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The effect of plant defence elicitor on *Costus igneus* for the enhanced phytochemical productivity

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ABSTRACT

Medicinal Plants have proven potential of treating and preventing various disorders and illness from the ancient time. Many Phytopharmaceuticals companies are emerging as a result of its popularity in both the developed as well as developing countries. Newer approaches are developed to protect the plant source and to enhance its productivity in terms of phytochemicals so that the demand and supply can be balanced. *Costus igneus*, a newer plant to India is gaining its popularity for its use as ornamental plant in southern part of the country to Diabetes treatment herbal remedy. Flavonoid diglycosides and sapogenins are mostly responsible for its anti-diabetic activity. Due to over exploitation of plant some alternative methods has to be developed in order to protect the plant from its natural habitat. Elicitors are the compounds that stimulating plant defence pathways and the secondary metabolites are released due to defence responses which are triggered and activated by elicitors. The present study was focused on the application of Salicylic acid (SA) as an elicitor in different concentration (0.50 mg/l, 0.100 mg/l, 0.150 mg/l), which was sprayed on the plants of *Costus igneus* grown using complete block design in controlled growth conditions provided in polyhouse and the quantitative estimation for phytochemicals was done. The results suggest that SA enhances the quantity of desired phyto pharmaceuticals, which also plays as essential role in plant defensive pathways.

Keywords: Medicinal plants, phyto-pharmaceuticals, *Costus igneus*, Elicitors, Salicylic acid (SA), plant defence mechanism.

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INTRODUCTION

Plants are the major source of drug in most of the developing countries. Factors like climate change are putting extra burden on existence of rich medicinal plant due to which many rare and common plant species are on the erg of extinction. Biotechnological approaches can contribute to preserve the beneficial effects of valuable plants. *Costus igneus* commonly known as insulin plant is newly introduced plant in our country. It is widely available in the regions of south and Central America. The leaves of the plant are consumed as dietary supplement for the management of diabetes. The scientific studies on various parts of the plant revealed many pharmacological activities including anti-diabetic, hypo-lipidemic, diuretic, ameliorative effect, antioxidant, putative activity, anti-cancerous etc[1]. The popularity and wide spread use of the plant have made it over-exploited. The need of an hour is some modern approaches have to be applied to preserve the plant and to increase the productivity. Application of plant elicitors can contribute to trigger the defence responses so as to make plant more disease resistant as well as it can also help in enhanced productivity of phytopharmaceuticals responsible for the activity[2]. The researchers are utilizing the concept of elicitors more in laboratory conditions in cell suspension form, and a little study in the practical application in the field is done specially for medicinal plants. So the present research work is an approach to analyse the practical effect of selected elicitor application in the field conditions in terms of quantity enhancement of secondary metabolites.

MATERIALS AND METHODS

Plant material: Healthy plants of Costus igneus was collected from office (Nursery), Forest department, Government of Madhya Pradesh, Indore, Salicylic acid purchased from Sun chemicals, Indore, Methanol, Fehling solution A & B, Ferric chloride, Mayer's reagent (Mercuric Chloride, Potassium Iodide), Ninhydrin solution, Sodium Hydroxide, Biuret Reagent, Conc. Sulphuric Acid, Acetic Acid, Dilute

Hydrochloric Acid, Folin-Ciocalteu reagent, Sodium carbonate.

Development of Poly-house/green house

To provide natural ventilated environmental control for the growth of *Costus igneus* and to assure the effect of chosen elicitor, Poly-house/hooped green house was constructed in the medicinal garden of the Institute (Picture#1). It was uniquely design by using eco-friendly materials of construction like bamboos so that the plant can be acclimatised easily. The green house was having the proper facility of irrigation and drainage.

Picture#1: Construction of green house in medicinal garden of Institute

Cultivation of plant in green house and application of elicitor

The procured plants were cultivated in the green house using randomized complete block design. The green house was divided in blocks having the separate divisions for cultivation of controlled and elicitor treated groups arrangement. The plants were irrigated at regular interval of time using drip system. After the acclimatization and when the plants started signs of growth, they were sprayed with varying concentration of salicylic acid (SA) in treated groups as shown in the scheme 1. The untreated group was irrigated regularly for their growth. The application of elicitor was continued for 45 days and the plant was subjected to SA application with the growth of every new apical leaf (Picture#2)

Picture#2: Cultivation of plant Costus igneus and application of Elicitor

Scheme: 1 Schematic representation of plant distribution and SA treatment during field study

SA (0.50 mg/l conc)	SA (0.100 mg/l conc)	SA (0.150 mg/l conc)	Free moving space	Untreated plants of *Costus igneus*
SA (0.150 mg/l conc)	SA (0.50 mg/l conc)	SA (0.100 mg/l conc)		Untreated plants of *Costus igneus*
SA (0.100 mg/l conc)	SA (0.150 mg/l conc)	SA (0.50 mg/l conc)		Untreated plants of *Costus igneus*

Collection, drying and solvent extraction of leaves of *costus igneus*

The leaves of plants was collected after 45 days of exposure of SA and was shade dried and the successive solvent extraction was performed using Methanol (Picture#3)

Picture#3: Collection shade drying and solvent extraction of leaves of Costus igneus

Phytochemical Examination of extract

Phytochemical examinations of the elicitor treated and untreated extract was carried out for all the extracts as per the standard methods for the identification of Alkaloids, Carbohydrates & glycosides, Phenolic compounds & tannins, Proteins & Amino acids, Flavonoids, Phytosterols, Terpenoids, gums and Saponins[3,4].

Determination of Total Phenolic Content.

The amount of phenolics in methanolic extract of *Costus igneus* was determined with the help of Spectrophotometric method using Folin-Ciocalteu reagent[5]. Added 2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of Na_2CO_3 (7.5% w/v) to 0.5 ml of each sample (3 replicates) of methanolic extract of plant under investigation (1 mg/ml). The blank was also

prepared by admixing 0.5 ml of methanol 2.5 ml Folin-Ciocalteu's reagent (10%, Prepared in water) and 2.5 sodium carbonate (7.5%). The resulting mixture was incubated at 45°C with constant shaking for 15 min. The absorbance of the samples was measured at 765 nm using spectrophotometer. The content of total phenol was calculated on the basis of the calibration curve of gallic acid and the results were expressed as mg of gallic acid equivalents (GAEs) per gram of extract. (Picture#4)

Picture#4: Determination of total phenolic and Flavonoid content

RESULTS AND DISCUSSION

The samples were analyzed for the pharmacognostic activity :-

Table 1: Organoleptic characteristics of dried leaves of *Costus igneus*

Scientific name	Common Name	Part of the Plant Used	Color	Odour	Taste	Texture
Costus igneus	Insulin Plant	Leaves	Light Cream	Sweet Aromatic	Sour	Granular

Table 2: Color of methanolic extract of leaves of *Costus igneus*

S. No.	Name of Reagent	Name of Drug	Colour of Extract
1	Methanol	***Costus igneus***	Brown

Table 3: Phytochemical investigation of extract

Phytochemical tests	Result
Carbohydrates	**-**
Tannins (Ferric Chloride test)	**-**
Alkaloids (Mayers Reagent)	**+**
Glycosides (Fehling Solution)	**+**
Phenolic compounds	**+**
Proteins & Amino acids	**-**
Flavonoids	**+**
Phytosterols	**+**
Terpenoids	**+**
Gums	**-**
Saponins	**+**

Quantitative Analysis of Extract for its Phenolic content:

Table 4: Absorbance of Phenol standard (Gallic acid)

S.No.	Concentration (µg/ml)	Absorbance
1	**50**	**0.213**
2	**100**	**0.532**
3	**150**	**0.856**
4	**200**	**1.156**
5	**250**	**1.489**
6	**300**	**1.711**
7	**350**	**2.013**
8	**400**	**2.389**

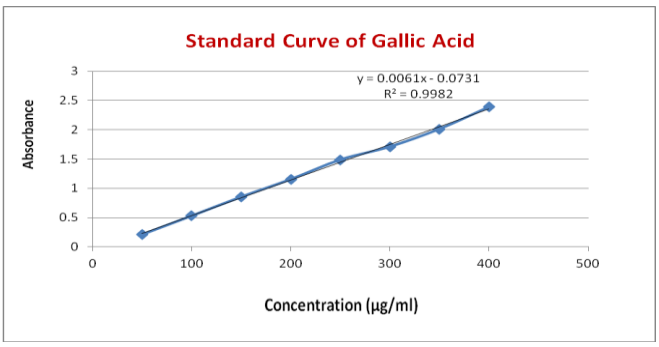

Figure 1: Standard curve of gallic acid

Table 5: Total Phenolic Content of the plant extracts (Methanolic extract of *Costus Igneus*) expressed in terms of gallic acid equivalent (mg of GA/g of extract) subjected to elicitor application (Salicylic acid):

S.No.	Concentration of Elicitor (SA) (mg/l)	Absorbance	Gallic acid equivalent (mg of GA/g of extract) (GAE)
1	0.50	0.089	25
2	0.100	0.189	43.66
3	0.150	0.111	30.66

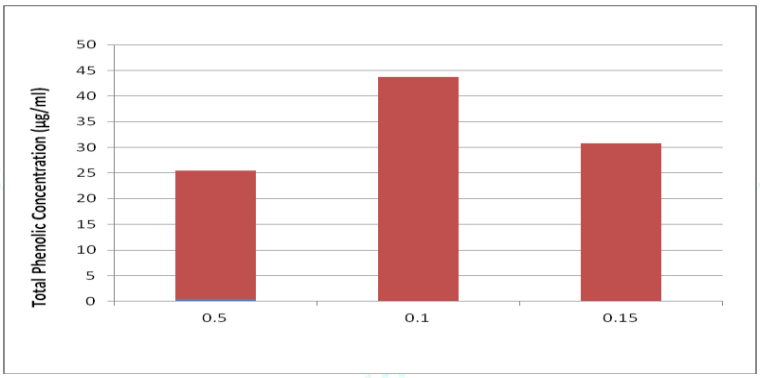

Figure 2: Total phenolic content at various concentration of Salicylic acid application

Elicitors are the compounds that stimulating plant defense pathways and the secondary metabolites are released due to defence responses which are triggered and activated by elicitors. The present study was focused on the application of Salicylic acid (SA) as an elicitor in different concentration (0.50 mg/l, 0.100 mg/l, 0.150 mg/l), which was sprayed on the plants of *Costus igneus* grown using complete block design in controlled growth conditions provided in polyhouse and the quantitative estimation for phytochemicals was done. The results suggest that SA enhances the quantity of phenolic compounds which plays as essential role in plant defensive pathways. Further study on quantitative analysis of other phytopharmaceuticals is needed along with qualitative analysis of enhanced phenolic compound to explore the complete effect of Salicylic acid as plant elicitor on *Costus igneus.*

CONCLUSIONS

Medicinal Plants have proven potential of treating and preventing various disorders and illness from the ancient time. Many Phytopharmaceuticals companies are emerging as a result of its popularity in both the developed as well as developing countries. Newer approaches are developed to protect the plant source and to enhance its productivity in terms of phytochemicals so that the demand and supply can be balanced. *Costus igneus*, a newer plant to India is gaining its popularity for its use as ornamental plant in southern part of the country to Diabetes treatment herbal remedy. Due to overexploitation of plant some alternative methods has to be developed in order to protect the plant from its natural habitat. In the present study elicitor technology was applied and its effect on enhancement of total phenolic content due to exposure of elicitor (Salicylic acid) was studied. The results suggested that Salicylic acid at the concentration of 0.1 mg/l shows maximum increase in total phenolic content as compared to 0.5 and 0.15 mg/l.

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