UNIVERSITI PUTRA MALAYSIA

THE HYPOGLYCAEMIC AND CHEMICAL STUDIES OF PARKIA SPECIOSA, Hassk

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THE HYPOGLYCAEMIC AND CHEMICAL STUDIES OF *PARKIA SPECIOSA*, Hassk

BY

FATHAIYA BTE JAMALUDIN

Thesis submitted in Fulfilment of the Requirements for the Degree of Master of Science in the Faculty of Food Science and Biotechnology Universiti Pertanian Malaysia

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*IN THE NAME OF ALLAH THE MOST

GRACIOUS AND MERCIFUL*

DEDICATED TO MY HUSBAND

AND CHILDREN

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LIST OF ABBREVIATIONS

IR	Infrared Spectra
^{1}H-NMR	Proton Nuclear Magnetic Resonance Spectra
$CDCL_3$	Deuterated Chloroform
TMS	Tetramethylsilance
s	singlet
d	doublet
t	triplet
q	quartet
m	multiplet
br-s	broad singlet
dd	doublet-doublet
^{13}C-NMR	Carbon-13 Nuclear Magnetic Resonance Spectra
BBD	Broad Band Decoupled
HPLC	High Performance Liquid Chromatography
UV	Ultra-violet Absorption
MS	Mass Spectra
TLC	Thin Layer Chromatography
std. dev.	Standard deviation
pet-ether	Petroleum ether
$CHCl_3$	Chloroform
$EtOA_c$	Ethyl acetate
CH_2Cl_2	Dichloromethane

NH_4CHCl_3	Ammonical chloroform
MeOH	Methanol
S	Seeds
P	Empty pods
T	Test
G	Standard
Fract	Fraction
Gliben	Glibenclamide
Sitos	Sitosterol
Stigma	Stigmasterol

Abstract of the thesis submitted to the Senate of Universiti Pertanian Malaysia as fulfilment of the requirement for the degree of Master of Science.

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July, 1991

Chairman : Dr. Suhaila Mohamed

Faculty : Food Science and Biotechnology

The administration of the chloroform extracts of Parkia speciosa (petai papan) to alloxan-treated diabetic rats produced a significant depression in blood glucose levels. Other solvent extracts failed to induce any reduction in the blood glucose levels of the diabetic animals. In normal rats serum glucose levels were not changed after the administration of chloroform extracats (400 mg/kg body weight) comparable to those administered with saline solution.

The determination of dose-response relationship of fresh and ground seeds and empty pods showed that the minimum effective dose of raw materils necessary to produce a significant ($p < 0.0001$) decrease in serum glucose concentration was 25 mg/kg body weight.

Extraction and isolation work on the active fractions of the mother liquors resulted in the isolation of pure compounds: S-4(A) from petai seeds, consisted of a mixture of β-sitosterol (66%) and stigmasterol (34%); P-7(A) from empty pods was proposed to be stigmast-4-en-3-one; P-9(4B) from petai pods was elucidated as a stigmasterol while P-9(3AB) was a compound from the pods which could not be identified. The S-4(A) and P-7(A) samples were found to have strong hypoglycaemic activity. The occurrence of β-sitosterol, stigmasterol and stigmast-4-en-3-one were reported for the first time in Parkia speciosa. These naturally occurring compounds were suggested for new potential orally hypoglycaemic agents.

A comparative study on the hypoglycaemic effect of glibenclamide (antidiabetic drug), with S-4(A) and P-7(A) showed glibenclamide to have 111% hypoglycaemic activity at 5 mg/kg body weight. A 2 : 1 mixture of β-sitosterol and stigmasterol displayed 83% hypoglycaemic activity while stigmast-4-en-3-one had 84% effect at a dose of 100 mg/kg. However, β-sitosterol or stigmasterol individually was found to possess no hypoglycaemic property when tested in isolation. This finding suggests S-4(A) produced a synergistic effect to cause hypoglycaemic activity. The behavioural synergism has not been reported before in work related to these sterols.

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains.

KAJIAN HIPOGLISAEMIK DAN KIMIA TERHADAP
PARKIA SPECIOSA, Hassk

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Julai, 1991

Pengerusi : Dr. Suhaila Mohamed

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Pentadbiran ekstrak klorofom dari Parkia speciosa (petai papan) melalui mulut kepada tikus yang dijadikan kencing manis dengan diracuni alloxan telah menunjukkan penurunan kandungan glukos dalam serum darah. Lain-lain ekstrak pelarut didapati tidak memberi apa-apa kesan terhadap tikus kencing manis. Bagi tikus normal didapati kandungan glukos serum tidak berubah selepas pentadbiran ekstrak klorofom (400 mg/kg berat badan) serupa dengan tikus yang diberi saline.

Penentuan sukatan biji petai mentah dan kulit petai mentah juga telah dijalankan. Dos minimum dari bahan mentah untuk menghasilkan penurunan glukos serum yang bererti ($p < 0.0001$) ialah 25 mg/kg berat badan.

Pengekstrakan dan pemisahan terhadap pecahan yang aktif telah menghasilkan komponen tulen : S-4(A), terkandung sebatian β-sitosterol (66%) dan stigmasterol (34%) daripada biji petai dan P-7(A) dikenali sebagai stigmast-4-en-3-one, P-9(4B) adalah stigmasterol dan akhir sekali P-9(3AB) daripada kulit petai tidak dikenali akan kimia dan nama strukturnya. Komponen S-4(A) dan P-7(A) telah menunjukkan kesan hipoglisaemik berbanding dengan kawalan. Penemuan β-sitosterol, stigmasterol dan stigmast-4-en-3-one di dalam Parkia speciosa adalah pertama kali direkodkan. Komponen-komponen terbitan semulajadi ini dicadangkan sebagai ubat kencing manis yang baru.

Seterusnya perbandingan kajian di antara glibenclamide (ubat kencing manis) dengan S-4(A) dan juga P-7(A) dijalankan. Keputusan menunjukkan glibenclamide telah menurunkan kandungan glukos dalam serum darah sebanyak 111% pada nilai dos 5 mg/kg berat badan. Sebatian β-sitosterol dan stigmasterol (2 : 1) menunjuk-kan 83% kesan hipoglisaemik sementara stigmast-4-en-3-one mempunyai 84% kesan hipoglisaemik pada nilai dos 100 mg/kg berat badan. Tetapi, β-sitosterol atau stigmasterol sahaja tidak memberi apa-apa kesan hipoglisaemik. Penemuan ini mencadangkan S-4(A) mempunyai kesan sinergistik untuk menyebabkan penurunan glukos dalam serum darah. Tingkah laku sinergisme ini adalah pertama kali direkodkan di dalam kajian yang berkaitan dengan komponen sterol ini.

CHAPTER 1

INTRODUCTION

The term natural product is referred to those organic compounds of natural origin that are unique to one organism or common to a small number of closely related organisms (Mann, 1977).

Primitive man found quite a number of plant extracts effective as medicines for the relief of pain or alleviation of the symptoms of disease, as poisons for the warfare and hunting uses as effective drugs for euthanasia and as narcotics, hallucinogens or stimulants to relieve the tedium or alleviate the fatigue and hunger. Today, such uses are still being practiced widely especially in remote areas but the acceptance of herbal medicine are continuously spreading into urban communities.

Between 1815 and 1860 (Mann, 1977), many of these active principles were isolated including morphine, strychnine, quinine, caffeine, nicotine, codeine, camphor and cocaine. It is worth noting that during 1950's structure elucidation and natural product research have become much more facile with the advent of nuclear magnetic resonance, mass spectrometry, and X-Ray crystallography.

Diabetes was first recognised almost 4,000 years ago. This disease has a tremendous impact on the health system of individuals in the world. The estimated number of patients who suffered from diabetes mellitus in Malaysia between 1984 to 1988 according to the Information Documentary System's are presented in Table 1. The incidence of diabetes is increasing every year. However, deaths in Malaysia appeared to be decreasing by 1% per year. According to the Malaysian Medical Institute, the total annual economic budget for diabetes was estimated at approximately 1/4 million ringgits in 1988.

Table 1

Number of Cases and Deaths Attributed to Diabetes Mellitus in Malaysia (1984 - 1988)

Year	No. of cases	Deaths
1984	10125	264
1985	14767	261
1986	17301	261
1987	17901	245
1988	17850	227

Source: Information Documentary System.

In 1976, the National Commission on Diabetes reported that 5% of the population of United States (approximately 10 million people) have diabetes. The incidence is increasing by 6% per year. Their total annual economic budget of diabetes was estimated at approximately 8 billion dollars in 1979 (Davidson, 1981).

In Malaysia, almost 6800 species of seed plants and 600 species of seedless plants have been recorded (Holtum, 1954 and Keng, 1970). In local traditional medical practices, many of these plants have been used for remedies of various illness including for diabetes. In fact, it was reported that at least 15% of these plants had been claimed by various people to have medicinal uses (Latif et al., 1984). Nevertheless, this area of research is still lacking in Malaysia. For instance, Kumis kucing (Orthosiphon stamineus), Akar putawali (Tinospora crispa), Lempedu tanah (Curanga amara) and Jering (Pithecellobium jeringa) which has been traditionally used to treat catarrh of the bladder, hypertension and diabetes have still not been tested biologically and investigated chemically.

On the other hand, eventhough there are many substances that have been isolated and characterized, the investigation of their potential biological effects are still not carried out (Kjaer, 1977).

Therefore, the implication of doing this kind of research is to optimize the cultivation and genetic selection of the food and plants. Additionally, new oral hypoglycaemic products can be synthesized commercially from the herbal plants. In the year 1988, about $74,230.5 \times 10^3$ amount of drugs have been synthesized by the private sector in the country.

There has been chemical investigation on seeds but not on empty pods of Parkia speciosa (Haask) (Gmelin et al., 1980). There are not much studies carried out regarding their medicinal chemistry. This research attempts to investigate the alleged hypoglycaemic activity of Parkia speciosa.

The objectives and scope of the research are:

i) to investigate the hypoglycaemic activity of P. speciosa on normal and alloxan diabetic rats.

ii) to isolate the active compounds from the active fractions.

iii) to elucidate the structures of the isolated active compounds.

The pharmacological investigations to study the effect of P. speciosa extracts on other systems in rats such as neuro-pharmacological studies, cardiovascular studies, morphological and biochemical changes, effect of extracts on smooth and skeletal muscles etc. were not done. Nevertheless these field of studies are of interest for future investigations.