EFFECT OF METHYL JASMONATE ON TRIGONELLINE CONTENT OF SUSPENSION CELL CULTURE OF *Abrus precatorius*

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To my beloved family

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ABSTRACT

Trigonelline was a plant hormone that is built up in a plant from the methylation of the nitrogen atom of niacin. It was responsible for induction of G2 phase arrest in the root apices of many plant species. Trigonelline was also capable of reducing the blood sugar level and thus treating diabetes type II. In this study, effect of methyl jasmonate, MeJa (50 µM, 100 µM and 200 µM) on trigonelline content in suspension cell culture of *Abrus precatorius* using High Performance –Liquid Chromatography for trigonelline was investigated. The effect of different inoculum size (0.1 g and 0.5 g) on cell biomass and trigonelline content of suspension cell culture of *A.precatorius* was studied. Suspension cell culture of *A. precatorius* was developed in a 100 mL conical flask containing 30 mL MS medium supplemented with 0.5 mg/L kinetin and 0.5 mg/L 2, 4- D. Results showed that 0.5 g inoculum had higher cell biomass with maximum biomass of 0.0795 g than 0.1 g inoculum that had biomass decrement throughout the culture. Furthermore, addition of 50 µM MeJa had multiplied trigonelline content to five times (6.62 mg/L) compared to the control (1.41 mg/L) for a period of 2 weeks. The correlation coefficient test indicated that cell biomass was not correlated with trigonelline content. Different increment of cell biomass with trigonelline suggested that trigonelline was not involved in regulating cell cycle of *A. precatorius*. In conclusion, 0.5 g inoculum size was a better size compared to 0.1 g inoculum suspension cell culture. The optimum trigonelline during cell growth of *A.precatorius* was also discovered.

ABSTRAK

Trigonelin adalah hormon tumbuh-tumbuhan yang terbina dalam tumbuh-tumbuhan dari proses metilasi atom nitrogen dari niasin. Ia bertanggungjawab dalam mendorong penangkapan fasa G2 dalam pucuk akar dari pelbagai pokok. Trigonelin juga berkemampuan untuk mengurangkan kandungan gula dalam darah dengan itu mengubati penyakit kencing manis jenis II. Dalam kajian ini, kesan 50µM, 100 µM dan 200 µM metil jasmonat terhadap kandungan trigonelin dalam kultur ampaian sel *Abrus precatorius* dikaji menggunakan kromatografi cecair berprestasi tinggi untuk mengesan trigonelin. Kesan saiz inokulum yang berbeza (0.1 g dan 0.5 g) terhadap biomas sel dan kandungan trigonelin kultur sel ampaian *A.precatorius* juga dikaji. Kultur ampaian sel *A.precatorius* disediakan dalam 100 mL kelalang kon dengan 30 mL media MS ditambah dengan 0.5 mg/L kinetin dan 0.5 mg/L 2, 4- D. Keputusan menunjukkan 0.5 g saiz inokulum mempunyai sel biomas lebih tinggi dari 0.1 g. Selain daripada itu, penambahan 50 µM metil jasmonat meningkatkan kadar trigonelin kepada lima kali ganda (6.62 mg/L) berbanding dengan kawalan (1.41 mg/L). Ujian korelasi menunjukkan biomas sel tidak berkorelasi dengan kandungan trigonelin. Kenaikan berbeza biomas sel dengan kadar trigonelin mencadangkan trigonelin tidak terbabit dalam regulasi kitaran sel *A.precatorius*. Kesimpulannya, penggunaan saiz inokulum sebanyak 0.5 g adalah saiz inokulum yang lebih baik berbanding penggunaan 0.1 g untuk kultur sel ampaian. Trigonelin optimum semasa tumbesaran sel juga ditemui.

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LIST OF SYMBOLS/ ABBREVIATIONS

±	-	plus minus
°C	-	degree celcius
%	-	percentage
pH	-	hydrogen concentration
g	-	gram
µM	-	micromolar
L	-	litre
mg/L	-	milligram per litre
µ	-	micro
mg/ml	-	milligram per millilitre
min	-	minute
rpm	-	revolutions per minute
2,4-D	-	2,4-Dichlorophenoxyacetic acid
MeJa	-	Methyl Jasmonate
FW	-	Fresh weight
FBG	-	Fasting Blood Glucose
OGTT	-	Oral Glucose Tolerant Test

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Disease is rapidly increasing worldwide. Hence, the development of new drugs for treating diseases undergoes a rapid phase. Unfortunately, the cost of commercial drugs is high and unaffordable for poor and moderate people. Diabetes mellitus that is a chronic disease that is caused by many factors such as inherited, acquired deficiency in insulin secretion and decreased responsiveness of the organs to secreted insulin (Ruiz *et al.*, 2015). The prevalence of diabetes is increasing globally for all age-groups. Number of people with diabetes has increased from 2.8% in 2000 to 4.4% in 2030 (Wild *et al.*, 2004). Example of drugs available in market for treating diabetes are sulphonylureas and biguanides (Waring, 2012). Since the cost of antidiabetic drugs are high and tendencies to adverse effect, the demand for traditional medicine has increased. A discovery of potential new compound for diabetes treatment i.e. identification of antidiabetic compound in a plant is exploited.

A.precatorius L. is a woody climber plant that commonly known as Crab's eye, rosary pea and in Malay known as Akar Saga is a perennial climber that can be found in tropical and subtropical regions (Yonemoto *et al.*, 2014). The seeds and flowers of

A.precatorius is found to be multicolour. The leaves are even-pinnate to 10cm long with 8-20 pairs. The plant usually grows in bushes and hedges upto 1000 m elevation (Balachandran & Rajendiran, 2015). *A. precatorius* is well known for its great medicinal purpose (Khare, 2004) as it is used in treatment of gonorrhoea, jaundice and skin diseases (Gul *et al.,* 2013a). The use of the plant worldwide has a negative impact on the plant as the amount of the plant in nature is decreasing (Perveen *et al.,* 2013).Example of the compound that has been discovered to possess antidiabetic property is trigonelline. Trigonelline is a secondary plant hormone that has been reported to have anticarcinogenic and antidiabetic activities (Ghule *et al.,* 2012). Cell cultures of *Trigonella foenum-graecum* had been found to increase the production of trigonelline compared with the differentiated plant (Radwan, 1980). Trigonelline increment was manipulated in *Pinellia Ternata* by elicitation (Liu *et al.,* 2010). Application of salicylic acid to peppermint increased the antidiabetic potential by increasing the trigonelline content (Figueroa-Perez *et al.,* 2015). Elicitation by using MeJa to *T. foenum-graecum* cell suspension cultures abled to increase the trigonelline production (Ahmed and Husam, 2011).

Trigonelline was successfully isolated from seeds of *A.precatorius*. (Ghosal and Dutta, 1970). Till now, there was no report on effect of elicitor on trigonelline content of *A.precatorius*. In this study, an attempt was made to enhance trigonelline content in *A.precatorius* cell suspension culture using methyl jasmonate.

1.2 Statement of the Problem

A.precatorius is popular medicinal plant in India (Khare, 2004). The plant had been discovered with the presence of trigonelline that is one of active compounds in diabetes treatment. However, the rapid use of the plant had decreased its population worldwide. The use of the plant without control for disease treatment will decrease the plant population and the true potential of the plant cannot be fully utilized. Karwasara

et al., (2011) has established a protocol for cell suspension culture of *Abrus precatorius* by using 2.0 g inoculum size.

The content of trigonelline in *A.precatorius* must be increased in quantity to increase the ability of the plant to produce these compounds. The existing population of *A.precatorius* must not be disturbed in increasing the trigonelline production in the plant. Therefore, the accurate protocol in increasing the cell biomass and increasing the amount of trigonelline must be established.

1.3 Objectives

1. To investigate the effect of inoculum size on cell biomass of suspension cell culture of *A. precatorius.*
2. To determine trigonelline content at different stages of *A. precatorius* cell culture.
3. To investigate the effect of methyl jasmonate on biomass and trigonelline contents of suspension cell culture of *A. precatorius.*

1.4 Scope of the Study

In this study, the best inoculum size for suspension cell culture for biomass accumulation identification was identified by using 0.1g and 0.5g of inoculum size in media supplied with 5mg/L kinetin and 5 mg/L 2, 4-D in a room temperature on a gyratory shaker. Other than that, the trigonelline content at different stages of *A.precatorius* cell growth was investigated. The amount of trigonelline in

A.precatorius suspension cell culture was manipulated by using MeJa as elicitor with concentration of 50µM, 100µM and 200 µM.

1.5 Significance of Study

Identification of inoculum size for obtaining the growth pattern of *A.precatorius* can make sure to understand the behaviour of the cells when different inoculum size was used. The relationship of biomass with the trigonelline content can be understand and the growth phase where the optimum trigonelline production can be identified.

This study was conducted in effort to find a better or alternative source of trigonelline in plant. The difference in content of trigonelline in *A.precatorius* with other plants reported with trigonelline content after elicitation was also investigated. The small scale of the research that was in 30mL MS media can be used as a guide in up-scaling the production of trigonelline in *A.precatorius*.

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