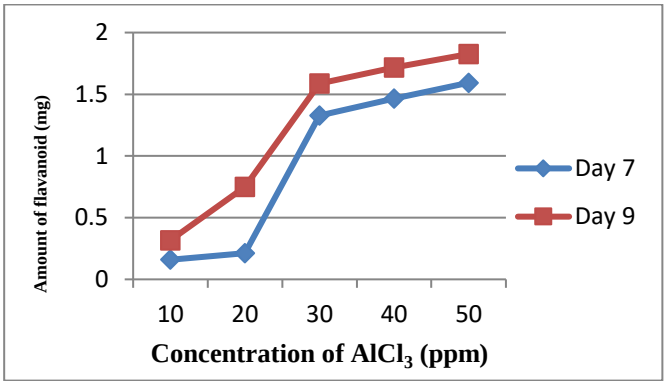


**Fig 3 Effect of heavy metal stress on flavonoid production in *Murraya koenigii***

Table 3 shows that the flavonoid amount increases with the increase in the concentration of aluminium chloride and there is significant difference between flavonoid amounts between the two days. Figure 3 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 aluminium chloride concentration when compared to the control.

## IV. CONCLUSION

Traditional medicine has been gaining more importance in recent years. A cheaper extraction procedure for pharmaceuticals, flavouring agents, dyes etc. has been a challenge for a long time. This study was aimed to increase the production of flavonoids by using heavy metal as elicitors in suspension cultures. Optimization of other physical and chemical parameters will be done in future. The cost of production will also be estimated.

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