



UNIVERSITI PUTRA MALAYSIA

***EFFECTS OF *Payena dasyphylla* (Miq.) ON HYALURONIDASE ACTIVITY
AND METALLOPROTEINASES EXPRESSION IN INTERLEUKIN-1 β
STIMULATED HUMAN CHONDROCYTES CELLS***

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FBSB 2012 55

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Masters of Science

EFFECTS OF *Payena dasyphylla* (Miq.) ON HYALURONIDASE ACTIVITY AND METALLOPROTEINASES EXPRESSION IN INTERLEUKIN-1 β STIMULATED HUMAN CHONDROCYTES CELLS

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August 2012

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Hyaluronidase is one of the target enzymes in the development of osteoarthritis (OA) disease that results from the disruption of the extracellular matrix structure in the cartilage. Although various treatments for OA have been identified, they suffer from numerous drawbacks. The drawbacks are significant as OA patients require the treatment for their entire lifetime. While there is still no curative treatment for this disease, recent studies for the prevention or treatment on OA were focused on the efficacy of natural products which are capable of ameliorating the symptoms with limited side effects. Therefore, this research aimed to study the effect of *Payena dasyphylla*, a selected Malaysian plants on the hyaluronidases activity, and certain matrix metalloproteinases (MMPs) expression. Prior to this, a total of 20 methanolic crude extracts (bark and leaf) were screened using a colorimetric anti-hyaluronidase enzymatic assay. Out of the plants tested, bark extracts of *Palaquium gutta*, *Pauteria*

obovatta and *Payena dasyphylla* (100 µg/ml) showed the highest inhibitory activity against bovine testicular hyaluronidase with 88.82 ± 0.15 , 90.47 ± 0.09 and 91.63 ± 0.21 percent respectively. Due to insufficient amount of samples, only *Payena dasyphylla* with an IC_{50} value of 48.75 ± 8.97 µg/ml was further studied for its effect on hyaluronidase activity in interleukin-1 β (100 ng/ml) stimulated human chondrocytes cells (NHAC-kn) using the zymography method. *Payena dasyphylla* was then fractionated into aqueous (Aq), ethyl acetate (EA) and hexane fractions where the EA fraction which showed the highest inhibitory activity against hyaluronidase was selected for further evaluation on its capability inhibiting the gene expressions of *HYAL1* and *HYAL2* using Reverse Transcription-Polymerase Chain Reaction (RT-PCR) technique. Besides evaluating the effect of *Payena dasyphylla* on the hyaluronidases, this plant extract was also tested on the expression of other mediators mainly on MMP-3 and MMP-13 using Western blotting method. *Payena dasyphylla* was then further investigated for its total phenolic and flavonoid content as well as antioxidant activity. The antioxidant activity was measured using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay whereas total phenolic content was determined using the Folin-ciocalteu's method. Results showed that *Payena dasyphylla* methanolic extract (100 µg/ml) inhibited hyaluronidase activity in the cultured human chondrocyte cells in response to IL-1 β (100 ng/ml) stimulation. Similarly, treatment with *Payena dasyphylla* EA fraction (100 µg/ml) also inhibited the *HYAL1* and *HYAL2* gene expressions. Cytotoxicity results revealed that both *Payena dasyphylla* methanolic and EA fraction did not show any concomitant effect on the NHAC-kn cell line at all concentrations tested. On the other hand, the *Payena dasyphylla* EA fraction also inhibited MMP-3 and MMP-13 protein expression. Interestingly, treatment without IL-1 β (10 ng/ml) stimulation

revealed a significant difference as MMP-3 and MMP-13 protein expressions were seen only with the presence of IL-1 β inducer. In addition, *Payena dasyphylla* EA fraction showed the highest amount of phenolic and flavonoid content with 168.62 ± 10.93 mg GAE/g and 95.96 ± 2.96 mg RE/g respectively compared to aqueous and hexane fractions. Besides, *Payena dasyphylla* EA extract exhibited antioxidant activity of 82.19% at the concentration of 100 μ g/ml with IC₅₀ value of 14.10 ± 1.11 μ g/ml. As a conclusion, these findings showed that *Payena dasyphylla* contained potential classes of compound inhibiting hyaluronidases, *HYAL1* and *HYAL2* gene expressions as well as MMP-3 and MMP-13 protein expressions that are responsible for the degeneration of the cartilage tissue. Therefore, this plant might serve as an alternative in formulating new generation of drugs in the management of cartilage damage.

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

KAJIAN TENTANG KESAN *Payena dasyphylla* (Miq.) TERHADAP AKTIVITI HYALURONIDASE DAN EKSPRESI METALLOPROTEINASES KE ATAS SEL KONDROSIT MANUSIA YANG DIRANGSANG DENGAN INTERLUKIN-1 β

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Hyaluronidase telah dikenal pasti sebagai salah satu target enzim yang menyebabkan penyakit osteoarthritis yang berpunca daripada gangguan di dalam struktur matriks extracellular di dalam rawan. Walaupun banyak rawatan untuk osteoarthritis telah dikenal pasti rawatan-rawatan ini mempunyai banyak kelemahan. Kelemahan ini adalah ketara kerana pesakit osteoarthritis memerlukan rawatan untuk seumur hidup mereka. Walaupun tiada lagi rawatan penyembuhan untuk penyakit ini, kajian masa kini untuk menghindari penyakit osteoarthritis berfokus terhadap penggunaan produk semulajadi yang mampu mengurangkan simptom dengan kesan sampingan yang minimum. Maka, kajian ini bertujuan untuk mengkaji kesan *Payena dasyphylla* pilihan pokok herba semulajadi Malaysia terhadap aktiviti hyaluronidase dan ekspresi MMP tertentu. Untuk tujuan itu, sebanyak 20 ekstrak methanol mentah pokok herba semulajadi (daun dan kulit) telah disaring dengan asai anti-enzim hyaluronidase. Daripada semua ekstrak yang diuji, ekstrak *Palaquium gutta*, *Pauteria obovatta* and *Payena dasyphylla* daripada sumber kulit (100 μ g/ml)

menunjukkan aktiviti perencatan yang paling tinggi terhadap hyaluronidase testis bovin sebanyak 88.82 ± 0.15 , 90.47 ± 0.09 and 91.63 ± 0.21 peratus. Ekstrak *Payena dasyphylla* dengan nilai IC_{50} sebanyak 48.75 ± 8.97 $\mu\text{g/ml}$ ini diuji selanjutnya untuk mengkaji kebolehan perencatan aktiviti enzim hyaluronidase dalam kultur sel kondrosit manusia yang dirangsang dengan IL-1 β menggunakan teknik *zymography*. Ekstrak methanol *Payena dasyphylla* ini kemudian dipecahkan kepada pecahan-pecahan kecil iaitu pecahan air, etil asetat (EA) dan hexane di mana pecahan etil asetat yang menunjukkan aktiviti perencatan enzim hyaluronidase tertinggi dipilih untuk kajian selanjutnya untuk menguji kebolehan pecahan ini merencat ekspresi gen-gen *HYAL1* dan *HYAL2* menggunakan teknik “*Reverse Transcription Polymerase Chain Reaction*” (RT-PCR). Selain menilai kesan *Payena dasyphylla* ke atas enzim hyaluronidase, pecahan ekstrak ini juga diuji ke atas rangsangan lain terutamanya MMP-3 dan MMP-13 menggunakan kaedah “*Western blot*”. Seterusnya, *Payena dasyphylla* dinilai untuk jumlah kandungan fenolik dan flavonoid serta kapasiti antioksidan dalam ekstrak. Kandungan kapasiti antioksidan di dalam ekstrak dinilai melalui kaedah daya pemulungan radikal 2, 2-diphenyl-1-picrylhydrazyl (DPPH), manakala jumlah kandungan fenolik dinilai melalui kaedah Folin-ciocalteu. Hasil eksperimen menunjukkan bahawa ekstrak mentah methanol *Payena dasyphylla* mampu merencat aktiviti enzim hyaluronidase di dalam kultur sel kondrosit manusia yang dirangsang dengan IL-1 β (100 ng/ml). Begitu juga dengan ekstrak EA *Payena dasyphylla*, ekstrak ini juga mampu merencat ekspresi gen-gen *HYAL1* dan *HYAL2* bergantung pada dos yang digunakan. Hasil kajian sitotoksik menunjukkan bahawa, ekstrak mentah metanol dan EA *Payena dasyphylla* tidak mempunyai sebarang kesan terhadap kultur sel kondrosit manusia pada semua kepekatan yang diuji. Di samping itu, rawatan dengan ekstrak EA *Payena dasyphylla*

terhadap MMP-3 dan MMP-13 juga mampu merencat ekspresi protein tersebut. Rawatan dengan atau tanpa kehadiran rangsangan IL-1 β menunjukkan perbezaan yang ketara di mana, ekspresi protein MMP-3 dan MMP-13 hanya wujud dengan kehadiran rangsangan IL-1 β . Selain itu, ekstrak EA *Payena dasyphylla* mencatatkan jumlah kandungan fenolik dan flavonoid yang tertinggi sebanyak 168.62 ± 10.93 mg GAE/g dan 95.96 ± 2.96 mg RE/g masing-masing berbanding ekstrak air dan hexane. Manakala, aktiviti anti-oksidaan *Payena dasyphylla* EA menunjukkan sebanyak 82.19% dengan nilai IC₅₀ sebanyak 14.10 ± 1.11 μ g/ml pada kepekatan 100 μ g/ml. Kesimpulannya, kajian ini telah menunjukkan bahawa *Payena dasyphylla* mengandungi kelas-kelas kompoun yang berpotensi untuk merencat aktiviti hyaluronidase, ekspresi gen- gen *HYAL1* dan *HYAL2* serta ekspresi MMP-3 dan MMP-13 yang bertanggungjawab dalam pendegradasian tisu rawan. Maka, *Payena dasyphylla* berpotensi untuk digunakan sebagai alternatif rumusan ubatan generasi baru dalam pengurusan kerosakan tulang rawan.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my deepest gratitude to my supervisor, Dr. Syahida Ahmad for her perceptiveness, advices and encouragement throughout the completion of my project. My deepest gratitude also goes to Assoc. Prof. Dr. Khozirah Shaari and Dr. Seema Nadeem for their constructive suggestions and invaluable help throughout the period of my study.

I would also like to express my sincere gratitude to all the postgraduates and officers from the Laboratory of Natural Products, Institute of Bioscience (IBS) as well as from the Faculty of Biotechnology and Biomolecular Science for their kind assistance and inspiring atmosphere that they have provided.

Most importantly, I am deeply indebted to my family and friends for their support, patience and encouragement during my study in UPM. Their endless support and motivation have been an enormous source of strength and inspiration in my life.

Last but not least, I would like to acknowledge my appreciation to The World Federation of Scientist and Mini Budget 2009 (Malaysia 2009 Second Stimulus Package) for their financial support throughout the completion of my course.

I certify that an Examination Committee has met on 8th August 2012 to conduct the final examination of Kamini Citalingam on her Master's of Science thesis entitled "Effects of *Payena dasyphylla* (Miq.) on hyaluronidase activity and metalloproteinases expression in interleukin-1 β stimulated human chondrocytes cells" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher degree) Regulations 1981. The Committee recommends that the student be awarded the relevant degree.

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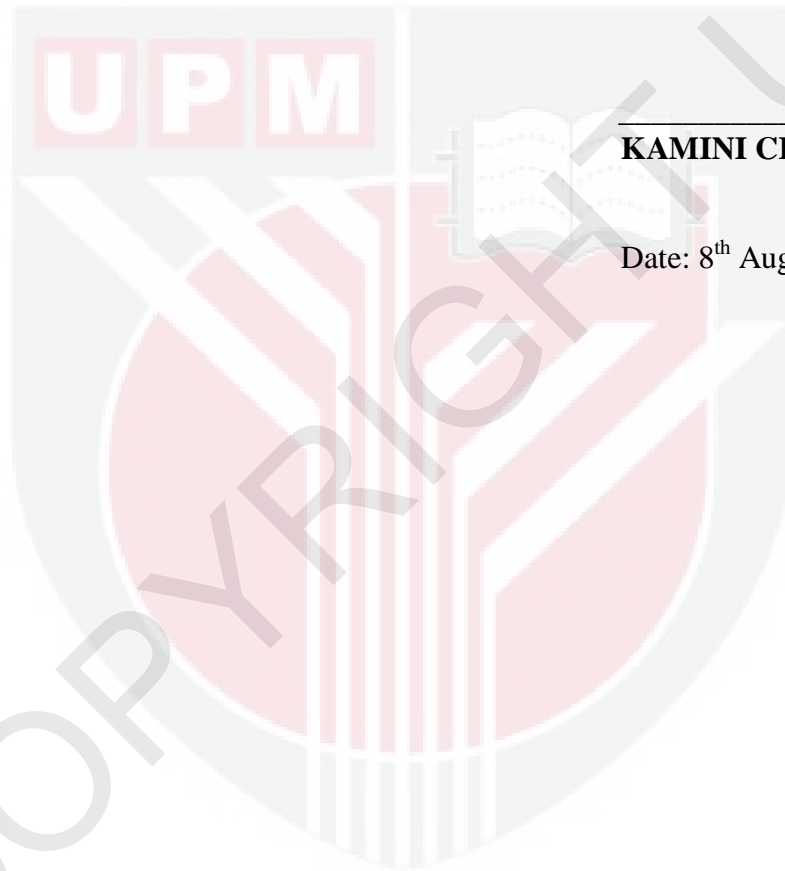
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DECLARATION

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



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Date: 8th August 2012



TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	v
ACKNOWLEDGEMENTS	viii
APPROVAL	ix
DECLARATION	xi
LIST OF TABLES	xv
LIST OF FIGURES	xvi
LIST OF APPENDICES	xviii
LIST OF ABBREVIATIONS	xix
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	
2.1 Osteoarthritis (OA)	6
2.2 Articular cartilage	9
2.2.1 Articular cartilage extracellular matrix (ECM) components	12
2.2.1.1 Hyaluronan (HA)	12
2.2.1.2 Chondrocytes	16
2.2.1.3 Hyaluronidase	17
2.3 Mechanism of hyaluronan degradation	19
2.4 Pathophysiology of Osteoarthritis	23
2.4.1 Other metabolic factors that contribute to the pathophysiologic changes in OA	24
2.5 Treatment	25
2.5.1 Conventional treatment	25
2.5.2 Natural products for the treatment of OA	28
2.6 <i>Payena dasyphylla</i>	30
2.6.1 Taxonomy and Nomenclature	31

2.6.2	Botanical background	31
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3 MATERIALS AND METHODS

3.1	General Materials	34
3.2	Test materials	34
3.3	Plant Extraction	36
3.4	Fractionation	36
3.5	Anti-hyaluronidase enzymatic assay	37
3.6	Cell culture	38
3.6.1	Dilution of plant sample	39
3.6.2	Cytotoxicity test	40
3.7	HA-Substrate zymography	41
3.7.1	Stimulation and treatment of NHAC-kn cells	41
3.7.2	SDS-PAGE and electrophoresis	42
3.7.3	Gel incubation, Staining and documentation	43
3.8	<i>HYAL 1</i> and <i>HYAL 2</i> gene expression analysis	43
3.8.1	Stimulation and treatment of NHAC-kn cells	43
3.8.2	Total RNA extraction	44
3.8.3	RNA Quantification	45
3.8.4	RT-PCR	45
3.8.5	Gel Electrophoresis	46
3.9	MMP-3 and MMP-13 protein expression	47
3.9.1	Stimulation and treatment of NHAC-kn cells	47
3.9.2	SDS-PAGE and Western Blotting	47
3.10	Total phenolic content	48
3.11	Flavonoid content	49
3.12	DPPH radical scavenging assay	50
3.13	Statistical Analysis	51

4 RESULTS

4.1	Effect of selected Malaysian plants on the Hyaluronidase enzyme inhibitory activity	52
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4.2	Effect of <i>Payena dasyphylla</i> on the NHAC-kn cell viability	55
4.3	Effect of <i>Payena dasyphylla</i> methanolic extract on the hyaluronidase enzyme activity in the conditioned-medium of cultured NHAC-kn cell line	56
4.4	Effect of different fractions of <i>Payena dasyphylla</i> on the bovine testicular hyaluronidase enzyme inhibitory activity	59
4.5	Effect of <i>Payena dasyphylla</i> ethyl acetate fraction on the NHAC-kn cell viability	61
4.6	Effect of <i>Payena dasyphylla</i> ethyl acetate fraction on the <i>HYAL1</i> and <i>HYAL2</i> mRNA gene expression	63
4.7	Effect of <i>Payena dasyphylla</i> ethyl acetate fraction on the MMP-3 and MMP-13 protein expression	66
4.8	Determination of total phenolic content	69
4.9	Determination of flavonoid content	69
4.10	Antioxidant activity of <i>Payena dasyphylla</i> EA	70
5	DISCUSSION	72
6	CONCLUSION AND RECOMMENDATIONS	81
	REFERENCES	82
	APPENDICES	93
	BIODATA OF STUDENT	108