

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfillment of the requirements for the degree of Master of Science

***IN VITRO AND IN VIVO ANTI-INFLAMMATORY ACTIVITIES OF
Hibiscus tiliaceus LEAVES EXTRACT***

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2020

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Inflammation has become the focus of global scientific research because of its implication in virtually all human and animal diseases. The anti-inflammatory drugs such as corticosteroids and non-steroidal anti-inflammatory drugs (NSAID) have their side effects. Therefore, the anti-inflammatory drugs from the natural product had been researched in this study. This study aimed to profile and determines the chemical constituents of *H. tiliaceus* leaves extract and fractions as well to determine the anti-inflammatory activities of methanol extract of *Hibiscus tiliaceus* leaves (HM) *in vitro* on lipopolysaccharide (LPS) stimulated RAW264.7 cell lines and *in vivo* using formalin-induced paw edema. HM was obtained by extraction process. Further fractionation of HM was done by using solvent-solvent partitioning and yielded four solvent fractions; hexane fraction (HH), dichloromethane fraction (HD), ethyl acetate fraction (HE) and water fraction (HW) of *Hibiscus tiliaceus* leaves. Antioxidant potency of HM and *H. tiliaceus* leaves fractions was tested by DPPH bio-autographic assay using thin layer chromatography (TLC) plate and 1, 1-

diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging at different concentrations ranging from 0.2 to 10 mg/ml. The antibacterial activity was determined using disc diffusion method against Gram-positive and negative bacteria. The inhibition zones of HM and *H. tiliaceus* leaves fractions were measured. The cytotoxicity effect of the extract on RAW264.7 cell lines was evaluated by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. *In vitro* of anti-inflammatory study, the reduction of nitric oxide (NO) production was observed by Griess reagent in LPS stimulated RAW264.7 cell lines. Meanwhile, *in vivo* study, the hind paws of 24 Sprague-Dawley rats were injected with 2.5% of formalin (100 μ L) solution. Then, the rats were treated with 100 mg/kg of HM and Ibuprofen (NSAID). The formalin-induced paw edema was determined by measuring the diameters of the injected paws. Results showed that HM and *H. tiliaceus* leaves fractions showed high antioxidant activity with the IC₅₀ values range from 0.6 to 1.95 mg/ml compare with Quercetin (positive control) at 0.2 mg/ml. Furthermore, HM and all fractions did not possess any antibacterial activity. Based on cytotoxicity results, HM and all fractions were non-toxic against RAW264.7 cell lines with the IC₅₀ > 30 μ g/mL and selected for anti-inflammatory assay. HM and all fractions also showed anti-inflammatory activity by reducing of NO production more than 80% compare to positive control, arginine. It is clearly indicated that *H. tiliaceus* leaves have anti-inflammatory potential agents against RAW264.7 cell lines. The *in vivo* study demonstrated that 100 mg/kg of HM have slightly reduction of formalin-induced rat edema. The findings clearly showed that *H. tiliaceus* leaves are safe and has potential as alternative drugs for inflammation disease.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu
sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

**AKTIVITI ANTI-RADANG EKSTRAK DAUN *Hibiscus tiliaceus* SECARA IN
VITRO DAN IN VIVO**

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Keradangan telah menjadi tumpuan penyelidikan saintifik global kerana implikasinya dalam hampir semua penyakit manusia dan haiwan. Ubat anti-radang seperti kortikosteroid dan ubat anti-radang bukan steroid (NSAID) mempunyai kesan sampingannya. Oleh itu, ubat-ubatan anti-radang dari produk semulajadi telah dikaji dalam kajian ini. Kajian ini bertujuan untuk membuat profil dan menentukan unsur kimia ekstrak dan pecahan daun *H. tiliaceus* serta menentukan aktiviti anti-radang ekstrak metanol daun *H. tiliaceus* (HM) *in vitro* pada “lipopolysaccharide” (LPS) yang dirangsang sel RAW264.7 dan *in vivo* menggunakan Eodema kaki yang dihasilkan oleh formalin kepada daun *Hibiscus tiliaceus*. HM diperolehi melalui proses pengekstrakan. Fraksinasi selanjut HM telah dilakukan menggunakan pembahagian pelarut dan menghasilkan empat pecahan pelarut; pecahan hexane (HH), pecahan diklorometan (HD), pecahan etil asetat (HE), dan pecahan air (HW).

Potensi antioksidan HM dan pecahan pelarut daun *H. tiliaceus* telah diuji oleh ujian “DPPH bio-autographic assay” menggunakan plat kromatografi lapisan nipis (TLC) dan 1, 1-diphenyl-2-picrylhydrazyl (DPPH) pembebasan radikal bebas pada kepekatan yang berbeza antara 0.2 hingga 10 mg/ml. Aktiviti antibakteria telah ditentukan menggunakan kaedah “disc diffusion” terhadap bakteria Gram-positif dan negatif. Zon perencatan HM dan pecahan daun *H. tiliaceus* dicatat. Kesan sitotoksiti ekstrak pada sel RAW264.7 telah dinilai dengan menggunakan 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT assay). Dalam kajian anti-radang, secara *in vitro* pengurangan pengeluaran nitrik oksida (NO) dilakukan menggunakan “Griess reagent” dalam LPS yang merangsang sel RAW 264.7. Sementara itu, dalam kajian secara *in vivo*, tapak kaki 30 ekor tikus Sprague-Dawley disuntik dengan 2.5% larutan formalin (100 μ L). Kemudian, tikus dirawat dengan 100 mg/kg HM dan Ibuprofen (NSAID). Edema kaki yang dihasilkan oleh formalin ditentukan dengan mengukur diameter kaki yang disuntik. Keputusan menunjukkan bahawa HM dan pecahan daun *H. tiliaceus* menunjukkan aktiviti antioksidan yang tinggi dengan nilai IC_{50} diantara 0.6 hingga 1.95 mg/ml berbanding dengan Quercetin (kawalan positif) pada 0.2 mg/ml. Selain itu, HM dan semua pecahan tidak mempunyai sebarang aktiviti antibakteria. Berdasarkan hasil sitotoksiti, HM dan semua pecahan adalah tidak toksik terhadap sel RAW264.7 dengan $IC_{50} > 30 \mu$ g/mL dan dipilih untuk ujian anti-radang. Mereka juga menunjukkan aktiviti anti-radang dengan mengurangkan pengeluaran NO lebih daripada 80% berbanding dengan kawalan positif, arginin. Ia jelas menunjukkan bahawa daun *H. tiliaceus* mempunyai agen berpotensi anti-radang terhadap sel RAW264.7. Kajian dalam secara *in vivo* menunjukkan bahawa 100 mg/kg HM mempunyai sedikit pengurangan eodema kaki tikus yang dihasilkan oleh formalin. Penemuan dengan jelas menunjukkan bahawa daun *H. tilaceus* adalah selamat dan mempunyai potensi sebagai drug alternatif untuk penyakit keradangan.