EFFECT OF SELECTED BACTERIA ON THE ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF CENTELLA ASIATICA

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THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

INSTITUTE OF BIOLOGICAL SCIENCES
FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR

2012
In the name of God, the most compassionate the merciful

Specially dedicated to:

My kind parents
Nader and Masoumeh
UNIVERSITI MALAYA

ORIGINAL LITERARY WORK DECLARATION

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Name of Degree: Doctor of Philosophy (PhD)

Title of Project Paper/Research Report/Dissertation/Thesis ("this Work"): Effect of selected bacteria on the antibacterial and antioxidant activities of *Centella asiatica*

Field of Study: Microbial Biotechnology

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The role of selected naturally distributed bacteria on the antibacterial and antioxidant activities and production of phenolic compounds in one of the most important Asian medicinal plants namely *Centella asiatica* was investigated based on the effects of beneficial and pathogenic bacteria on the metabolic pathways of plants as reported in literature. Firstly, antibacterial and antioxidant properties and production of phenolic compounds of two subspecies of *C. asiatica* which are commonly used in Malaysia (University of Malaya Herbarium Voucher Specimens: KLU047364 and KLU047552) were evaluated and the most potent of them was chosen for the rest of the study. Different antioxidant assays were used to investigate the antioxidant activities of different samples while a disc diffusion method was applied to measure the antibacterial activity of these samples. Total phenolic contents of different parts of *C. asiatica* were also evaluated using Folin-Ciocalteu method. The selected *C. asiatica* subspecies was then treated with selected pathogenic and beneficial bacteria. Antibacterial and antioxidant activities and production of phenolic compounds in different parts of the plant inoculated with the pathogenic bacteria (*Enterobacter sp.*) were studied at early and late stages of infection. The antibacterial and antioxidant capacities as well as production of phenolic compounds were also investigated in different parts of the selected subspecies of *C. aisiatica* after treatment with the beneficial bacteria (*Pseudomonas sp.*). Production of phenolic compounds in the treated plants was also analyzed using high performance liquid chromatography (HPLC) and then compared to the non-treated plants (controls). Based on the importance of endophytic bacteria and their interactions with the host plant, some endophytic bacteria
associated with *C. asiatica* were also isolated and identified using 16S rDNA sequencing method. Antibacterial and antioxidant potential of the cell-free supernatant of these endophytic bacterial cultures were also evaluated. A multiple callus-subculturing method was used to produce endophytic bacteria-free calli after optimization of an *in vitro* callus induction protocol for *C. asiatica*. The results confirmed the promising antibacterial and antioxidant activities of both examined subspecies of *C. asiatica*. However, the subspecies KLU047364 showed better antibacterial and antioxidant activities compared to KLU047552 and thus selected for the rest of the study. *C. asiatica* in early response to *Enterobacter sp.* infection increased the production of phenolic compounds. Both antibacterial and antioxidant activities of the plant were also enhanced in early stage of infection while the production of phenolic compounds and antioxidant activity of plant was reduced in late stage of infection. Antibacterial and antioxidant activities as well as total phenolics production of *C. asiatica* were increased after treatment with the beneficial *Pseudomonas sp.* bacteria. HPLC results showed irregular changes in amount of phenolic compounds produced in *C. asiatica* after treatment with the bacteria. All isolated endophytic bacteria could inhibit the growth of *Pseudomonas aeruginosa* except *Bacillus gibsonii*. The isolated endophytic bacteria also showed fair antioxidant potentials. The best result for callus production from leaf explant of *C. asiatica* was obtained from the combination of 6-benzylaminopurine at concentration of 3mg/ml and 1-naphthaleneacetic acid at a concentration of 3mg/ml. The multiple callus-subculturing method resulted in 75% endophytes-free callus.
ABSTRAK

ACKNOWLEDGEMENTS

All praises to the Almighty God for affording me the strength and determination to complete this study.

It is my pleasure to express my sincere gratitude and appreciation to my supervisor Associate Professor Dr. Koshy Philip from the Department of Microbiology, Faculty of Science, not only for his guidance and valuable advice but for his constant encouragement and the freedom that he provided to work and think throughout my research.

I am thankful to Prof. Dr. Sekaran Muniandy as my co-supervisor from the Department of Molecular Medicine, Faculty of Medicine for his valuable suggestions and useful discussions during my research.

I am also grateful to Assoc. Prof. Dr. Kamaruzaman Bin Sijam from the Department of Plant Pathology and Mohammad Bagher Javadi from the Department of Agriculture Technology, University Putra Malaysia for kindly providing the Enterobacter sp. and Pseudomonas sp. bacteria respectively.

I would like to thank University of Malaya for the use of laboratory facilities and for providing research grant PS151/2009A.

It's my pleasure to offer my thanks to all my laboratory mates, colleagues and friends especially Wuen Yew Teoh, Jeffery Saravana Kumar, Abdul-Muhsin M. Shami, Arash
Khorasani, Chee Poi Leng, Reza Ebrahimi, Behrooz Banisalam, Rajib Imdadul Haq, and Fazilia Mohd Hatta for maintaining a pleasant research atmosphere and making my stay in Malaysia an enjoyable one.

Last but not least, it is difficult to word my gratitude towards my family members for their encouragement and support during this period. I am grateful to my parents and my brother for supporting me in every possible ways.
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<td>ANOVA</td>
<td>analysis of variance</td>
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<tr>
<td>BAP</td>
<td>6-benzylaminopurine</td>
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<tr>
<td>BHT</td>
<td>Tert-butylated hydroxytoluene</td>
</tr>
<tr>
<td>bp</td>
<td>base pairs</td>
</tr>
<tr>
<td>cfu</td>
<td>colony forming unit</td>
</tr>
<tr>
<td>CRD</td>
<td>completely randomized design</td>
</tr>
<tr>
<td>cv.</td>
<td>cultivar</td>
</tr>
<tr>
<td>ddH₂O</td>
<td>distilled deionized water</td>
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<tr>
<td>DNA</td>
<td>deoxy ribonucleic acid</td>
</tr>
<tr>
<td>DNMRT</td>
<td>Duncan New Multiple Range Test</td>
</tr>
<tr>
<td>DPPH</td>
<td>2,2 –diphenyl-1-picrylhydrazil</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylene diamine tetra acetic acid</td>
</tr>
<tr>
<td>et al.</td>
<td>et alia</td>
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<tr>
<td>IAA</td>
<td>indole-3-acetic acid</td>
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<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>µmol m² s⁻¹</td>
<td>micromole per meter square per second</td>
</tr>
<tr>
<td>MS</td>
<td>Murashige and Skoog</td>
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<tr>
<td>NAA</td>
<td>a-naphthaleneacetic acid</td>
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<td>OD</td>
<td>optical density</td>
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<td>PCR</td>
<td>polymerase chain reaction</td>
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<td>pH</td>
<td>-log [H⁺]</td>
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<tr>
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<td>Full Form</td>
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<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
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<tr>
<td>rpm</td>
<td>revolutions per minute</td>
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<td>SOD</td>
<td>superoxide dismutase</td>
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<td>sp.</td>
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<td>UV</td>
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