



UNIVERSITI PUTRA MALAYSIA

**DETERMINING HYPOGLYCAEMIC AND ANTIADIPOGENIC
PROPERTIES OF PHENOLIC-RICH SOYA HUSK EXTRACT
USING IN-VITRO AND IN-VIVO MODELS**

TAN SEOK TYUG

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**DOCTOR OF PHILOSOPHY
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By

TAN SEOK TYUG

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

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fulfilment of the requirement for the degree of Doctor of Philosophy

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OF PHENOLIC-RICH SOYA HUSK EXTRACT USING *IN-VITRO* AND
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May 2012

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Faculty : Medicine and Health Sciences

The growing incidence of obesity and diabetes seem to be an unavoidable trend in both developed and developing countries. Emerging evidence derived from epidemiological studies claim that sufficient intake of phenolics may effective in protecting human against the oxidative-stress related diseases such as cancers, cardiovascular diseases, diabetes, obesity and osteoporosis. Besides, numerous literatures reported that there is considerable concentration of health-promoting compounds in the outer layers of cereal grains and legumes, or specifically in the bran or hull (husk). However, the bran or hull from cereal grains and legumes are often considered as by-products in food processing industries, and is currently used only for animal feed or as fertilizer. Therefore, the present study aimed to explore the possibility and potentiality of soya husk in improving obesity-diabetes parameters. The present study begun with proximate composition determination and antioxidant analysis in grade A soya milk powder (GASP), grade B soya milk powder (GBSP) and soya husk powder (SHP). Findings from the present

study showed that SHP had a significant higher fibre contents ($74.41 \pm 0.19\%$) of the powders tested, which in turn, also deposited its potentiality to appear as an interfering component for obesity-diabetes studies. Following, the three powders were subjected for antioxidant capacity analyses, free phenolics and isoflavones identification. Soya by-products, for instance, GBSP and SHP in present study, have reported to contain significant amounts of bioactive compounds such as daidzein, gallic acid, vanilic acid, syringic acid, ferulic acid and chlorogenic acid. The hypoglycaemic of phenolic-rich soya husk powder extract (SHPE) were determined *in-vitro* with three different parameters whereas murine 3T3-L1 adipocytes were employed for evaluating the anti-adipogenic properties of SHPE. Collectively, the hypoglycaemic properties of SHPE were successfully demonstrated in three parameters studied. In addition, SHPE with lower concentrations (25-100 $\mu\text{g/ml}$) were also regarded as potential anti-adipogenic agent due to their ability in reducing lipid droplet accumulation (48.48 ± 0.03 to $102.02 \pm 2.00\%$), triglyceride accumulation (5.68 ± 0.07 to 11.17 ± 0.14 mg/dl), GPDH activity (49.13 ± 0.15 to $91.44 \pm 0.04\%$) and subsequently down-regulating PPAR γ expression in 3T3-L1 adipocytes. Since *in-vitro* model has provided promising findings, evaluation on the hypoglycaemic and anti-adipogenic properties of SHPE was further expanded using an *in-vivo* model. It is showed that short-term of SHPE supplementations (250 mg/kg BW or 500 mg/kg BW) improved the biomarker parameters (fasting plasma blood glucose, insulin level and lipid profiles) of obese-diabetic rats. Additionally, the supplementations were also shown to slightly up-regulated PPAR γ expression (4.81 ± 1.31 to $6.69 \pm 2.71\%$) in adipocytes of experimental rats. Therefore, SHPE could be considered as potential hypoglycaemic and anti-adipogenic agents. In line with the

reported promising effects, the present study has revealed the possibility of soya husk to be used for the development of hypoglycaemic and anti-adipogenic nutraceuticals.



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

PENENTUAN HIPOGLISEMIK DAN ANTI-ADIPOGENIK DARI EKSTRAK KULIT SOYA YANG KAYA DENGAN FENOLIK DI MODEL *IN-VITRO* DAN *IN-VIVO*

Oleh

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Insiden obesiti dan diabetik yang kian meningkat seolah-olahnya merupakan satu isu yang tidak dapat dielakkan di negara maju mahupun yang sedang membangun. Hasil kajian dari epidemiologi mendakwa bahawa pengambilan fenolik yang secukupnya mampu melindungi manusia terhadap penyakit yang berkaitan dengan tekanan oksida seperti kanser, penyakit kardiovaskular, diabetik, obesiti dan osteoporosis. Selain itu, kajian sebelumnya turut menunjukkan bahawa lapisan luaran atau khususnya kulit (sekam) dari bijirin dan kekacang mempunyai sumber sebatian yang berfaedah terhadap kesihatan manusia. Namun, kulit atau sekam dari bijirin dan kekacang sering dianggap sebagai produk sampingan dalam industri pemprosesan makanan, dan kini ia hanya digunakan sebagai makanan haiwan atau baja. Oleh itu, kajian ini bertujuan untuk menerokai kemungkinan dan potensi penggunaan kulit soya dalam memperbaiki parameter obesiti-diabetik. Kajian ini dimulai dengan pengajian komposisi proksimatif dan analisis antioxidan di serbuk susu soya gred A (GASP), serbuk susu soya gred B

(GBSP) dan serbuk kulit soya (SHP). Hasil kajian menunjukkan SHP merupakan sumber gentian (fiber) yang lebih baik ($74.41 \pm 0.19\%$) di kalangan serbuk yang dikaji; tetapi, hasil kajian ini juga mendedahkan kemungkinan SHP sebagai komponen yang berupaya mempengaruhi hasil kajian obesiti-diabetik. Ketiga-tiga serbuk tersebut juga ditujukan kepada analisis kapasiti antioxidan, pengajian fenolik dan isoflavon yang bebas. Produk sampingan soya, misalnya, GBSP dan SHP dalam kajian ini, juga dilaporkan mengandungi sebatian bioaktif seperti daidzein, asid galik, asid vanilik, asid syringik, asid ferulik dan asid klorogenik. Ciri-ciri hipoglisemik di ekstrak serbuk kulit soya yang kaya dengan fenolik (SHPE) dianalisiskan melalui tiga parameter yang berbeza manakala 3T3-L1 sel adiposit mencit digunakan untuk penilaian ciri-ciri anti-adipogenik dari SHPE. Secara keseluruhan, ciri-ciri hipoglisemik SHPE telah berjaya ditunjukkan di dalam ketiga-tiga parameter yang dikaji. Selain itu, SHPE dengan kepekatan yang lebih rendah ($25-100 \mu\text{g/ml}$) juga berpotensi sebagai ejen anti-adipogenik kerana kemampuannya dalam mengurangkan pengumpulan titisan lipid (48.48 ± 0.03 ke $102.02 \pm 2.00\%$), pengumpulan trigliserida (5.68 ± 0.07 ke $11.17 \pm 0.14 \text{ mg/dl}$), aktiviti GPDH (49.13 ± 0.15 ke $91.44 \pm 0.04\%$) dan menurunkan transkripsi PPAR γ di 3T3-L1 adiposit. Disebabkan penemuan yang menggalakkan daripada model *in-vitro*, penilaian terhadap ciri-ciri hipoglisemik dan anti-adipogenik di SHPE dilanjutkan kepada model *in-vivo*. Supplementasi SHPE (250 mg/kg BW atau 500 mg/kg BW) dalam jangka masa pendek mampu memperbaiki parameter obesiti-diabetik (paras glukose darah, paras insulin dan profit lipid dalam plasma) di tikus. Tambahan pula, SHPE juga meningkatkan transkripsi PPAR γ (4.81 ± 1.31 ke $6.69 \pm 2.71\%$) pada adiposit tikus kajian. Oleh itu, SHPE adalah berpotensi sebagai agen

hipoglisemik dan adipogenik. Kajian ini telah mendedahkan kemungkinan kulit soya untuk digunakan dalam pemrosesan nutraseutikal yang berkaitan dengan hipoglisemik dan anti-adipogenik.



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I certify that an Examination Committee has met on 8 May 2012 to conduct the final examination of Tan Seok Tyug on her Doctor of Philosophy thesis entitled “Hypoglycaemic and anti-adipogenic properties of phenolic-rich soy husk powder extract using *in-vitro* and *in-vivo* models” 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 Doctor of Philosophy.

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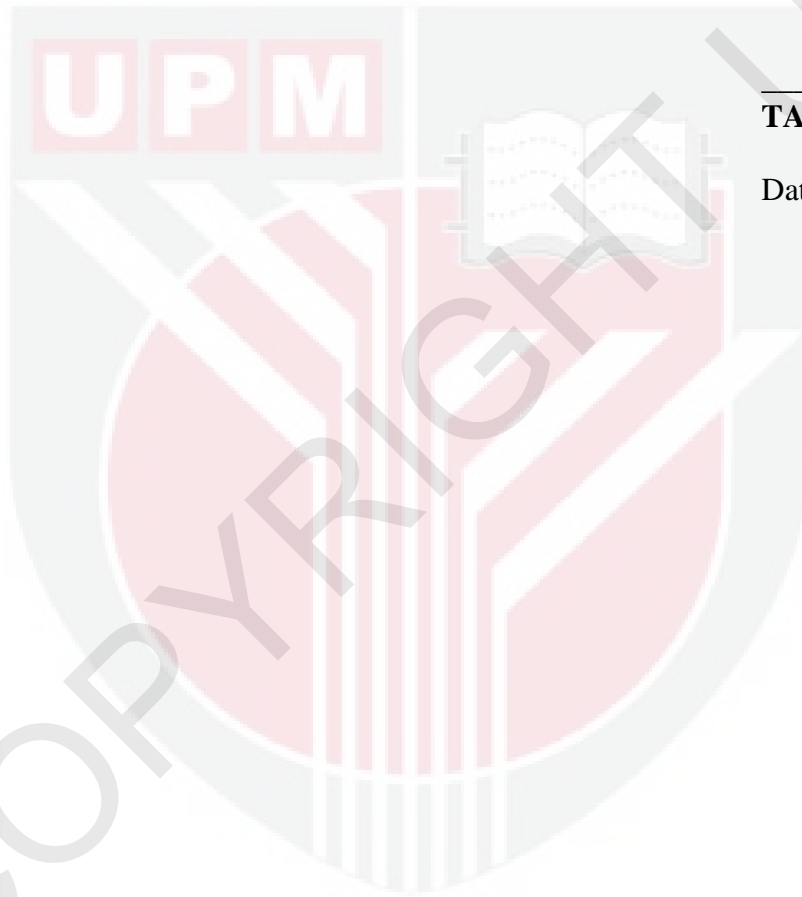
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DECLARATION

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



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Date: 8 May 2012

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