DETERMINATION OF FLAVONOIDS IN *Centella asiatica* (L.) URBAN AND THEIR UTILIZATION IN HERBAL NOODLE

By

MOHAMAD KHAIRI MOHD ZAINOL

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

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Dedicated especially to……

Mak and Ayah
Kak Ya, Kak Chik, Kakak and Adik,
Kak Na and Abang Zaini
Abang Naim and Kak Liza
Zamzahaila Mohd Zin
and last but not least
Nur Fatin Aliya

Thank you …..

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'Pegaga' or *Centella asiatica* has been medically recognized to be effective in the treatment of skin diseases and many other illnesses. Due to the presence of abundant amount of active compounds, *C. asiatica* is potentially a good source to alleviate or prevent the development of chronic diseases, cancer and others. The objective of this study was to develop and optimize a functional food namely *C. asiatica* herbal noodle containing flavonoids from *C. asiatica.* The study included determination and quantification of flavonoids (bioactive compounds) and selection of the best formulation using RSM to develop the product containing *C. asiatica.* The first stage of the study was conducted to evaluate the highest antioxidative activity and total phenolic compounds in different parts namely leaf, root and petiole among four different accessions of *C. asiatica* namely Ca 10, CA 05 Ca 08 and CA 11 with the objective to determine the best accession that would then be used in the developed products. Methanol was used as an extraction solvent and antioxidative assay was biochemically done using ferric thiocyanate method (FTC) and thiobarbituric acid test (TBA) while total phenolic compounds (TPC) was determined using the Folin-

Ciocalteu phenol method. Results from FTC and TBA test showed that no significant (p<0.05) difference was exhibited in antioxidative activity between leaf extract of *C. asiatica* especially CA 05 as compared to that of α-tocopherol and BHT while petiole extracts showed somewhat negligible activity. On the other hand, the antioxidative activities measured correlated well with total phenolic compounds (TPC) for leaf extract of CA 05. Therefore leaf of CA 05 was used for further analysis to determine the compound responsible for the high antioxidative activities. The results also suggested that more than one compound attributed to antioxidative activity of different parts of *C. asiatica* and flavonoids are one of phenolic compounds that were known to be potent antioxidant that might be responsible for the antioxidative activities in *C. asiatica*. Further study was done to determined the stability of flavonoids (catechin, quercetin and rutin) during drying and to identify the optimum drying method that could sustained the most flavonoids in *C. asiatica* which were then incorporated into food product. Reverse-phase high-pressure liquid chromatography (RP-HPLC) with a Symmetry C_{18} column (Waters, USA) and a water/methanol mobile phase was used to determine individual flavonoids in leaf *C. asiatica* CA 05. The results showed that vacuum oven drying method was the optimum treatment among the different drying techniques based on factors of cost and yield of flavonoids sustained. The next area of study was focused on incorporating *C. asiatica* into the product, analyzed for bioactive compound present in it and tested for consumer acceptance. Response surface methodology (RSM) using Central Composite Design with quadratic model was used to in the product formulation, development and optimization. Three independent variables were chosen (*C. asiatica* extract, salt and sodium hydroxide or 'air abu') in the development and optimization of the product. Finally, the products were analyzed

for the presence of flavonoids and the sensory evaluation (dependent variables). *C. asiatica* herbal noodles containing pegaga extract (formulation of: 10% *C. asiatica* extract, 5g salt and 5g 'air abu') was the most optimum product based on the dependent variables collected. This research would then be able to serve as reference for new products in the food industry and wider the selection of consumption of natural products.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGENALPASTIAN BAHAN FLAVONOID DARI *Centella asiatica* (L.) URBAN DAN PENGGUNAANNYA DALAM MEE HERBA

Oleh

MOHAMAD KHAIRI MOHD ZAINOL

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'Pegaga' atau *Centella asiatica* telah dikenali sebagai tumbuhan yang efektif untuk mengubati penyakit kulit, hipertensi dan lain-lain. Kajian ini dijalankan untuk menghasilkan makanan berfungsi yang mengandungi flavonoid daripada *C. asiatica.* Kajian ini mencangkupi penentuan dan mengenalpastian kuantiti flavonoid di dalam *C. asiatica* serta pemilihan formulasi terbaik untuk menghasilkan makanan berfungsi tersebut. Peringkat awal kajian telah melibatkan penentuan tahap aktiviti antioksida dan kandungan bahan fenol dalam daun, akar dan batang pegaga daripada empat jenis pegaga yang berbeza iaitu CA 01, CA 05, CA 08 dan CA 11, untuk mengenalpasti bahagian dan jenis pegaga terbaik yang akan digunakan dalam penghasilan produk herba iaitu mee herba pegaga. Analisis antioksida dijalankan dengan menggunakan kaedah tiocianat ferik (FTC) dan ujian asid tiobarbiturik (TBA). Aktiviti antioksida daripada ekstrak-ekstrak ini kemudiannya akan dibandingkan dengan antioksidaan piawai α-tokoferol dan toluena hidroksida butilat (BHT). Manakala kandungan bahan fenol (TPC) dapat ditentukan dengan

menggunakan kaedah Folin-Ciocalteu fenol. Keputusan daripada kaedah FTC and TBA menunjukkan tiada perbezaan yang signifikan (p<0.05) ditunjukkan dalam aktiviti antioksida antara ekstrak daun pegaga terutamanya CA 05 berbanding α-tokoferol dan BHT. Selain itu, aktiviti antioksida yang telah diukur juga menunjukkan korelasi yang baik dengan kandungan bahan fenol (TPC) terutamanya untuk bahagian daun CA 05. Keputusan ini mencadangkan daun CA 05 boleh digunakan sebagai sumber bahan aktif dalam penghasilan makanan berfungsi. Selain itu, didapati bahan fenol juga mungkin bertangungjawab dalam aktiviti antioksida yang tinggi dalam pegaga terutamanya didalam daun CA 05. Kajian ini mencadangkan bahawa kemungkinan lebih daripada satu bahan aktif yang bertanggungjawab dalam aktiviti antioksida didalam bahagian-bahagian berbeza pegaga manakala flavonoid, bahan fenolik yang diketahui mempunyai tahap aktiviti antioksidaan yang tinggi, berkemungkinan merupakan bahan yang paling memainkan peranan dalam aktiviti antioksidaan *C. asiatica*. Kajian seterusnya melibatkan tahap kestabilan flavonoid (katekhin, kuersetin dan rutin) di dalam pegaga terhadap pengeringan dan mengenalpasti teknik pengeringan yang dapat meminimakan kehilangan flavonoid, yang seterusnya akan dimasukkan kedalam produk. Kromatografi cecair tekanan tinggi fasa terbalik (RP-HPLC) dengan kolum Symmetry C_{18} (Waters, USA) dan fasa gerak air/metanol telah digunakan untuk mengenalpasti kehadiran flavonoid didalam daun *C. asiatica* CA 05. Pengeringan dengan bervakum dikenalpasti sebagai teknik terbaik untuk pengeringan daun pegaga dalam usaha untuk mengurangkan kehilangan flavonoid berdasarkan kepada faktor kos dan banyaknya hasil. Kajian seterusnya melibatkan penghasilan makanan berfungsi (mee herba) yang ditambah bahan aktif daripada *C. asiatica* dan seterusnya menganalisa kandungan biomarkernya dan juga tahap penerimaan pengguna. Kaedah

metodologi respon permukaan (RSM) menggunakan teknik *Central Composite Design* (CCD) dengan persamaan quadratik digunakan dalam formulasi, penghasilan dan pengoptimunan produk. Tiga pembolehubah tetap telah dipilih iaitu eksrak *C. asiatica*, garam and natrium hidroksida atau 'air abu') dalam penghasilan mee herba pegaga tersebut. Seterusnya, produk yang telah disediakan dianalisa untuk mengenalpasti kandungan bahan aktif, dan kajian ujirasa terhadap produk-produk yang dihasilkan telah dijalankan untuk mengukur kandungan nutrien dan tahap penerimaan produk. Berdasarkan ciri-ciri fizikal dan sensori mee yang dianalisa, mee yang menggunakan formulasi (10% ekstrak pegaga, 5g garam dan 5g air abu) menunjukkan hasil yang optima berdasarkan kepada data-data daripada ujikaji (analisis biomarker dan tahap penerimaan pengguna). Kajian ini seterusnya boleh dijadikan sebagai rujukan dalam usaha menghasilkan produk-produk baru dalam industri makanan terutamanya produk herba.

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I certify that an Examination Committee met on 28th August 2004 to conduct the final examination of Graduate Student on her Master of Science thesis entitled "Determination of flavonoids in *Centella asiatica* (L.) Urban and their utilization in herbal noodle" 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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DECLARATION

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 degree at UPM or other institutions.

_____

MOHAMAD KHAIRI MOHD ZAINOL

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