



Methyl Jasmonite Induced Elicitation of the Biosynthesis of Bonducellin – A Homo Isoflavanoid in the Cell Suspension Culture of Leaf Derived Callus of *Caesalpinia bonducella* (L) Roxb

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Caesalpinia bonducella (L) Roxb., a significant ethno medicinal plant is a repository of bioactive compounds. Bonducellin is an important secondary metabolite found in *Caesalpinia bonducella* (L) Roxb., which possess antioxidant, anti-inflammatory, anticancer, antispasmodic, antihelminthic, anti-sterility and also used in the treatment of Polycystic Ovarian Syndrome (PCOS). The over exploitation of the plant is drastically reducing the natural population. Hence, the conservation

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strategies should be implemented by providing an *in vitro* alternative for the production of pharmacologically important compounds like bonducellin to retain the present status of the plant. The main objective of the present study is to enhance the *in vitro* production of bonducellin using abiotic elicitor methyl jasmonite. The leaf derived callus obtained in the Murashige and Skoog (MS) medium treated with 1 ml methyl jasmonite for 10 days produced $3.42 \pm 0.02 \mu\text{g/g DW}$ bonducellin. Thus, the abiotic elicitor methyl jasmonite can be utilized for the large-scale industrial production of bonducellin.

Keywords: Bonducellin; Methyl jasmonite; LCMS; HPLC; *Caesalpinia bonducella* (L) Roxb.

1. INTRODUCTION

Medicinal plants are the treasure trove of numerous pharmaceutically important secondary metabolites. *Caesalpinia bonducella* (L.) Roxb. is an indigenous medicinal plant with antiperiodic, febrifuge and anti-helminthic properties. The powder of the roasted pod of this plant has been used as a substitute for quinine. The root bark is used to treat fever, intestinal worms, tumours, amenorrhoea, cough and also removing the placenta after child birth. Recently the plant has been reported to be effective in the treatment of Polycystic Ovarian Syndrome or PCOS [1].

Therapeutic value of *Caesalpinia bonducella* (L.) Roxb. is assigned to an important secondary metabolite bonducellin - a homoisoflavanoid present in the family Caesalpinaceae [2]. Bonducellin [7-Hydroxyl (E)-3-phenylmethelene Chroman-4-one] is a better constituent of the seed kernel. The structure, extraction and isolation of bonducellin were studied extensively [3]. The objective of the present study is to provide an alternative tool for the enhanced *in vitro* production of bonducellin in cell suspension culture of leaf derived callus without disturbing the plants of *Caesalpinia bonducella* (L) Roxb. in nature.

2. METHODOLOGY

Caesalpinia bonducella (L) Roxb. was collected from Manjiri and authenticated in BSI, Regional office, Western circle, Pune (BSI/WRC/Cert./2015). The authenticated plant is used as the source of explant to establish *in vitro* culture. Murashige and Skoog (MS) medium and phyto hormones were prepared according to the standard procedures. The cell suspension culture was established in liquid Murashige and Skoog (MS) medium supplemented with 1 mg/l 2,4-D was inoculated with 1 g callus. The abiotic elicitor methyl jasmonite was directly added to the cell suspension culture. The cell suspension culture was maintained in a gyratory shaker for 30 days at 110 ± 20 rpm in dark at $25 \pm 2^\circ\text{C}$. The

observations were recorded in 5 days intervals. The cells obtained after filtration of cell suspension culture were dried, powdered and then used for the extraction. The methanolic extract obtained after cold maceration is centrifuged and the supernatant is used for LCMS and HPLC analysis for the identification and quantification of bonducellin respectively.

3. RESULTS AND DISCUSSION

The liquid Murashige and Skoog (MS) medium supplemented with 0.5 ml methyl jasmonite produced 0.63 ± 0.17 g callus compared to 0.44 ± 0.02 g callus obtained in 1ml of methyl jasmonite. The weight of the callus was increased to 0.89 ± 0.46 g after 5 days of inoculation when the conc. of methyl jasmonite was raised to 1 ml. The weight of the callus was reduced to 0.46 ± 0.28 g after 10 days of inoculation in liquid Murashige and Skoog (MS) medium supplemented with 1 mg/l 2,4-D (Fig. 1). Thus, the weight of the callus produced in the presence of abiotic elicitors was found to be decreasing with increase in age of the culture as well as increase in the conc. of the elicitors - methyl jasmonite. The growth of the cells and callus was found to be inhibited with high levels of elicitors.

The weight of the dry callus was noted and then bonducellin extraction and quantification was carried out through HPLC using the standard calibration curve of pure bonducellin (Fig. 2). The amount of bonducellin was $1.02 \pm 0.41 \mu\text{g/g DW}$ in callus in 0.5 ml methyl jasmonite after 5 days while $0.29 \pm 0.01 \mu\text{g/g DW}$ was produced after 10 days. The amount of bonducellin was increased to $1.20 \pm 0.08 \mu\text{g/g DW}$ in cell suspension culture with 1 ml of methyl jasmonite after 5 days. The amount of bonducellin was increased to $3.42 \pm 0.01 \mu\text{g/g DW}$ in cell suspension culture with 1 ml methyl jasmonite after 10 days (Fig. 3). Even though the dry weight of the callus was reduced to half with the increase in the conc. of methyl jasmonite, the amount of bonducellin increased almost three times.



Fig. 1. Leaf induced callus on 2,4 – D mg/l Murashige and Skoog (MS) medium

Table 1. Effect of Methyl Jasmonite on the production of bonducellin in the cell suspension culture of *Caesalpinia bonducella* (L) Roxb

Sr. No.	Abiotic Elicitors	Duration of treatment	Amount of bonducellin ($\mu\text{g/g DW}$)
1.	Methyl jasmonate (0.5 ml)	5 days	1.02 ± 0.41
		10 days	0.29 ± 0.01
2.	Methyl jasmonate (1 ml)	5 days	1.20 ± 0.08
		10 days	3.42 ± 0.00

The values represented as the mean \pm SE calculated on three independent experiments. The p - value was < 0.05 in all experiments

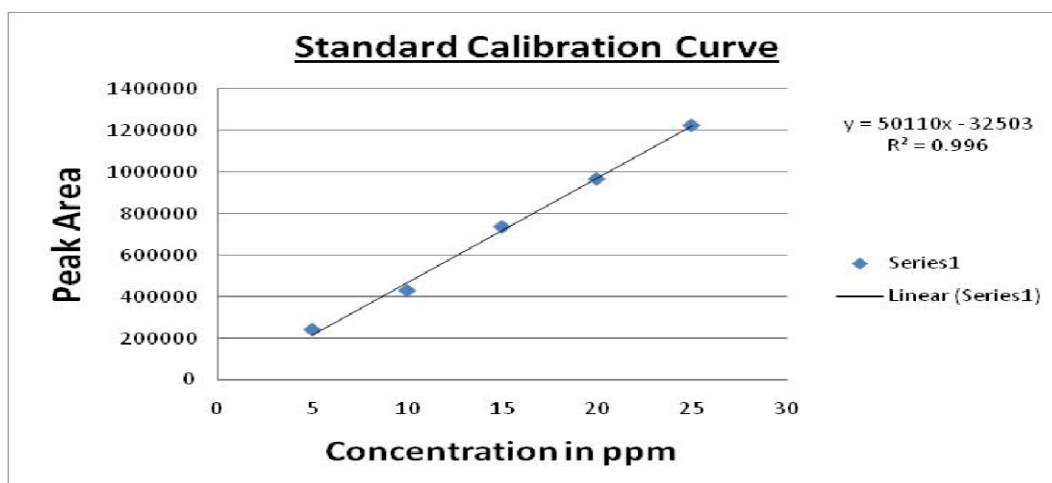


Fig. 2. Standard Calibration Curve of Bonducellin

Veerporte et al., [4] studied the importance of plant tissue culture and metabolic engineering in the *in vitro* synthesis of secondary metabolites. Naik and Alkhayri [5] studied the effect of biotic and abiotic elicitors in the production of pharmacologically significant secondary metabolites in the medicinal plants. Purushothaman et al., [6] reported the molecular

structure of bonducellin - a homoisoflavanoid in *Caesalpinia bonducella* (L) Roxb. Bonducellin imparts most of the bioactive properties of *Caesalpinia bonducella* (L) Roxb. which led to an array of studies in the natural and artificial synthesis of bonducellin especially from the seeds of *Caesalpinia bonducella* (L) Roxb. Das et al., [2] reported the isolation, synthesis and

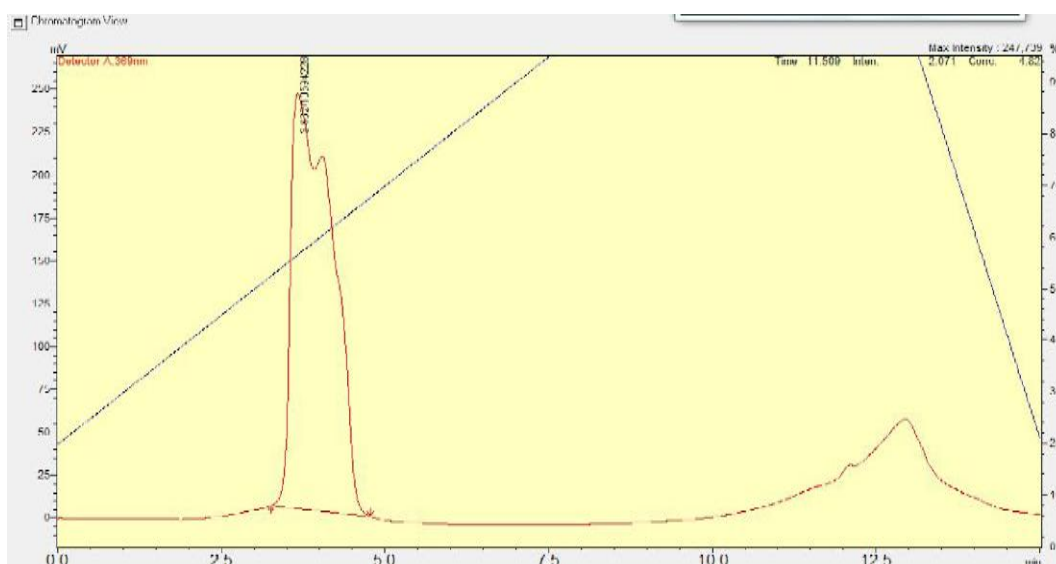


Fig. 3. HPLC Chromatogram of 1 ml Methyl jasmonate treatment after 10 days on suspension culture

bioactivity of bonducellin from *Caesalpinia pulcherrima*. Thus, it is evident that bonducellin can be extracted from the members of the genus *Caesalpinia*. Recently Mannivanan et al., [1] reported the glutathione induced biosynthesis of bonducellin in *Caesalpinia bonducella* (L) Roxb. leaf callus [7,8]. The present study also proved the enhancement in the biosynthesis of bonducellin in leaf derived callus suspension culture fortified with methyl jasmonite [9]. Hence methyl jasmonite 1ml in cell suspension culture of *Caesalpinia bonducella* (L) Roxb. can be utilised for the establishment of bioreactor for the industrial production of bonducellin [10].

4. CONCLUSION

To conclude, the present study throws light on the *in vitro* alternative for the production of pharmacologically important bioactive compound bonducellin. The leaf derived callus obtained on the MS medium treated with 1 ml methyl jasmonite for 10 days produced $3.42 \pm 0.02 \mu\text{g/g}$ DW bonducellin. Thus, the abiotic elicitor methyl jasmonite can be utilized for the industrial production of bonducellin through bioreactor. Thus, bonducellin can be produced industrially without disturbing the natural population of *Caesalpinia bonducella* (L) Roxb.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Manju Rakesh and Neeta M Patil hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT,

etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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