



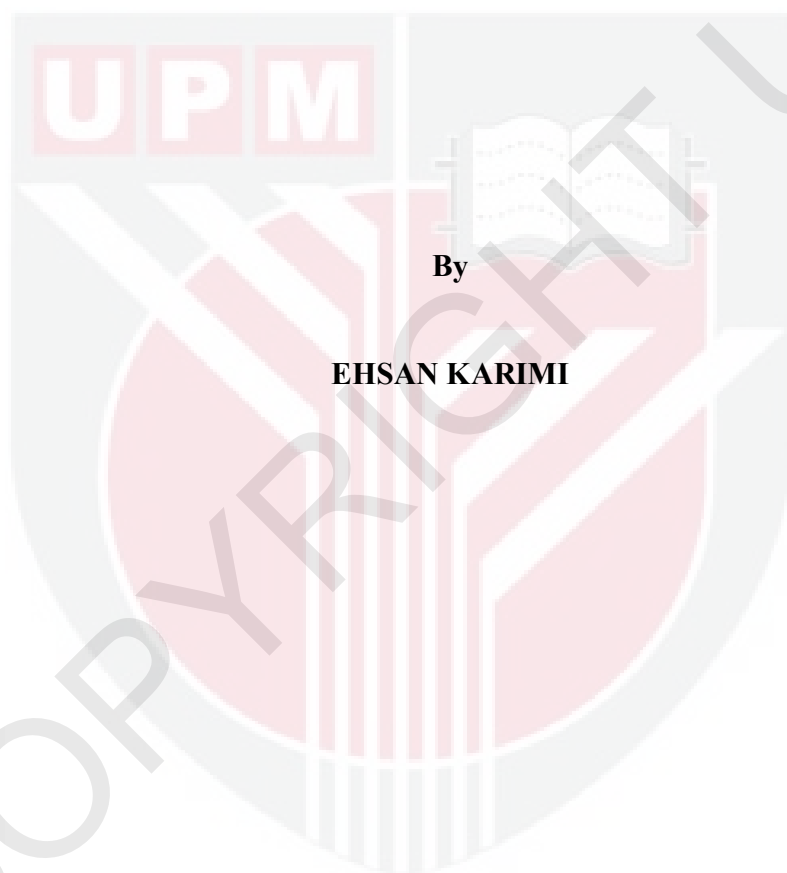
UNIVERSITI PUTRA MALAYSIA

**PHYTOCHEMICAL ANALYSES AND BIOLOGICAL ACTIVITIES OF
DIFFERENT PARTS OF THREE VARIETIES OF *LABISIA PUMILA* BENTH.**

EHSAN KARIMI

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**PHYTOCHEMICAL ANALYSES AND BIOLOGICAL ACTIVITIES OF
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By

EHSAN KARIMI

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

December 2011

DEDICATIONS

This thesis is dedicated to my:

My dear mother (Zahra) and father (Ali Asghar)

With innermost and everlasting affection and love.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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DIFFERENT PARTS OF THREE VARIETIES OF *LABISIA PUMILA* BENTH.**

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December 2011

Chairman : Associate Professor Hawa Binti Jaafar, PhD

Faculty : Agriculture

Labisia pumila (Myrsinaceae), also known as "Kacip Fatimah" (KF), has been used by many generations to induce and facilitate childbirth. There are three varieties of KF in Malaysia, namely, *L. pumila* var. *alata*, *L. pumila* var. *pumila* and *L. pumila* var. *lanceolata*. Recently, researches have demonstrated its estrogenic activity and high concentration of phenols. Due to ethnopharmacological application and lack of scientific information about bioactive compounds and possible biological activities of *Labisia pumila* Benth. with the potential to be applied in pharmaceutical, cosmetic and food industries, this study was conducted to evaluate the leaf, stem and root parts of three varieties of *L. pumila* for the presence of flavonoids, phenolics and antioxidant activities in extracts obtained by three solvents (methanol, ethyl acetate and water) with different polarities. The antimicrobial properties of extracts were assessed in reflux and microwave obtained extracts. Furthermore, qualitative and quantitative compositions of flavonoids

and phenolics in all the extracts were analyzed using High-performance liquid chromatography (HPLC) system. Apart from that the anti-inflammatory and anticancer activities were determined in microwave methanolic extract. Finally, different levels of glasshouse irradiance were tested in order to examine possible changes in the total phenolic and flavonoid contents and antioxidant activities of all varieties of *L. pumila*. Similar results were obtained from two different methods (reflux and microwave) of extraction on biological activities but the microwave result was more effective to increase the yield in a shorter period while at the same time, using less solvent compared to the reflux extraction. Meanwhile, in all the three varieties of *L. pumila* Benth. the methanolic extract showed higher contents of phenolics and flavonoids as compared to ethyl acetate and boiling water extract. Results on the phenolics and flavonoids contents in the leaves, stems and roots of the three varieties showed significant difference ($p < 0.05$). In all the varieties, the leaves contained higher phenolics and flavonoids as well as higher antioxidant activity compared to the roots and stems. Antioxidant activities of all varieties also showed significant difference ($p < 0.05$) by DPPH and FRAP analyses. *L. pumila* var. *alata* contained higher antioxidants compared to var. *pumila* followed by var. *lanceolata*. Meanwhile, methanolic extract from the leaf of *L. pumila* var. *pumila* had higher total flavonoids content (1.53 and 2.77 mg rutin equivalent /g dry weight (DW) than *L. pumila* var. *alata* (1.32 and 2.49 mg rutin equivalent /g DW) and var. *lanceolata* (1.28 and 2.29 mg rutin equivalent /g DW) while the leaf of *L. pumila* var. *alata* recorded higher total phenolics content (2.65 and 3.48 mg gallic acid equivalent (GAE)/g DW) than *L. pumila* var. *pumila* (2.56 and 3.37 mg GAE/g DW) and var. *lanceolata* (2.43 and 3.23 mg GAE/g DW) using both the reflux and microwave extractions methods,

respectively. High performance liquid chromatography (HPLC) analyses of phenolics and flavonoids in all three varieties revealed the presence of gallic acid, caffeic acid, rutin, quercetin, kaempferol and myricetin in all plant parts. Crude methanolic extract of all varieties had exhibited antibacterial activities against both Gram positive (*Micrococcus luteus*, *Bacillus subtilis* B145, *Bacillus cereus* B43, *Staphylococcus aureus* S1431) and Gram negative (*Enterobacter aerogenes*, *Klebsiella pneumonia* K36, *Escherichia coli* E256, *Pseudomonas aeruginosa* PI96) pathogens at low concentrations but with lower activity than kanamycin. The antifungal activity of methanolic extracts of all varieties against *Fusarium* sp., *Candida* sp. and *Mucor* sp. using the agar diffusion disc was moderate compared to streptomycin. The anti-inflammatory activity was determined using microwave extract of the leaf and root by *in vitro* nitric oxide (NO) inhibitory activities in a murin macrophage cell line (RAW 264.7). The results demonstrated strong inhibitory activity in NO inhibition by all varieties. Cell exposed to the extracts showed viability with a range of 50 to 90%. *In vitro* anticancer activities of extracts against two human cancer cell lines (MCF-7; MDA-MB-231) and Chang cell as a normal human hepatocyte could be categorized as moderate to weak by leaf and root methanolic extracts. The compounds present in the extracts were non-toxic, which render them as suitable potential therapeutics to be developed into anticancer drug. Exposure to different levels of light intensity had affected the phenolics, flavonoids and antioxidant activity of *L. pumila* where the highest concentration and activity were demonstrated under high irradiance of 630 compared to 310 $\mu\text{mol m}^{-2} \text{s}^{-1}$, suggesting that the efficacy of *L. pumila* can be altered and enhanced under increasing light intensity. The highest amount of these components accumulated mainly in the leaves followed by the roots and then the stems.

In conclusion, three varieties of *L. pumila* Benth. are a potential source of bioactive compounds endowed with useful biological activities such as antioxidant, antimicrobial, anti-inflammatory and anticancer properties.



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

**ANALYSIS FITOKIMIA DAN AKTIVITI BIOLOGI BAHAGIAN-BAHAGIAN
BERBEZA TIGA VARIETI LABISIA PUMILA BENTH.**

Oleh

EHSAN KARIMI

Disember 2011

Pengerusi : Profesor Madya Hawa Binti Jaafar, PhD

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Labisia pumila (Myrsinaceae), juga dikenali sebagai “Kacip Fatimah”(KF), telah digunakan oleh banyak generasi untuk mempercepatkan dan memudahkan proses bersalin. Terdapat tiga varieti KF di Malaysia iaitu dengan nama *L. pumila* var. *alata*, *L. pumila* var. *pumila* and *L. pumila* var. *lanceolata*. Baru-baru ini, pengkaji telah menunjukkan aktiviti estrogenik dan kepekatan fenol yang tinggi di dalamnya. Disebabkan oleh aplikasi etnofarmokologikal dan kekurangan maklumat saintifik berkenaan dengan sebatian bioaktif dan aktiviti-aktiviti biologi *Labisia pumila* Benth. tambahan lagi ianya berpotensi untuk diaplikasikan dalam industri farmaseutikal, kosmetik dan makanan; kajian ini telah dijalankan untuk menilai bahagian-bahagian daun, batang dan akar pada ketiga-tiga jenis *L. pumila* dalam menentukan kehadiran flavonoid, fenolik dan aktiviti antioksidan dalam ekstrak yang diperoleh dari ketiga-tiga jenis pelarut (metanol, etil asetat dan air) dengan kutub yang berlainan. Ciri-ciri anti mikrobal juga dinilai dalam ekstrak yang diperoleh dari refluks dan gelombang mikro.

Selanjutnya, kualitatif dan kuantitatif komposisi flavanoid dan fenolik dalam semua ekstrak telah dianalisis dengan menggunakan sistem RP-HPLC. Selain dari itu, anti radang dan anti kanker juga ditentukan dalam ekstrak gelombang mikro metanolik. Akhir sekali, tahap perbezaan iridiasi rumah kaca telah diuji bagi menentukan kesan iridiasi pada pengumpulan fenolik dan flavonoid serta aktiviti antioksidan bagi kesemua varieti *L. pumila*. Keputusan yang hampir sama telah diperoleh dari dua kaedah pengestrakan yang berbeza (refluks dan gelombang mikro) pada aktiviti biologi; namun keputusan gelombang mikro adalah lebih efektif dalam meningkatkan hasil pada jangka masa yang lebih pendek; pada masa yang sama penggunaan pelarut adalah kurang dibandingkan dengan pengekstrakan refluks. Sementara itu, dalam ketiga-tiga varieti *L. pumila* Benth., ekstrak metanolik menunjukkan kandungan fenolik dan flavonoid yang tinggi jika dibandingkan dengan pengekstrakan menggunakan etil esetat dan air didihan. Keputusan kandungan fenolik dan flavanoid dalam daun, batang dan akar bagi ketiga-tiga varieti *L. pumila* menunjukkan perbezaan signifikan ($p < 0.05$). Dalam kesemua varieti, daun mengandungi lebih banyak fenolik, flavanoid dan aktiviti antioksidan berbanding dengan batang dan akar. Aktiviti antioksidan untuk kesemua varieti juga menunjukkan perbezaan signifikan ($p < 0.05$) bagi analisis DPPH dan FRAP. *L. pumila* var. *alata* mengandungi lebih banyak antioksidan jika dibandingkan dengan var. *pumila* kemudian diikuti dengan var. *lanceolata*. Pada masa yang sama, ekstrak metanolik dari daun *L. pumila* var. *pumila* mempunyai jumlah kandungan flavonoids yang lebih tinggi (1.53 dan 2.77 mg rutin bersamaan (E)/g DW) berbanding *L. pumila* var. *alata* (1.32 dan 2.49 mg rutin E/g DW) dan var. *lanceolata* (1.28 dan 2.29 mg rutin E/g DW) walaupun daun *L. pumila* var. *alata* mempunyai total kandungan fenolik yang lebih tinggi (2.65 dan 3.48

mg Galic acid bersamaan (GAE)/g DW) berbanding *L. pumila* var. *pumila* (2.56 dan 3.37 mg GAE/g DW) dan var. *lanceolata* (2.43 dan 3.23 mg GAE/g DW) masing-masing dengan menggunakan kaedah pengekstrakan refluks dan gelombang mikro. Analisis HPLC bagi fenolik dan flavanoid dalam ketiga-tiga varieti menunjukkan kehadiran asid galik, asid caffeic, rutin, quercetin, kamperol dan myricetin dalam semua bahagian pokok. Ekstrak mentah metanolik bagi kesemua varieti menunjukkan kesan aktiviti anti bakteria terhadap kedua-dua patogen gram positif (*Micrococcus luteus*, *Bacillus subtilis* B145, *Bacillus cereus* B43, *Staphylococcus aureus* S1431) dan gram negatif (*Enterobacter aerogenes*, *Klebsiella pneumonia* K36, *Escherichia coli* E256, *Pseudomonas aeruginosa* PI96) adalah pada kepekatan yang rendah; dalam masa yang sama juga aktiviti adalah lebih rendah daripada kanamaisin. Kesan aktiviti anti kulat ekstrak metanolik bagi kesemua jenis varieti terhadap *Fusarium* sp, *Candida* sp. dan *Mucor* sp. dengan menggunakan cakera resapan agar adalah sederhana berbanding dengan streptomisin. Aktiviti anti radang ditentukan dengan menggunakan gelombang mikro yang diperoleh dari ekstrak daun dan akar dengan nitrik oksida (NO) invitro; aktiviti perencatan dalam garisan sel makrofaj murin (RAW 264.7). Keputusan menunjukkan semua varieti mempunyai aktiviti perencatan yang kuat dalam perencatan NO. Dedahan sel terhadap ekstrak menunjukkan viabiliti dalam julat antara 50 dan 90%. Kesan aktiviti anti kanser in vitro ekstrak terhadap dua garisan sel kanser manusia (MCF7; MDA-MB-231) dan sel Chang sebagai hepatocyte manusia normal; boleh dikategorikan sebagai sederhana kepada lemah dengan menggunakan metanolik ekstrak bagi daun dan akar. Sebatian yang terdapat dalam ekstrak adalah tidak toksik; menyebabkan ia sesuai sebagai therapeutik yang berpotensi untuk berkembang menjadi

ubat antikanser. Pendedahan kepada keamatan cahaya yang berbeza memberi kesan kepada fenolik, flavanoid dan aktiviti antioksidan bagi *L. pumila* di mana kepekatan dan aktiviti paling tinggi ditunjukkan di bawah iridiasi yang tinggi $630 \mu\text{mol m}^{-2} \text{s}^{-1}$ jika dibandingkan dengan $310 \mu\text{mol m}^{-2} \text{s}^{-1}$, ini mencadangkan bahawa kemujaraban *L. pumila* boleh diubah dan ditingkatkan di bawah keamatan cahaya yang tinggi. Kandungan komponen yang terkumpul adalah paling tinggi terutamanya dalam daun diikuti dengan akar dan batang. Secara kesimpulannya, ketiga-tiga varieti *L. pumila* Benth adalah merupakan sumber sebatian bioaktif yang berpotensi dan berkeupayaan dalam aktiviti-aktiviti biologi seperti antioksidan, anti radang dan ciri-ciri anti kanser.

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Finally, I wish specially thank to my dear mother and father for their spiritual, financial and moral support.

Sincerely

EHSAN KARIMI

I certify that a Thesis Examination Committee has met on September 2011 to conduct the final examination of Ehsan Karimi on his thesis entitled "**PHYTOCHEMICAL ANALYSIS AND BIOLOGICAL ACTIVITIES OF DIFFERENT PARTS OF THREE VARIETIES OF *LABISIA PUMILA BENTH.*** " 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 relevant degree of Doctor of Philosophy.

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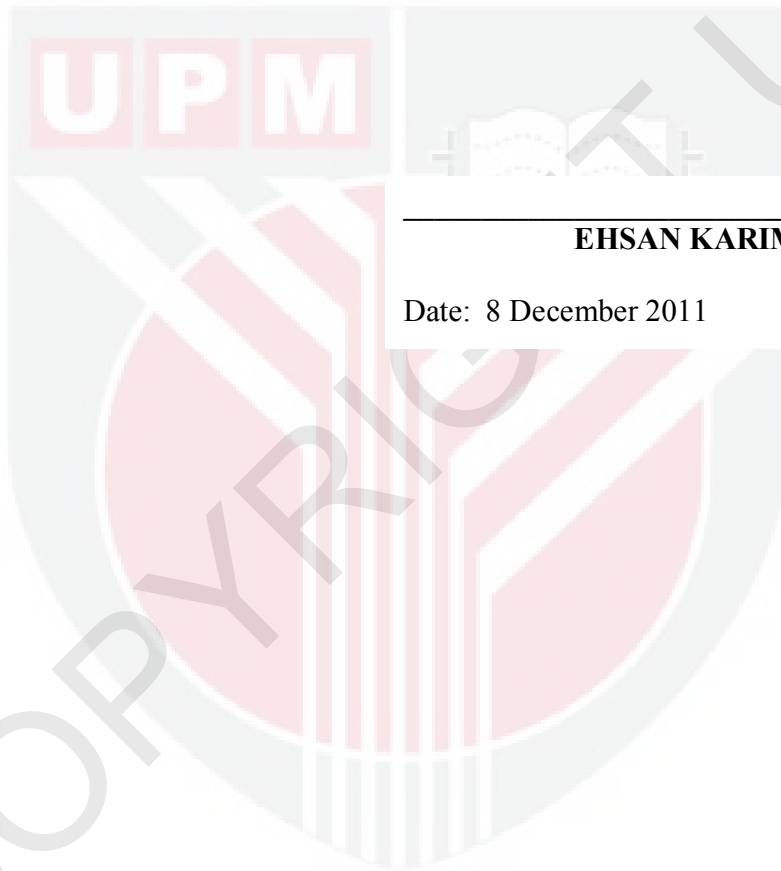
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DECLARATION

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 other degree at University Putra Malaysia or at any other institution.



EHSAN KARIMI

Date: 8 December 2011

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