UNIVERSITI PUTRA MALAYSIA

ANTIOXIDATIVE CONSTITUENTS OF *CENTELLA ASIATICA* L. URBAN

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ANTIOXIDATIVE CONSTITUENTS OF *CENTELLA ASIATICA* L. URBAN

By

NORHAYATI BINTI YUSUF

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

September 2011

DEDICATION

To my husband: Azman Bin Jali
To my children: Akmal, Amirul and Alia
To my Parents: Haji Yusuf Haji Adam and Hjh Aishah @ Halimah Awang Mat
To my brothers and sisters
To all who 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

ANTIOXIDATIVE CONSTITUENTS OF *CENTELLA ASIATICA* L. URBAN

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Chair: Misri Bin Kusnan, PhD

Faculty: Faculty of Science

Antioxidants present in fruits and vegetables are well known to contribute to the defense mechanisms against oxidative stress. *Centella asiatica*, locally known as pegaga is claimed to have high antioxidant as well as medicinal properties. In spite of extensive studies made on the triterpenes production associated with the healing properties of this plant, the protective natural antioxidative constituents have rarely been studied. The influence of the medium composition and physical factors on the production of antioxidant activity has not been reported. Thus, this study was carried out to determine the antioxidative constituents and antioxidative properties of twelve accessions of *C. asiatica* intact plants as well as callus cultures in response to treatment with 0, 10, 25 and 50 μM of paraquat (PQ) for five days. Apart from that, the production of α-tocopherol, ascorbic acid and carotenoid content were also studied by manipulating the biochemical and physical culture growth conditions.

Results obtained from the studies revealed that the antioxidative constituents of *C. asiatica* intact plants and cultures vary significantly between the accessions.

Accession CA03 exhibited the highest concentrations of ascorbic acid and glutathione while CA10 produced the highest concentration of α-tocopherol and carotenoid. The antioxidants studied were also successfully detected in cultures of *C. asiatica* with CA08 callus being most dominant in ascorbic acid and α-tocopherol, while CA12 callus was highest in carotenoid content; no significant differences were observed in the glutathione content of the calluses. The amounts of the non-enzymatic antioxidants were significantly higher in cell cultures compared to the leaf tissues except for carotenoid content. Specific activities of superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) were high in CA03, but they had lower ascorbate peroxidase (APX) specific activity. On the other hand, large variation was observed in the specific activities of the antioxidative enzymes in callus cultures with CA09 showing higher specific activities of SOD and CAT while CA01 and CA11 demonstrated higher activities of GR. Moreover, the APX specific activity was found to be higher in CA02 and CA08 compared to other accessions. CAT, GR and APX specific activities were found to be higher in callus cultures while contrasting results were observed for SOD. Assays on total antioxidants, scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals and superoxide radicals showed that both *C. asiatica* intact leaves and calluses possessed lower activities compared to control. In contrast, almost all leaf accessions showed excellent scavenging effect on hydroxyl radicals compared to the calluses. In response to PQ treatment, the leaf chlorophyll content decreased subsequently with the progress of necrosis. Hydrogen peroxide in CA03 leaf and callus were significantly induced at early phase of treatment and further decreased at the end of experiment, while PQ treatment had no

significant effect on the level of malondialdehyde (MDA) and electrolyte leakage. Contrasting patterns were observed in other accessions. Furthermore, the viability and growth of cell cultures decreased significantly with increased PQ concentrations and time of exposure. The above results indicated that CA03 was the most robust accession and considered as a tolerant accession. Further studies on the effect of PQ on the antioxidants using CA03 concluded that oxidative stress caused by PQ triggered the production of α-tocopherol and carotenoid content as well as CAT and GR specific activities while significantly lowered the production of ascorbic acid and APX specific activity. Manipulating the physical and biochemical culture conditions managed to produce higher concentrations of ascorbic acid, α-tocopherol and carotenoid content of *C. asiatica* calluses. Further studies concluded that combination of 3.0 mg/l 2,4-Dichlorophenoxyacetic acid (2,4-D) with 1.0 mg/l kinetin was found to be the best plant growth regulator (PGR) combination for inducing ascorbic acid, α-tocopherol and carotenoid productions. Studies on the effect of medium composition revealed that full strength MS medium with 30-40 g/l sucrose, with addition of 0.5 to 1.0 g/l casein hydrolysate was the most favorable medium composition in enhancing the productions of all antioxidants studied. Varying the pH of the medium from 5.1 to 5.9 did not significantly affect the antioxidants produced. Higher light intensities with continuous illumination increased the production of ascorbic acid and α-tocopherol while calluses incubated in dark condition were able to trigger carotenoid production. The above results indicated that the antioxidants produced can be modulated by manipulating the medium composition, PGR combination and environmental conditions in *in vitro* cultures of *C. asiatica*.

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

KANDUNGAN BAHAN-BAHAN ANTIOKSIDA *CENTELLA ASIATICA* L. URBAN

Oleh

NORHAYATI BINTI YUSUF

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Antioksidan yang terdapat di dalam buah-buahan dan sayur-sayuran diketahui menyumbang kepada mekanisma pertahanan terhadap tegasan oksidatif. *Centella asiatica*, dikenali sebagai pegaga oleh masyarakat tempatan, dipercayai tinggi dengan nilai antioksidatif dan juga perubatan. Kajian secara meluas telah dijalankan terhadap penghasilan triterpena yang berkaitan dengan unsur-unsur penyembuhan dalam tumbuhan ini, walaubagaimanapun, kajian terhadap kandungan antioksidan semulajadi di dalam tumbuhan ini masih berkurangan. Pengaruh komposisi media dan juga faktor fizikal ke atas aktiviti penghasilan antioksidan juga belum dilaporkan. Oleh itu, kajian ini dijalankan untuk menentukan kandungan dan sifat antioksidatif dua belas aksesi pokok induk dan kultur kalus *C. asiatica* sebagai tindakbalas terhadap rawatan dengan 0, 10, 25 and 50 μM paraquat (PQ) selama lima hari. Selain daripada itu, penghasilan α-tokoferol, asid askorbik dan karotenoid juga dikaji dengan memanipulasi faktor biokimia dan fizikal keadaan pengkulturan.

Keputusan kajian menunjukkan kandungan antioksidan di dalam pokok induk dan kultur kalus adalah berbeza di antara aksesi. Aksesi CA03, mengandungi kepekatan asid askorbik dan glutathion yang paling tinggi manakala CA10 tinggi dengan α-tokoferol dan karotenoid. Kandungan antioksidan juga telah berjaya dikesan di dalam kultur kalus dimana kalus CA08 dominan dengan asid askorbik dan α-tokoferol manakala CA10 tinggi dengan karotenoid, tiada perbezaan direkodkan untuk kandungan glutathion kultur kalus. Amaun antioksidan bukan enzim adalah lebih tinggi di dalam kultur sel berbanding dengan tisu daun kecuali untuk kandungan karotenoid. CA03 tinggi dengan aktiviti spesifik enzim superoksid dismutase (SOD), katalase (KAT) dan glutathion reduktase (GR), walaubagaimanapun, aktiviti spesifik enzim askorbat peroksida (APX) adalah berkurangan. Selain itu, terdapat perbezaan yang besar pada aktiviti spesifik enzim antioksidan kultur kalus dimana CA09 tinggi dengan aktiviti spesifik enzim SOD dan KAT manakala CA01 dan CA11 menghasilkan aktiviti enzim GR yang lebih tinggi. Sebagai tambahan, aktiviti spesifik enzim APX adalah lebih tinggi di dalam aksesi CA02 dan CA08 berbanding dengan aksesi yang lain. Aktiviti spesifik enzim KAT, GR dan APX adalah lebih tinggi di dalam kultur kalus, keputusan yang berbeza di dapati untuk aktiviti spesifik enzim SOD. Asai untuk jumlah antioksidan, kesan pemerangkapan radikal DPPH dan superoksida menunjukkan kedua-dua pokok induk dan kultur kalus mempunyai aktiviti pemerangkapan yang rendah berbanding kawalan. Hampir kesemua tisu daun menunjukkan kesan pemerangkapan radikal hidroksil yang lebih baik berbanding dengan kultur kalus. Sebagai tindakbalas terhadap rawatan PQ, kandungan klorofil daun berkurangan dengan peningkatan nekrosis. Kandungan hidrogen peroksida di

dalam daun dan kalus CA03 meningkat pada awal fasa rawatan dan semakin berkurangan pada akhir fasa eksperimen, manakala rawatan PQ tidak memberi kesan ke atas paras malondialdehida (MDA) dan kebocoran elektrolit. Corak penghasilan yang berbeza di dapati pada aksesi yang lain. Viabiliti dan pertumbuhan kultur sel semakin menurun dengan peningkatan kepekatan dan masa pendedahan kepada PQ. Keputusan di atas menyimpulkan bahawa CA03 adalah aksesi yang paling tegar dan rintang terhadap rawatan PQ. Kajian selanjutnya mengenai kesan PQ ke atas enzim antioksidan menggunakan CA03 menunjukkan tegasan oksidatif yang dicetuskan oleh PQ meransang penghasilan α-tokoferol dan karotenoid serta aktiviti spesifik enzim KAT dan GR manakala penghasilan asid askorbik dan APX direncatkan. Manipulasi faktor biokimia dan fizikal keadaan pengkulturan berupaya meninggikan penghasilan asid askorbik, α-tokoferol dan karotenoid. Kombinasi 3.0 mg/l 2,4-Dichlorophenoxyacetic acid (2,4-D) dengan 1.0 mg/l kinetin merupakan kombinasi hormon tumbuhan terbaik untuk menggalakkan penghasilan asid askorbik, α-tokoferol dan karotenoid. Kajian terhadap kesan komposisi media menunjukkan media MS yang mengandungi 30-40 g/l sukrosa, dengan penambahan 0.5 hingga 1.0 g/l casein merupakan komposisi media terbaik untuk mengaruh penghasilan kesemua antioksidan yang dikaji. Mempelbagaikan pH media dari 5.1 hingga 5.9 tidak memberi kesan ke atas penghasilan antioksidan. Intensiti cahaya yang tinggi dengan pencahayaan berterusan meninggikan penghasilan asid askorbik dan α-tokoferol manakala kalus yang dieramkan dalam keadaan gelap berupaya meningkatkan kandungan karotenoid. Keputusan kajian di atas menunjukkan bahawa penghasilan

antioksidan boleh diubahsuai dengan memanipulasikan komposisi media, kombinasi hormon dan keadaan persekitaran kultur *in vitro C. asiatica.*

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I certify that a Thesis Examination Committee has met on 12 September 2011 to conduct the final examination of Norhayati Binti Yusuf on her thesis entitled "Antioxidative Constituents of *Centella asiatica* L. Urban" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

NORHAYATI BINTI YUSUF

Date: 12 September 2011

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