

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfillment of the requirement for the degree of Doctor of Philosophy

**EFFECTS OF HONEY ON FACTORS RELATING TO  
CARDIOVASCULAR DISEASE BASED ON  
IN VITRO AND IN VIVO STUDIES**

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Along history, honey has been used not only as a nutrient, but also as a medicine. Honey is rich in phenolic compounds, which act as natural antioxidants and are increasingly popular due to their potential in improving the human health. Thus, this study was designed to analyse some physico-chemical characteristics of two different types of honey, namely honey bee honey (Acacia) and stingless bee honey (Kelulut), to study the anti-inflammatory effects of polyphenols on the atherosclerosis modulation in human umbilical vein endothelial cells (HUVECs) and investigate the effects of these two honey for supplementation in subjects with cardiovascular risk factor. Ash, colour, water activity, viscosity, Brix, moisture, acidity, hydroxymethyl furfural (HMF), diastase activity and sugar analyses of the honey samples were assessed for chemical characterisation. Total phenolic compound, ferric reducing antioxidant capacity (FRAP) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity were measured as antioxidant determinants. Acacia honey in this study showed significantly higher content of colour (redness and yellowness), viscosity, Brix, sugar content, FRAP and total phenolic compared to those of Kelulut honey. The results also showed that Acacia honey had significantly lower content of water activity, moisture, acidity, HMF and diastase activity as compared to Kelulut honey. In this study on anti-inflammatory effects of polyphenols on the atherosclerosis modulation in HUVECs, secondary preventive effects of polyphenols (caffeic acid phenyl ester [CAPE], kaempferol and quercetin) and their mixture were

investigated via inhibition of ICAM-1 and VCAM-1 expressions in (HUVECS) through enzyme-linked immunosorbent assay (ELISA) at 5, 10, 25, 50 and 100  $\mu$ M with 24, 48 and 72 incubation period for a single compounds and ratios of 2:1, 1:1, 1:2 for the mixtures. Result showed that mixture of kaempferol and quercetin significantly enhance the anti-inflammatory effects by suppressing the ICAM-1 and VCAM-1 expressions up to 132% and 101%, respectively. Based on the intervention study in subjects with cardiovascular risk factors, it was revealed that 10 g/day Acacia honey supplementation significantly decreased blood glucose, total cholesterol, TG, LDL-c by 7.1%, 7.6%, 16% and 5.4%, respectively. Meanwhile, 30 gram per day of Acacia honey supplementation significantly decreased blood glucose (7.1%), total cholesterol (4.9%) and LDL-c (12.5%). On the other hand, Kelulut honey supplementation only showed beneficial effects in high doses (30 g/day) by decreasing the total cholesterol (4.8%) significantly. Based on the lipid profile data, it can be concluded that Acacia honey intervention at 10 g/day exhibits better lipid lowering action. Thus, this study concluded that low dose of Acacia honey can be proposed as a nutritional dietary supplementation for those with cardiovascular disease risk modulation.

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**KESAN MADU KE ATAS FAKTOR-FAKTOR YANG BERKAIT DENGAN  
PENYAKIT KARDIOVASKULAR BERDASARKAN KAJIAN *IN VITRO*  
DAN *IN VIVO***

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Madu lebah digunakan sejak berzaman bukan sahaja sebagai makanan, tetapi juga sebagai ubat. Madu kaya dengan kandungan fenolik yang bertindak sebagai antioksidan semulajadi dan popular dalam meningkatkan kesihatan manusia. Oleh itu, kajian ini dijalankan untuk menganalisis beberapa sifat-sifat fizikokimia dari dua jenis madu iaitu madu lebah (Akasia) dan madu lebah tanpa sengat (Kelulut), mengkaji kesan anti-inflamasi polifenol dalam modulasi aterosklerosis dalam *human umbilical vein endothelial cells* (HUVECs) dan mengkaji kesan suplementasi kedua-dua madu lebah ini kepada subjek yang mempunyai faktor risiko penyakit kardiovaskular. Sifat-sifat fizikokimia dijalankan melalui analisis abu, warna, aktiviti air, kepekatan, Brix, kelembapan, keasidan, hidroksimetil furfural (HMF), aktiviti *diastase* dan analisis gula. Manakala komponen fenolik, kapasiti antioksidan (FRAP) dan aktiviti pengurangan radikal bebas 2,2-diphenyl-1-picrylhydrazyl (DPPH) dijalankan sebagai penentu antioksidan. Dalam kajian ini, madu Akasia mempunyai kandungan warna (kemerahan dan kekuningan), kepekatan, Brix, kandungan gula, FRAP dan kandungan fenolik yang tinggi secara signifikan berbanding madu Kelulut. Keputusan juga menunjukkan bahawa Akasia mempunyai kandungan aktiviti air, kelembapan, keasidan, HMF dan *diastase* yang rendah secara signifikan berbanding Kelulut. Dalam kajian kesan anti-inflamasi polifenol terhadap modulasi aterosklerosis dalam HUVECs, kesan pencegahan sekunder polifenol (*caffeic acid phenyl ester* [CAPE], *kaempferol* dan *quercetin*) dan campuran polifenol dijalankan

melalui pengurangan ekspresi ICAM -1 dan VCAM-1 dalam sel HUVECS dengan menggunakan penetapan kadar absorben taut-enzim (ELISA) pada 5, 10, 25, 50 dan 100  $\mu$ M dengan masa inkubasi selama 24, 48 dan 72 jam untuk sebatian tunggal dan nisbah 2: 1, 1: 1, 1: 2. Keputusan menunjukkan bahawa campuran kaempferol dan quercetin dapat meningkatkan kesan anti-inflamasi dengan menurunkan ekspresi ICAM-1 dan VCAM-1 sebanyak 132% dan 101% secara signifikan. Berdasarkan kajian terhadap subjek yang mempunyai faktor risiko penyakit kardiovaskular, didapati bahawa 10g/ hari madu Akasia berjaya menurunkan masing-masing kandungan glukosa darah, kandungan kolesterol, TG, LDL-c sebanyak 7.1%, 7.6%, 16% dan 5.4%, secara signifikan. Manakala 30 gram sehari pemberian madu Akasia juga mengurangkan glukosa darah (7.1%), kandungan kolesterol (4.9%) dan LDL-c (12.5%) secara signifikan. Madu Kelulut hanya menunjukkan kesan dalam dos yang tinggi (30g/ hari) dengan menurunkan kandungan kolesterol (4.8%) secara signifikan. Berdasarkan data profil lipid, dapat disimpulkan bahawa madu Akasia pada 10g/ hari menunjukkan penurunan profil lipid yang lebih baik. Sebagai kesimpulan, dos madu Akasia yang rendah boleh dicadangkan sebagai suplemen pemakanan bagi mereka yang mempunyai risiko penyakit kardiovaskular.