



UNIVERSITI PUTRA MALAYSIA

**POTENTIAL ANTI-INFLAMMATORY, ANTI-PYRETIC AND
ANTIULCERATIVE EFFECTS OF HEXANE FRACTION OF *ARDISIA
CRISPA* (THUMB.) A. DC.**

LAU MOI FONG

FPSK(m) 2011 12

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UNIVERSITI PUTRA MALAYSIA**

2011

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(THUMB.) A. DC.**



By

LAU MOI FONG

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

March 2011

Abstract of thesis presented to the Senate of University Putra Malaysia in fulfilment of the requirement for the degree of Master of Science.

POTENTIAL ANTI-INFLAMMATORY, ANTI-PYRETIC AND ANTI-ULCERATIVE EFFECTS OF HEXANE FRACTION OF *ARDISIA CRISPA* (THUMB.) A. DC.

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March 2011

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Faculty: Faculty of Medicine and Health Sciences

Ardisia crispera has been claimed by local villagers to have medicinal properties, and it is widely used in treating dysmenorrhoea, rheumatism, orchitis, skin problem, coughs, fractured bones, sprains and for treatment women afterbirth. This study was conducted to investigate the possible anti-inflammatory and anti-pyretic effects of hexane fraction of *A. crispera* root (HFAC) in various experimental animal models. For toxicity screening, ACHE at the dose range of 300 - 1800 mgkg⁻¹ was tested to determine its LD₅₀. For antipyretic activity study, brewer's yeast was injected into mice to induce fever and later, HFAC at dose ranging from 10 to 300 mgkg⁻¹ was administered to the rats orally. For anti-inflammatory activity study, 12-O-tetradecanoylphorbol-13-acetate (TPA) was applied to ear of mice to induce oedema and treated with 0.5, 1 and 2 mgmL⁻¹ of HFAC topically. Cotton-pellet-induce granuloma in sub chronic study, treated groups have received 3, 10, 30 and 100

mgkg⁻¹ of HFAC administered orally for 7 days. Then pellets are dissected out and weighed. For anti-arthritic activity study, Complete Freund's adjuvant (CFA) was injected onto plantar aponeurosis of right paw of rat to induce chronic arthritis. The following day onwards, HFAC at 3, 10, 30, and 100 mgkg⁻¹, and indomethacin were administered to the rats. Paw volume of rat was measured with a plethysmometer for 14 days. Ankle tissue was collected for ELISA test of tumor necrosis factor alpha (TNF- α) and interleukin-1 beta (IL-1 β). In anti-ulcerogenic study, the rats were divided into 3 groups: L-NAME pre-treatment group, NEM-pretreatment group and saline pre-treatment group. Each group has 6 sub-groups with 6 animals were fasted for 48 hours with free access of water. Groups were orally administered with 1 ml absolute ethanol to induce ulcer. One hour after ethanol administration, animals were sacrificed by cervical dislocation and stomach was removed to determine the ulcer lesion areas and photographs were taken. The stomach biopsies were kept in 10% formalin for histological analysis. The LD₅₀ of HFAC was 836.12 mgkg⁻¹. Results obtained showed that HFAC showed significant anti-pyretic effect at all doses (10, 30, 100 and 300 mgkg⁻¹). At 30, 100 and 300mgkg⁻¹, HFAC exhibited even higher efficacy when compared with 100 mg/kg acetaminophen. In TPA induced ear oedema model, 1 and 2 mg/ear of HFAC produced significant suppression by 19.9% and 20.2% of ear oedema, respectively. HFAC also elicited a significant ($P<0.05$) inhibition of granuloma tissue and exudate formation in cotton-pellet induced granuloma. The result exhibited that HFAC at all doses (3, 10, 30 and 100 mgkg⁻¹) have significant ($p<0.05$) inhibition of oedema of 45.3%, 64.3%, 73.1% and 49.6%, respectively. In arthritic model, during the first phase after CFA-injection, oedema was increased and then reduced at 4 days after. The second onset of action started at day 9 for certain doses of HFAC treated groups (10 and 30 mgkg⁻¹) and

indomethacin. At 3, 10 and 30 mgkg⁻¹, HFAC significantly reduced TNF- α by 45.2%, 45.7% and 25.1%, respectively when compared with control. For IL-1 β , only 10 mgkg⁻¹ and 30 mgkg⁻¹ of HFAC elicited a significant ($p < 0.05$) inhibition of this mediator in local tissue by 45.9% and 36.5%, respectively. The efficacies of those doses were comparable to the effect of indomethacin (34.6%). The gastroprotective effect of HFAC was observed in the groups pre-treated with L-NAME, which was very similar to cytoprotective response on lesion induced in pre-treatment saline groups. At 30, 100, and 300 mgkg⁻¹ HFAC exhibited its cytoprotective effect by 95.6%, 99.3% and 99.1% respectively. At 30 and 100mgkg⁻¹, animals pre-treated with saline received HFAC, have exhibited reduction in gastric lesion by 95.6% and 99.3% compared to NEM pre-treatment group (57.1% and 52.6%) and thus it showed significant gastroprotection ($p < 0.001$). At 300 mgkg⁻¹ with saline pre-treated group, HFAC significantly suppressed the gastric lesion ($p < 0.05$) by 99.1% when compared to NEM group (69.9%). Thus, HFAC may possess anti-ulcerogenic effect via SH group. In histological analysis, all doses of HFAC treated in NEM pre-treated animals at 10 mgkg⁻¹, 30 mgkg⁻¹, 100 mgkg⁻¹ and 300 mgkg⁻¹ have shown a significant increase in the mean score of haemorrhage and blood congestion features ($p < 0.05$) indicating the attenuation of ulcer inhibition in NEM pretreated group. Thus, based on the results of the present studies, it can be concluded that *A. crispa* possesses anti-inflammatory, antipyretic and anti-ulcerogenic effect in rats and this may possibly due to the flavonoid and triterpenoid content in HFAC. However, further studies should be done to determine the exact mechanisms underlying those pharmacological effects especially in the fraction's bioactive compounds.

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

**KESAN POTENSI ANTI-INFLAMASI, ANTI-PIREKSIA DAN ANTI-ULSER
DARI HAKSANA EKSTRAK DARIPADA AKAR *ARDISIA CRISPA* (THUMB.)**

A.DC.

By

LAU MOI FONG

March 2011

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Ardisia crispa telah lama digunakan oleh masyarakat tempatan dalam rawatan perubatan tradisional. Tumbuhan ini telah digunakan secara meluas untuk merawat dismenorea, reumatisme, orkitis, masalah kulit, batuk, tulang retak, terseliuh dan rawatan untuk perempuan selepas bersalin. Maka, tujuan kajian ini dijalankan ialah untuk menentukan kesan ekstrak heksana dari akar *Ardisia crispa* (HFAC) ke atas ujian anti-inflamasi dan anti-piretik di dalam pelbagai model uji kaji. Untuk ujian anti-piretik, HFAC diuji ke atas mencit yang diaruh dengan yis Brewer untuk menghasilkan kesan pireksi. Sebelumnya, rawatan menggunakan HFAC dari julat dos 10-300 mgkg⁻¹ di beri secara oral kepada mencit. Manakala, untuk aktiviti anti-inflamasi, 12-O-tetradekanoilphorbol-13-asetat (TPA) yang mampu menyebabkan edema, diaruh pada telinga mencit yang sebelum itu telah dirawat dengan HFAC sama ada pada kepekatan 0.5, 1.0, atau 2.0

mgmL⁻¹ secara topikal. Untuk kajian granuloma yang diaruh dengan pelet kapas, tikus-tikus diberikan dos samada 3, 10, 30 atau 100 mgkg⁻¹ HFAC selama 7 hari. Selepas itu, pelet dikeluarkan and ditimbang. Dalam kajian inflamasi kronik, adjuvant lengkap Freund di aruh pada plantar aponeusis di kaki kanan tikus. Sebelum itu, HFAC sama ada pada dos 3, 10, 30 atau 100 mgkg⁻¹ dan kawalan positif, indometasin pada 100 mgkg⁻¹ dirawat selama 14 hari . Tisu pergelangan kaki tikus diuji untuk menentukan kehadiran faktor nekrosis tumor alfa (TNF- α) dan interleukin 1-beta (IL-1 β). Manakala aktiviti ulser ke atas HFAC dilakukan terhadap tiga kumpulan tikus masing masing dirawat terlebih dahulu samada dengan L-NAME, NEM atau salina. Ulser di dalam tikus diaruh menggunakan 1ml etanol mutlak. Selepas satu jam, perut tikus dibedah untuk menentukan luas permukaan lesi gaster dan gambar ulser juga turut diambil. Biopsi perut disimpan dalam 10% formalin untuk analisis histologi. Hasil kajian menunjukkan semua dos HFAC (10-300 mgkg⁻¹) mempunyai kesan anti-piretik yang signifikan. Pada dos 30, 100 dan 300mgkg⁻¹, HFAC mempunyai kesan yang lebih baik berbanding dengan acetaminophen pada dos 100mgkg⁻¹. Untuk model edema telinga mencit diaruh oleh TPA, pada kepekatan 1.0 dan 2.0 mgml⁻¹, HFAC berjaya mengurangkan pembentukan edema masing-masing sebanyak 19.9% dan 20.2%. Selain itu, HFAC juga merencat secara signifikan ($p < 0.05$) terhadap pembentukan eksudat dan tisu granuloma dalam kajian granuloma yang diaruh oleh pelet kapas. Disamping itu, kajian artritis menunjukkan bahawa semua dos HFAC iaitu 3, 10, 30 dan 100 mgkg⁻¹ mampu mengurangkan edema secara signifikan ($p < 0.05$) masing-masing sebanyak 45.3%, 64.3%, 73.1% dan 49.6%. Semasa fasa pertama, edema meningkat pada tahap optimal dan kemudiannya merencat pada hari ke-3. Untuk fasa sekunder, HFAC pada

dos 10 dan 30 mgkg⁻¹ serta kawalan positif, indomethacin bermula pada hari ke 9. Pada dos 3, 10 dan 30 mgkg⁻¹, ACHE menurunkan penghasilan TNF- α masing-masing sebanyak 45.2%, 45.7% dan 25.1% . Di samping itu, pada dos 10 dan 30mgkg⁻¹ , HFAC mampu merencat penghasilan IL-1 β di dalam tisu lokal secara signifikan, masing-masing sebanyak 45.9% dan 25.1% berbanding dengan kawalan negatif. Kesan HFAC adalah setara dengan kesan yang dihasilkan oleh indometasin sebanyak 34.6%. Untuk kajian ulser di dalam haiwan yang dirawat terlebih dahulu dengan L-NAME, HFAC memberikan kesan perlindungan gaster yang hampir sama di dalam haiwan yang dirawat terlebih dahulu dengan salina. Manakala, pada dos 30, 100 dan 300 mgkg⁻¹ , HFAC menunjukkan kesan perlindungan sito (sitoprotektif), yang menghasilkan perencatan ulser yang signifikan, masing-masing sebanyak 95.6%, 99.3% dan 99.1%. Bagi kumpulan haiwan yang dirawat terlebih dahulu dengan salina, kemudiannya dirawat dengan HFAC pada dos 30 dan 100 mgkg⁻¹ , mereka menunjukkan perencatan pada luas permukaan lesi gaster, masing-masing sebanyak 95.6% dan 99.3% apabila dibandingkan dengan haiwan yang dirawat terlebih dahulu dengan NEM, masing-masing memberikan perencatan sebanyak 57.1% dan 52.6%. Dengan itu, HFAC menunjukkan kesan perlindungan gaster yang signifikan ($p < 0.001$). Di dalam kumpulan haiwan yang di rawat terlebih dahulu dengan salina, HFAC iaitu pada dos 300 mgkg⁻¹ juga mengurangkan secara signifikan luas permukaan lesi gaster ($p < 0.05$) sebanyak 99.1% berbanding dengan kumpulan yang diberi pra-rawatan dengan NEM iaitu sebanyak 69.9%. Dengan itu, adalah dicadangkan bahawa kumpulan sulfhidril (-SH) mungkin terlibat di dalam aktiviti anti-ulser ke atas HFAC. Analisis histopatologi mempamerkan semua dos HFAC dari kumpulan pra-rawatan NEM menghasilkan

peningkatan yang signifikan dalam ciri-ciri seperti pendarahan dan kongesi ($p < 0.05$). Akhirnya, kajian ini menyimpulkan bahawa *A. crista* mempunyai kesan anti-inflamasi, anti-pireksia dan anti-ulser, kemungkinan dari kandungan flavonoid dan triterpenoid yang terdapat dalam HFAC. Walau bagaimanapun, kajian lanjut ke atas kompaun bioaktif yang diasingkan dari HFAC perlu dijalankan untuk menentukan mekanisme sebenar aktiviti-aktiviti farmakologi tersebut.



ACKNOWLEDGEMENTS

This is an appropriate chance to thank and remember all those who have guided me throughout this process, and contributed in making this research project a success. Finally, I have completed this master research thesis. During this study, I have gain a lot of knowledge while I was facing problems. In addition, each step in order to fulfill this project have been supported by many individuals and also institutes.

First of all, I would like to express my sincere appreciation and deepest gratitude to the following person for their support during the research. With most sincere praise and appreciation to my supervisor Dr. Roslida Binti Abd Hamid @ Abdul Razak because giving me the guidance, advices, encouragement and support throughout the process of experimentation and thesis writing.

My deepest gratitude is extended to co-supervisors Professor Dr. Muhammad Nazrul Hakim Bin Abdullah, Associate Professor Dr. Sabrina Bt Sukardi and Dr. Nik Musaadah Mustapha who have generously assisted in contributing their idea and protocols for animal studies and analysis. I also like to thank Forest Research Institute Malaysia (FRIM) for proving facilities and equipments to complete this research. Thank you to En. Ramli from Animal House of UPM who help me to manage animal and laboratory staff for all the help that was provided to me. Not forgetting to thank my all beloved friends for their friendship and encouragement. Last, but never least, to my family especially my husband for their love, company and financial support for making life in here bearable.

I certify that a Thesis Examination Committee has met on 22 March 2011 to conduct the final examination of Lau Moi Fong on her thesis entitled “Potential Anti-inflammatory, Anti- pyretic and Anti-ulcerative Effects of Hexane Fraction of *Ardisia crispa* Thumb. A.DC.” 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 award the Master of Science.

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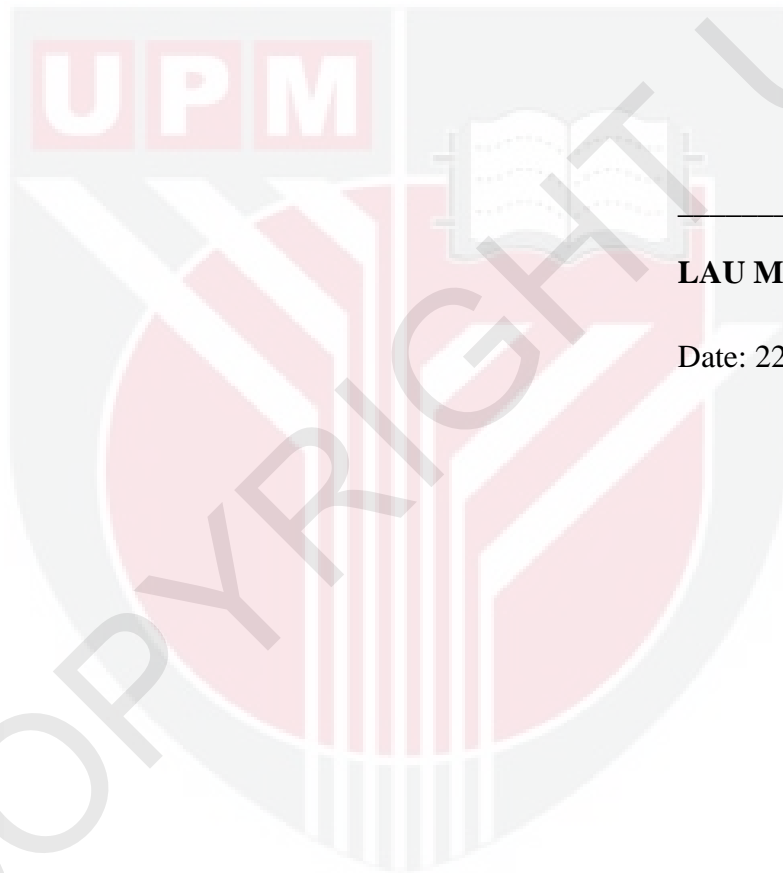
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DECLARATION

I declare that the thesis is my original work except for quotations and citation which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for other degree at Universiti Putra Malaysia or at any other institution.



LAU MOI FONG

Date: 22 March 2011

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