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CHEMICAL CONSTITUENTS FROM Ficus deltoidea var. kunstleri Corner AND ITS ANTIDIABETIC PROPERTY

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THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE (NATURAL PRODUCT) (MASTER BY RESEARCH)



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Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah



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ABSTRACT

The aims of this study are to determine the chemical structures of the isolated compounds from the leaves and stem bark of *Ficus deltoidea* var. kunstleri by using various type of spectroscopy and to evaluate their antidiabetic activities. The isolation process of isolated compounds involved extraction, separation and purification by using column chromatography and preparative thin layer chromatography techniques. These compounds were structurally identified by using various spectroscopic techniques such as 1D (¹H, ¹³C and DEPT) and 2D-NMR (HMBC, HMQC and COSY), UV, IR and mass spectrometry; and also by comparison with previous studies. Study on *in-vitro* antidiabetic activities were evaluated by using α -glucosidase inhibitory assay with 4nitrophenyl-a-D-glucopyranoside (pNPG) as substrate. As findings, chromatographic purification of F. deltoidea var. kunstleri afforded eleven chemical compounds: α tocopherol, β -amyrin cinnamate, triglycerides fatty acids, hexadecyl palmitate, bergapten which are first time reported from this species and two mixture of compounds; mixture of pentacyclic triterpenes; lupeol, α -amyrin, β -amyrin and a mixture of sterols; stigmasterol and β -sitosterol together with a new ester triterpene; euphol ester. The bioactivity of α -glucosidase inhibition on the crude extracts and isolated compounds from this variation was first time reported. As conclusion, all crude 05-4506 extracts and the isolated compounds were inactive against the in-vitro α-glucosidase bupsi activity and have no potential as antidiabetic drugs. The implication is the results from this study can be used as future references to discover possible potential new drugs.















KANDUNGAN SEBATIAN KIMIA DARIPADA Ficus deltoidea var kunstleri CORNER DAN SIFAT ANTIDIABETIKNYA

ABSTRAK

Kajian ini bertujuan menentukan struktur kimia sebatian yang dipencilkan daripada daun dan kulit kayu *Ficus deltoidea* var. *kunstleri* dengan mengunakan pelbagai jenis spektroskopi dan menguji aktiviti antidiabetik. Proses pemencilan bagi sebatian yang dipencilkan dalam kajian ini melibatkan pengekstrakan, pemisahan dan pemurnian dengan menggunakan teknik kromatografi turus dan kromatografi lapisan nipis preparatif. Semua struktur sebatian ini dikenalpasti melalui pelbagai teknik spektroskopik iaitu 1D (¹H, ¹³C dan DEPT) dan 2D-NMR (HMBC, HMQC dan COSY), UV, IR dan spektrometri jisim; dan juga perbandingan dengan data daripada kajian lepas. Kajian ke atas aktiviti antidiabetik *in-vitro* diuji menggunakan rencatan cerakinan α-glukosida dengan 4-nitrofenil-α-D-glukopiranosida (pNPG) sebagai substrat. Dapatan kajian daripada penulenan kromatografik *F. deltoidea* var. *kunstleri* menghasilkan sebelas sebatian kimia iaitu α-tokoferol, β-amirina cinnamata, asid lemak trigliserida, heksadesil palmitat dan bergaptena yang merupakan kali pertama dilaporkan daripada spesies ini dan dua sebatian bercampur iaitu campuran triterpena pentasiklik; lupeol, α-amyrina, β-amyrina dan campuran sterol iaitu stigmasterol dan β-

os sitosterol bersama-sama dengan satu ester triterpena baharu iaitu ester euphol. Bioaktiviti rencatan cerakinan α -glukosida ke atas ekstrak mentah dan sebatian yang dipencilkan daripada variasi ini adalah kali pertama dilaporkan. Kesimpulannya, semua ekstrak mentah dan sebatian kimia yang dipencilkan tidak aktif terhadap α -glukosida *in vitro*. Implikasinya, hasil kajian ini boleh digunakan sebagai bahan rujukan dan pemangkin penemuan ubat yang baharu.

















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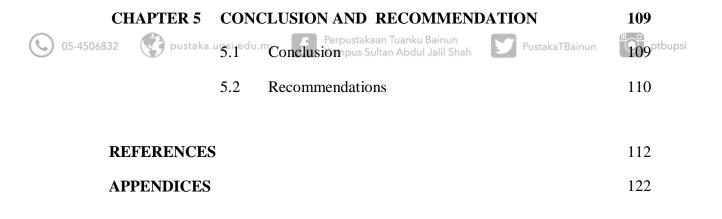








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LIST OF SYMBOLS AND ABBREVIATIONS

	α	Alpha
	β	Beta
	γ	Gamma
	λ	Maximum wave length
	δ	Chemical shift
	Kg	Kilogram
	mg	Miligram
	g	Gram
	Hz	Hertz
	ppm	Part per million
) 05-45068	1D-NMR 32 2D-NMR	One Dimension Nuclear Magnetic Resonance ka.upsi.edu.my Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jeli Shah Two Dimension Nuclear Magnetic Resonance
	¹ H NMR	Proton NMR
	¹³ C NMR	Carbon NMR
	NMR	Nuclear Magnetic Resonance
	HMBC	Heteronuclear Magnetic Bond Correlation
	HMQC	Heteronuclear Magnetic Quantum Coherence
	COSY	Correlation Spectroscopy
	DEPT	Distortionless Enhancement of Polarisation Transfer
	UV	Ultra violet
	IR	Infrared
	MS	Mass spectrometry
	LC-MS	Liquid Chromatography-mass spectrometry

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	ELISA	Enzyme-Linked Immunosorbent Assay
	CC	Column Chromatography
	TLC	Thin Layer Chromatography
	PTLC	Preparative Thin Layer Chromatography
	DCM	Dichloromethane
	R_{f}	Retention factor
	CDCl ₃	Chloroform-D
	MHz	Mega Hertz
	nm	Nanometer
	μL	Microliter
	pNPG	4- nitrophenyl-α-D-glucopyranoside
05-45068	DMSO 32 cm ⁻¹ pusta	Dimethyl Sulfoxide ka.upsi.edu.my f Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah PustakaTBainun ptbupsi Per centimeter
	J	Coupling constant
	d	Doublet
	dd	Doublet of doublet
	t	triplet
	S	Singlet
	br s	Broad singlet
	m	Multiplet











CHAPTER 1

INTRODUCTION



1.1 **Research Background**

Malaysia is one of the world's mega diverse country and placed on the rank of 12th in the world, according to National Biodiversity Index, which based on estimates of country richness and endemism in four terrestrial vertebrate classes and vascular plants. Moreover, Malaysia also has a living heritage of various herbal species that may contain medicinal and healing properties. It has been estimated to have more than 2000 herbal plants in Malaysia and most of them are left unexplored yet that have healing qualities and highly potential to be commercialized (MARDI, 2011). Malaysian Agricultural Research and Development Institute (MARDI) recorded a gross profits of more than RM 5.4 billion a year from herbal related products (Ahmad & Othman, 2014).

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Plant has its own popularity among traditional healer and old folks as medicine. Ethnobotany is a study of how people of particular culture and region make use of indigenous plants or in other words, study of traditional human uses of plants. It is recognized as an effective way to discover future medicines. According to World Health Organization (WHO), over 80% of the global populations relies on traditional medicine since the products of western pharmaceutical industries is not affordable and also influenced by lack of health care facilities (Kamala Pranuthi et al., 2014).

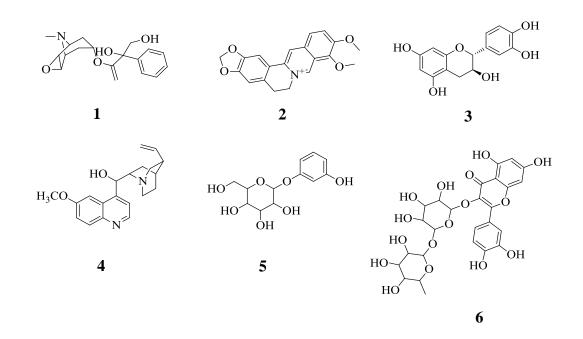
Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend against attack from predators, for example insects. Many of the phytochemicals have beneficial effects on 05-4506 long-term health when consumed by humans and can be used effectively to treat the bupsi human ailments.

Chemical compounds in plants arbitrate their effects on the human body. About 199 chemical substances derived from plants that can be considered as important drugs which currently used in one or more countries all over the world (Farnsworth, Akerele, Bingel, Soejarto, & Guo, 1985). For examples, anisodine **1** that acts anticholinergic agents, berberin **2** that can be used in the treatment of eye infections and diarrhea, catechin **3** that acts as antioxidants, quinine **4** that acts as anthelmintic which acts against infections caused by parasitic worms, rutin **5** helps strengthen the capillaries and salicin **6** that used as pain reliever.









1.2 Objectives

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^{O5-4506} This study aimed on investigating the chemical constituents of *Ficus^B deltoidea* var^{bupsi} *kunstleri*. The objectives guiding this inquiry to:

- i. extract, isolate and purify chemical constituents from different crude extract using various chromatographic techniques.
- ii. elucidate and identify the chemical structure of the isolated compounds using various spectroscopic techniques such as NMR, UV, IR and MS.
- iii. determine the antidiabetic property of crude extracts and the isolated compounds.







1.3 Significance of Research

Naturally occurring substance had been used by humans in long term for medical purposes. Plants consisted second metabolites have played a leading medical role in most cultures among the anchestors and traditional folks. With the development of science at the beginning of the 19th century, plants begans to be examined more closely for understanding their pharmacological properties.

Diabetes mellitus, is a well known metabolic disorders of carbohydrates and characterized by an abnormal postprandial increase of blood glucose level. The enzyme, α -glucosidase secreted from intestinal chorionic epthelium is responsible for the degradation of carbohydrates. Inhibitors of α -glucosidase will slow down the process of digestion and absorption of carboydrates by blocking the activity of glucosidase. Several α -glucosidase inhibitors, for example, acarbose obtained from natural sources and have been used clinically in treatment of diabetes mellitus. But, they have been associated with serious gastrointestinal side effects. Therefore, it is necessary to search for alternatives that can display α -glucosidase inhibitory activity without side reaction. More than 400 plant species having hypoglycemic activity have been available in literature, however, searching for new antidiabetic drugs from natural plants is still attractive since they are containing substances which demonstrate alternative and safe effects on diabetes mellitus (Patel, Prasad, Kumar, & Hemalatha, 2012).

The epiphytic plant, *Ficus deltoidea* form Moraceae family has its own popularity been among traditional healer and old folks for treating ailment such as diarrhea, cold and fever and also claimed to have antidiabetic property as studies have







been carried out to date. Phytochemicals study of *F. deltoidea* var. *kunstleri* is important in finding natural active compounds that potentially useful in medicine practice rather than uses of artificial compounds that may contain hazardous chemicals. Thus, in present study, hopefully, relevant bioactive compounds will be identified from the leaves and stem barks of *F. deltoidea* var. *kunstleri* since lacks of bioactive compounds studies on this plant as for discoverable of potential new drugs that leading to α glucosidase inhibitory activity.

1.4 Family of Moraceae



Moraceae family belongs to order of Rosales and a major group in angiosperm or flowering plant comprising 40 genera and with more than 1000 species that evergreen or shrubs and can be found in tropical and subtropical region. There are 10 genera with 359 species in Malaysia (Flora Singapura, 2014; Natalini nova, 2009). The Moraceae contain important food plants such as *Ficus* (Figs), *Morus* (Mulberry) and *Artocarpus* (Breadfruit and Jackfruit) (Dixon, 2011). According to Watson & Dallwitz (1992), *Moraceae* is classified below.

Domain	: Eukaryote
Kingdom	: Plantae
Phylum	: Tracheophyta
Subphylum	: Euphyllopytina

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		Class	: Magnolio	psida		
		Order	: Rosales			
		Family	: Moraceae			
		Genus	:			
	1.	Antiaris		22.	Naucleopsis	
	2.	Antiaropsis		23.	Olmedia or Trophis	
	3.	Artocarpus		24.	Olmediopsis	
	4.	Bagassa		25.	Parartocarpus	
	5.	Batocarpus,		26.	Perebia	
	6.	Bosqueiopsis		27.	Poulsenia	
	7.	Brosimum		28.	Prainea	
	8.	Broussonetia		29.	Pseudolmedia	
	9.	Castilla		30.	Scyphosyce	
	10.	Clarisia		31.	Sorocea	
	11.	Craterogyne		32.	Sparattosyce_	
05-45068	³² 12. (Cudrania / Mad	<i>flura</i>	Perpustakaan Tuan Kampus Sultar <u>3/3</u> bo	ku Bainun	iun 🚺 ptbupsi
	13.	Dorstenia		34.	Treculia	
	14.	Fatoua		35.	Trilepisium	
	15.	Ficus		36.	Trophis	
	16.	Helianthostylis		37.	Trymatococcus	
	17.	Helicostylis		38.	Utsetela	
	18.	Hullettia		<i>39</i> .	Morus	
	19.	Maclura		40.	Metatrophis	
	20.	Maquira		41.	Milicia	
		-				

21. Mesogyne

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1.4.2 **Botanical Description**

Moraceae are monoecious or dioecious trees shrubs, climbers and herbs and characterized by milky latex in all parenchymatous tissue. Most of the genera in Moraceae family are wind-pollinated by male flowers. Many species are dioecious with male and female flowers on separates male and female tress, while, monoecious species with male and female flowers on the same tree. Flowers are small, unisexual and apetalous (having no petals). It have alternate or opposite leaves and small petalless male or female flowers (Dixon, 2011).

Each minute fruit has an outer fleshy layer (exocarp and mesocarp) and a stony inner layer (endocarp) surrounding the seed. Due to this reason, it is called as drupelets 05-4506 or achene. The fruit enveloped by an enlarged calyx or immersed in a fleshy receptacle, tousi connate in fleshy syncarps (Dixon, 2011).

Uses of Moraceae 1.4.3

Genera Morus and Maclura are important in the production of silk. Some species in Broussonetia, Maclura, and Morus are important for paper making and some species in Artocarpus, Ficus, and Morus have edible fruit.

Moraceae contains a number of latex-producing plants. For example, latex from Ficus species and Artocarpus altilis is employed in chewing gums, glues, caulking compounds, and birdlime (a sticky substance used to ensnare birds).







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Brosimum galactodendron produces an abundance of latex similar in taste to ordinary milk. *F. elastica* was used as an early source of rubber before synthetic rubber was invented. *Antiaris toxicaria* is a valuable source of poison for arrows and darts in Tropical Asia. This is because, its latex containing an extremely toxic cardiac glycoside, which has the effect of increasing the force of contraction of the muscles of the heart. Besides that, *Ficus* species in Fiji and China are used to treat toothache. (Encyclopedia Britannica, 2013)

Several genera in the Moraceae family are valuable sources of timber; for example some species of Artocarpus and Broussonetia which are used for furniture or timber. Wood of Antiaris which not highly durable, is suitable for veneer and plywood, furniture components, and light construction used by African. Species of Artocarpus 05-4506 produce woods of variable durability used in joinery, furniture and cabinetwork, and burst musical instruments in Southeast Asia and Oceania. In the American tropics, Bagassa, Brosimum, Clarisia, Helicostylis, and Poulsenia are all sources of wood for general construction. Brosimum guianense also called leopardwood or snakewood is employed in inlays, turnery, fancy handles for cutlery, and violin bows (Encyclopedia Britannica, 2008)

1.5 The Genus *Ficus*

1.5.1 Botanical Descriptions of Ficus





Ficus is one of the genus in the Moraceae family and member of tribe Ficeae. It comprises one of the largest genera of angiosperms. There are about more 800 species of trees, shrubs, climbers and creepers (Frodin, 2004). The plants are distributed throughout the world tropics, native primarily to tropical of East Asia and it is rare in temperate regions. Based on the Plant List 2013, there are total of 830 species are recognized, for examples, *F. abelii* Miq, *F. bakeri* Elmer, *F. calcarata* Corner, *F. deltoidea* Jack, *F. elmeri* Merr., *F. fiskei* Elmer, *F. geniculata* Kurz, *F. hahliana* Diels, *F. immanis* Corner, *F. jansii* Boutique, *F. katendei* Verdc, *F.lamponga* Miq., *F. macilenta* King, *F. nana* Corner, *F. oleracea* Corner, *F. pachyclada* Baker, *F. racemosa* Linn., *F. sabahana* Kochummen, *F. tilifolia* Baker, *F. ulmifolia* Lam, *F. vasta* Forssk *F. wassa* Roxb and others.



1.5.2 Ficus deltoidea

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One of the species in the genus is *Ficus deltoidea*. It is commonly known as "Mas Cotek", "Serapat Angin", "Telinga Beruk" or "Sempit-Sempit" in Malaysia due to fine spots with gold colour on the surface of each leaves (Nashriyah et al., 2012) and called as kangkalibang by African can be recognized by very distinctive inflorescence.

The plant is an evergreen shrub that reaches a height of two meters, with whitish grey bark. The leaves are broadly spoon-shaped to obovate and the leaf length between 4 cm and 8 cm with bright green coloured above and rust-red to olive-brown beneath (Hamidun, Noriha, Siti Noraini, Syarul Nataqain, & Normah, 2014). The fruit a seedlike achene with a width of 1 to 1.5 cm and has yellow to orange-red coloured,







usually enclosed within syncarp formed which form from an enlarged hollow fleshy receptacle.

It is distributed in Malesia that include Thailand, Indonesia and Malaysia (Lansky & Paavilainen, 2011). It can be found growing on bris soil along the costal areas, peat soils and most forest habitats except the mangrove swamps (Musa, 2006). Seven varieties of *F. deltoidea* can be found in Malaysia, namely var. *intermedia* Corner, var. *bilobata* Corner, var. *kunstleri* (King) Corner, var. *angustifolia* (Miq.) Corner, var. *deltoidea*, var. *motleyana* (Miq.) Corner, and var. *trengganuensis* Corner (Kochummen, 1978).

F. deltoidea is a one of the common traditional medicinal plants in Malaysia. It **O5-4506832 Destate upsi-edu my** is believed that different parts of plants can cure different type of diseases, for example, the fruits are chewed to relieve headache, toothache and cold. The leaves of the plant are used traditionally for treating diabetes, high blood pressure, heart problems, gout, diarrhea, pneumonia and skin diseases. Decoction of the whole plant has been used as tonic by women afterbirth for strengthen their uterus (Sulaiman et al., 2008).

1.5.3 Ficus deltoidea var kunstleri

Leaf morphology of *Ficus deltoidea* variation the most variable and shows heterophylly in this species (Nashriyah et al., 2012). Figure below showed the characteristics of *F*. *deltoidea* var *kunstleri*.

