TRITERPENE PRODUCTION IN *CENTELLA ASIATICA* (L.) URBAN (PEGAGA) CALLUS AND CELL SUSPENSION CULTURES

By

ANNA LING PICK KIONG

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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Especially dedicated to:

My parents: Joseph Ling and Teresa Lau

Brothers and Sisters: Martin, Peter, Catherine, Angela, Cecilia & Paul

Nieces and Nephews: Teresa, Anna, Grace, James, John, Emi, Henry, Stephen, Austin and Justin

To all the **Fathers** and **Sisters**, **my love ones** and all those who has sacrificed and supported me throughout my studies

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the Degree of Doctor of Philosophy

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Chairman: Professor Maziah Mahmood, Ph.D.

Faculty: Science and Environmental Studies

Centella asiatica or locally known as 'Pegaga' is one of the most common medicinal plants used by diverse ancient cultures and tribal groups. Its medicinal values are mainly attributed to the presence of the triterpene constituents. As there is still no information available on the triterpene production in cultured tissues, studies were carried out in determining the triterpene distribution particularly asiatic acid, madecassic acid, asiaticoside and madecassoside in intact plants of the twelve accessions of *C. asiatica* collected throughout Malaysia as well as in the callus and cell suspension cultures.

Results obtained from the studies revealed that twelve accessions of *C. asiatica* differed both in their morphologies and their triterpene contents. The triterpenes constituents were detected at a range of 0.134 to 1.655 mg/g dry weight in the whole plant intact tissues. Triterpenes were also successfully detected in the callus (0.014 to 0.773 mg/g dry weight) and cell suspension cultures (0.005 to 0.084 mg/g dry weight), the amount that were lower than that produced in the intact

tissues. However, manipulating the physical culture conditions, feeding of precursor, elicitation as well as amino acid addition managed to increase the triterpenes content in cultured tissues. Studies on the effects of the medium composition show that full strength of the basal Murashige and Skoog medium supplemented with B5 vitamins and sucrose (3-4%) increased the triterpenes content in both callus and cell suspension cultures. An interaction of auxin-cytokinin has observed being important for both callus and cell suspension cultures in enhancing triterpenes production. Higher triterpenes content was obtained in callus treated with 2,4-D and kinetin while the combination of kinetin and dicamba enhanced the triterpenes production in cell suspension cultures. The precursor-feeding studies revealed that lower concentrations of squalene (0.16 mg/L in callus and 0.8 mg/L in cells) were preferred for triterpenes production. Squalene at 0.16 mg/L had successfully triggered the production of madecassoside, asiaticoside and madecassic acid in callus cultures while asiatic acid and madecassic acid content was increased in cells treated with 0.8 mg/L squalene. The elicitor studies exhibited that the different elicitors showed distinctive effects on triterpenes production. Nevertheless, supplementation of succinic acid at 3 and 4 mg/L was found the best in increasing the triterpenes production in callus and cell suspension cultures, respectively. Addition of amino acid into the culture media was also found to promote the triterpenes production in *in vitro* cultures. The study further concluded that the combinations of the optimized factors namely medium composition, precursor feeding, elicitation and amino acid addition is a very useful strategy in enhancing the triterpenes

production particularly the asiatic acid and madecassic acid in *in vitro* cultures of *C. asiatica*.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PENGHASILAN TRITERPENA DALAM KULTUR KALUS DAN SEL AMPAIAN *CENTELLA ASIATICA* (L.) URBAN (PEGAGA)

Oleh

ANNA LING PICK KIONG

Januari 2004

Pengerusi : Profesor Maziah Mahmood, Ph.D.

Fakulti : Sains dan Pengajian Alam Sekitar

Centella asiatica atau dikenali sebagai pegaga oleh masyarakat tempatan adalah antara tumbuhan ubatan yang biasa digunakan oleh pelbagai suku kaum. Nilai perubatannya adalah disebabkan oleh kehadiran unsur-unsur triterpena. Memandangkan masih tidak terdapat informasi tentang penghasilan triterpena dalam tisu yang dikulturkan, kajian ini dijalankan untuk mengenalpasti taburan triterpena terutamanya asid asiatik, asid medikasik, asiatikosida dan medikasosida dalam 12 aksesi pokok induk *C. asiatica* yang telah dikumpul dari seluruh Malaysia serta dalam kultur kalus dan sel ampaian.

Keputusan yang diperolehi dalam kajian ini mendedahkan bahawa 12 aksesi *C. asiatica* adalah berbeza dari segi morfologi dan kandungan triterpena. Unsur triterpena telah dikesan pada julat di antara 0.134 ke 1.655 mg/g berat kering dalam keseluruhan pokok induk. Triterpena juga berjaya telah dikesan di dalam kultur kalus (0.014 ke 0.773 mg/g berat kering) dan sel ampaian (0.005 ke 0.084 mg/g berat kering) di mana kuantiti ini adalah lebih rendah daripada pokok induk.

Namum begitu, dengan memanipulasikan keadaan pengkulturan fizikal, pembekalan prekursor, penggunaan elisitor serta penambahan asid amino telah berupaya meningkatkan kandungan triterpena dalam kultur tisu. Kajian tentang kesan komposisi media menunjukkan media basal Murashige dan Skoog yang telah dibekalkan dengan vitamin B5 dan sukrosa (3-4%) dapat meningkatkan kandungan triterpena dalam kedua-dua kultur kalus dan sel ampaian. Interaksi auksin-sitokinin diperhatikan amat penting dalam meninggikan penghasilan triterpena dalam kedua-dua jenis kultur. Kandungan triterpena yang lebih tinggi diperolehi dalam kalus yang telah dirawat dengan 2,4-D dan kinetin manakala kombinasi kinetin dan dicamba membawa kepada penghasilan triterpena yang lebih tinggi dalam kultur sel ampaian. Kajian pembekalan prekursor mendedahkan kepekatan skualen yang rendah (0.16 mg/L dalam kalus dan 0.8 mg/L dalam sel) cenderung dalam penghasilan triterpena. Skualen pada 0.16 mg/L berjaya mengaruh penghasilan medikasosida, asiatikosida dan asid mekasik dalam kultur kalus manakala kandungan asid asiatic dipertingkatkan dalam sel yang dirawat dengan 0.8 mg/L skualen. Kajian elisitor menunjukkan elisitor yang berbeza memberikan kesan yang jelas dalam penghasilan triterpena. Namun begitu, pembekalan asid suksinik masing-masing pada kepekatan 3 dan 4 mg/L adalah terbaik dalam meningkatkan penghasilan triterpena dalam kultur kalus dan sel ampaian. Penambahan asid amino ke dalam media pengkulturan juga didapati menggalakkan penghasilan triterpena dalam kultur *in vitro*. Kajian ini seterusnya menyimpulkan bahawa kombinasi faktor optimum iaitu komposisi media, pembekalan prekursor, penggunaan elisitor dan penambahan asid amino

merupakan strategi yang amat berguna dalam meninggikan penghasilan triterpena terutamanya asid asiatik dan asis medikasik dalam kultur *in vitro C. asiatica*.

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I certify that an Examination Committee met on 19th January 2004 to conduct the final examination of Anna Ling Pick Kiong on her Doctor of Philosophy thesis entitled 'Triterpene production in *Centella asiatica* (L.) Urban (Pegaga) callus and cell suspension cultures' in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of University Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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DECLARATIAON

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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ANNA LING PICK KIONG

Date:

TABLE OF CONTENTS