

CHEMICAL CONSTITUENTS FROM THE CALYCES OF *HIBISCUS*
SABDARIFFA LINN. AND ITS α -GLUCOSIDASE
INHIBITORY ACTIVITY

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ABSTRACT

The purpose of this study was to investigate the chemical compounds from the calyces of *Hibiscus sabdariffa* Linn. and their α -glucosidase inhibitory activity. Samples were collected from Balik Pulau, Pulau Pinang. The total of three kilogram of samples were air dried at room temperature, grinded and serially extracted by solid liquid extraction technique using hexane, dichloromethane, methanol, ethanol and water. Compounds were isolated and purified by several chromatographic techniques. Their structures were elucidated with spectroscopic methods including Nuclear Magnetic Resonance (1D-NMR and 2D-NMR), Liquid Chromatography-Mass (LCMS) spectrometry, infrared (IR) spectroscopy, ultraviolet (UV) spectroscopy and compared with data reported in the literature. The α -glucosidase inhibitory activity on the crude extracts and pure compounds was conducted using α -glucosidase inhibitory assay. The finding of this phytochemical study has led to the isolation of seven pure compounds namely squalene, triglyceride fatty acid, ethyl stearate, 4-ethoxy-3-((ethoxycarbonyl)oxy)-4-oxobutanoic acid, 2-(1-ethoxy-4-methoxy-1,4-dioxobutan-2-yl)-1,5-dimethyl-3-(2-ethoxy-2-oxoethyl)-4-(2-methoxy-2-oxoethyl-3-(methoxycarbonyl)oxy)pentane-1,2,5-tricarboxylate, 2-(2-ethoxy-2-oxoethyl)-1,5-dimethyl-3,4-bis-(2-methoxy-2-oxoethyl)-3-((methoxycarbonyl)oxy)pentane-1,2,5-tricarboxylate, 7-((1,5-dimethoxy-1,4-dioxo pentan-3yl)oxy)-3,11-bis(ethoxycarbonyl)-7-(3-methoxy-1-((methoxycarbonyl)oxy)-3-oxopropyl)-5,9-dioxo-4,6,8,10-tetraoxatridecane-1,8-dioic acid along with two mixture compounds; mixture of stigmasterol with β -sitosterol and mixture of 3-hydroxy-4-oxopentanal with 5-hydroxy methylfurfural. The methanol crude extract showed the highest activity among other crude extracts towards α -glucosidase compared to acarbose and quercetin. In addition, the selected isolated compounds were not active against the α -glucosidase inhibitory activity. As a conclusion, inactive compounds which isolated from the calyces of *Hibiscus sabdariffa* Linn. did not show any α -glucosidase inhibitory activity. Implication of this study is it can be used as future references for phytochemical studies of this species to discover possible potential new drugs in pharmaceutical.

KANDUNGAN KIMIA DARIPADA KELOPAK *Hibiscus sabdariffa* Linn. DAN AKTIVITI PERENCATAN A-GLUKOSIDANYA

ABSTRAK

Tujuan kajian ini ialah untuk mengkaji kandungan kimia daripada kelopak *Hibiscus sabdariffa* Linn. dan aktiviti perencatan α -glukosidanya. Sampel dikumpul dari Balik Pulau, Pulau Pinang. Sebanyak tiga kilogram sampel dikeringkan pada suhu bilik, dikisar dan diekstrak secara bersiri dengan teknik pengekstrakan cecair-pepejal menggunakan heksana, diklorometana, metanol, etanol dan air. Sebatian telah dipencilkan dan dituliskan melalui beberapa teknik kromatografi. Struktur sebatian telah dikenalpasti dengan kaedah spektroskopik termasuk Resonans Magnetik Nuklear (1D-RMN dan 2D-RMN), kromatografi cecair spektroskopi jisim (LCMS), spektroskopi inframerah (IR) dan spektroskopi ultraviolet (UV) dan dibandingkan dengan kajian literatur. Aktiviti perencatan α -glukosida pada ekstrak mentah dan sebatian tulen telah dijalankan menggunakan ujian perencatan aktiviti α -glukosida. Hasil dapatan daripada kajian fitokimia ini membawa kepada pemencilan tujuh sebatian tulen iaitu squalena, asid lemak trigliserida, etil stearat, asid 4-etoksi-3-(etoksi karbonil)oksi-4-oksobutanoik, 2-(1-etoksi-4-metoksi-1,4-dioksobutan-2-il)-1,5-dimetil-3-(2-etoksi-2-oksoetil)-4-(2-metoksi-2-oksoetil-3-(metoksikarbonil)oksi)pentana-1, 2,5-trikarboksilat, 2-(2-etoksi-2-oksoetil)-5-dimetil-3,4-bis-(2-metoksi-2-oxoetil)-3-(metoksikarbonil)pentana-1,2,5-trikarboksilat, asid 7-dimetoksi-1,4-dioksopentana-il)-3, 11-bis(etoksikarbonil)-7-(3-metoksi-1-(metoksikarboniloksi)-3-oksopropil)-5,9-diokso-4,6,8,10-tetraoksatri dekana-1,8-dioik bersama-sama dengan dua sebatian campuran stigmasterol dengan β -sitosterol dan campuran 3-hidroksi-4-oksopentanal dengan 5-hidroksimetilfurfural. Ekstrak mentah metanol menunjukkan aktiviti yang paling tinggi dalam kalangan ekstrak mentah yang lain terhadap α -glukosida berbanding dengan akarbos dan kuersetin. Tambahan pula, sebatian terencil yang terpilih tidak aktif terhadap aktiviti perencatan α -glukosida. Sebagai kesimpulan, sebatian tidak aktif yang terencil daripada kelopak *Hibiscus sabdariffa* Linn. tidak menunjukkan sebarang aktiviti perencatan α -glukosida. Implikasi kajian ini ialah ia boleh digunakan sebagai rujukan bagi kajian fitokimia bagi spesies ini untuk penemuan ubat yang baru dalam bidang farmaseutikal.

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LIST OF ABBREVIATIONS

°C	Degree Celcius
μL	Microliter
μm	Micrometer
¹³ C NMR	Carbon Nuclear Magnetic Resonance
1D NMR	One Dimension Nuclear Magnetic Resonance
¹ H NMR	Proton Nuclear Magnetic Resonance
2D NMR	Two Dimension Nuclear Magnetic Resonance
ACN	Acetonitrile
CC	Column Chromatography
CDCl ₃	Deuterated Chloroform
CH ₃	Methyl group
CHCl ₃	Chloroform
cm	Centimeter
cm ⁻¹	Per centimeter
COSY	Correlation Spectroscopy
<i>d</i>	Doublet
DCM	Dichloromethane
<i>dd</i>	Doublet of doublet
DEPT	Distortioness Enhancement by Polarization Transfer
FTIR	Fourier Transform Infrared
g	Gram
H ₂ O	Water
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Correlation
HPLC	High Performance Liquid Chromatography
HSQC	Heteronuclear Single Quantum Correlation
Hz	Hertz
IC ₅₀	Inhibition Concentration

IR	Infrared
<i>J</i>	Coupling Constant
K ₂ HPO ₄	Dipotassium Hydrogen Phosphate Anhydrous
kg	Kilogram
KH ₂ PO ₄	Potassium Dihydrogen Phosphate Anhydrous
LC-MS	Liquid Chromatography-Mass Spectrum
m	Meter
M	Molar
<i>m</i>	Multiplet
<i>m/z</i>	Mass per charge
MeOD	Deuterated Methanol
MeOH	Methanol
mg	Milligram
MHz	Mega Hertz
min	Minute
ml	Milliliter
mm	Millimeter
mM	Milli Molar
Na ₂ CO ₃	Sodium Carbonate
nm	Nanometer
NMR	Nuclear Magnetic Resonance
OCH ₃	Methoxyl group
OH	Hydroxyl group
PBS	Phosphate buffer solution
pNPG	4-nitrophenyl- α -D-glucoopyranoside
ppm	Part per million
PTLC	Preparative Thin-Layer Chromatography
Recycled-HPLC	Recycle High Performance Liquid Chromatography
<i>s</i>	Singlet
<i>t</i>	Triplet
TLC	Thin Layer Chromatography
UV	Ultra Violet
α	Alpha

β	Beta
γ	Gamma
δ	Chemical shift
λ	Wavelength



CHAPTER 1

INTRODUCTION



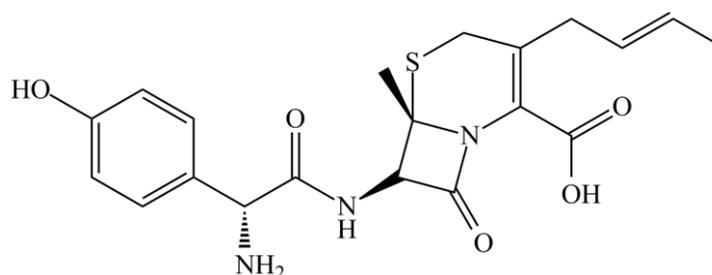
1.1 General

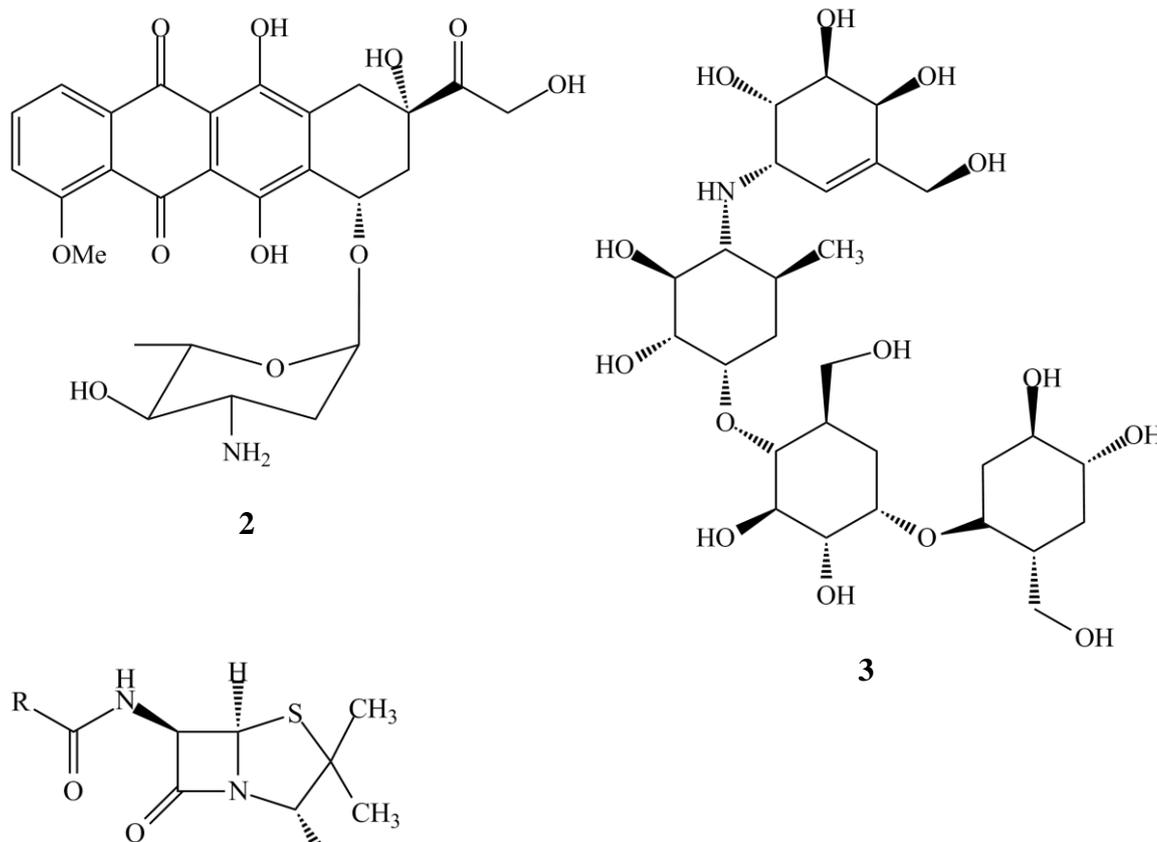
Biodiversity play an important role in human daily life. Biodiversity affect us in many ways like in food security, health, fisheries, tourism, forestry, biotechnology and others. Biodiversity can be defined as the variety of life on earth and includes variation at all levels of biological organization from genes to species to ecosystems. Genetic, organism and ecological diversity are all elements of biodiversity with each including a number of components (Gaston & Spicer, 2004; Vere, 2008). According to the World Development Indicators, Malaysia has only 0.2% of the world land mass, but its diversity of flora and fauna species makes it one of the richest countries in the world in terms of biodiversity per unit area (Ministry of Natural Resources and Environment,



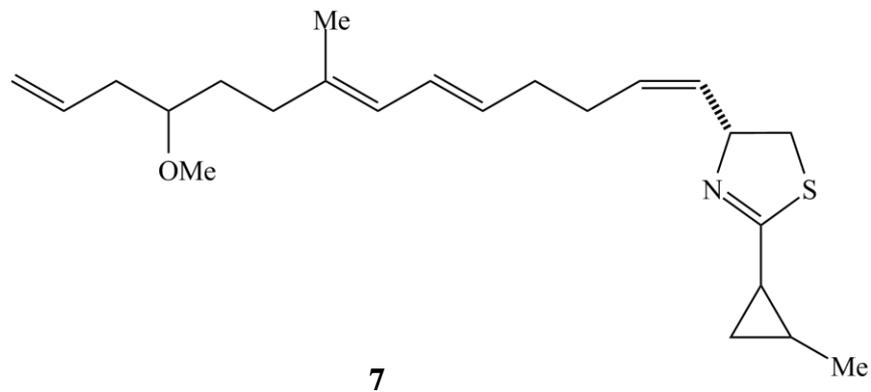
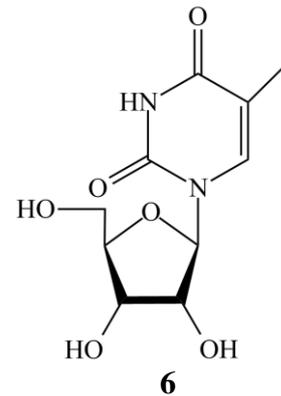
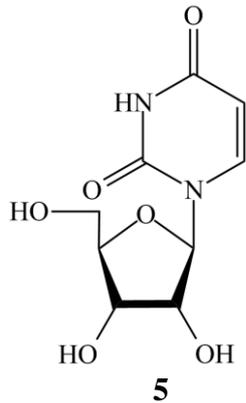
2006). The 2001 Global Diversity Outlook recognized Malaysia as one of the 12 mega-diversity countries in the world. There are 12,500 species of flowering plants and more than 1,100 species of ferns and fern allies in Malaysia (Hafidh et al., 2009). The basis of most traditional medicines come from the species of plants, animals and micro-organism and there are rural communities still depends on it for their health care needs. Research institutes see the rich in biodiversity especially the tropics as a source of new drugs (Ministry of Natural Resources and Environment, 2006).

From the early findings, a lot of drugs discovered in pharmaceutical based on natural products. Natural products can be defined as chemical compounds from nature that usually have a pharmacological or biological activity. (Natural Products Chemistry & Research, 2014). Natural products can be derived from microbial, plant, animal and marine categories. For example, antibacterial agents (cephalosporins **1**), anticancer agents (epirubicin **2**) and antidiabetic agent (acarbose **3**) are derived from microorganism (Chin, Balunas, Chai & Kinghorn, 2006). Natural product derived from microorganism was discovered since the discovery of penicillin **4**, in year 1929.

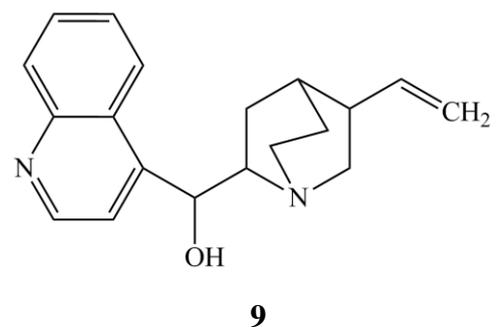
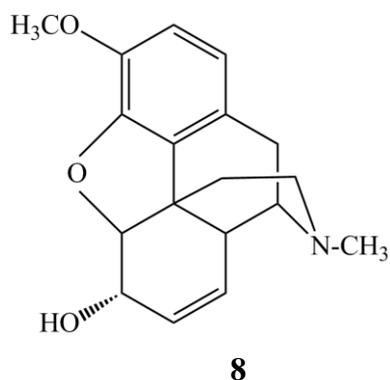
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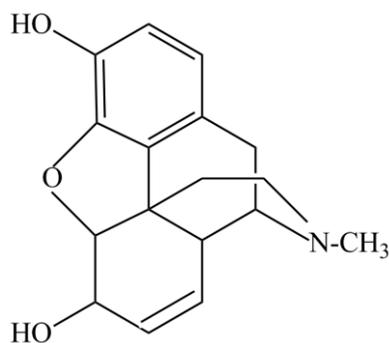


Besides that, natural products also can be derived from marine like coral, sponges, fish and marine microorganism. A study carried out by Heafner B. in 2003, found that compounds from marine potent in antiviral, anticancer and inflammatory activities. For example, spongouridine **5**, and spongothymidine **6**, derived from *Cryptotheca crypta* give potential as anticancer and antiviral agents while curacin A **7**, shows potent antitumor activity that obtained from a marine cyanobacterium (Nautiyal, 2013).

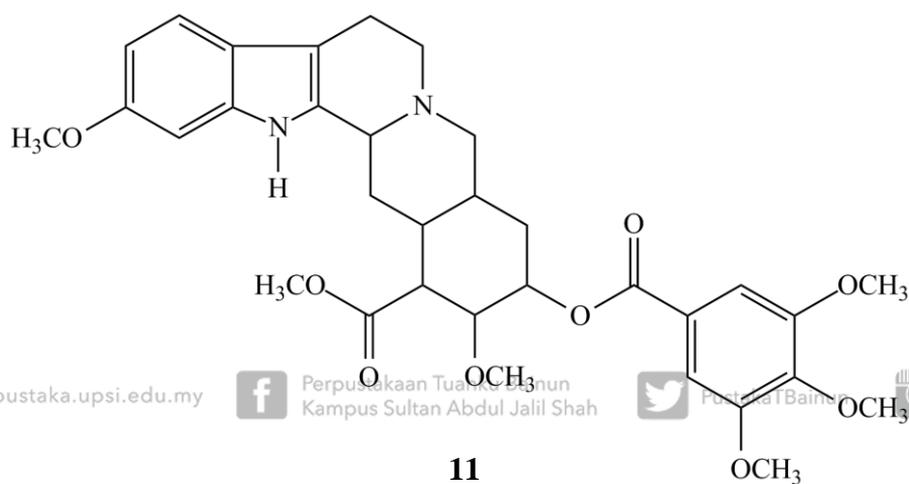


Morphine **8**, quinine **9**, codeine **10**, and reserpine **11** are example of natural product derived from plant used in medical (Pelletier, 1983). Morphine **8** and codeine **10** that isolated from *Papaver somniferum* are used as pain relief while quinine **9** and reserpine **11** are used for the treatment of malaria and antihypertensive, respectively.





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1.2 Objectives

This study generally aimed at investigating the chemical constituent from the calyces of *Hibiscus sabdariffa* Linn. and its α -glucosidase inhibitory activity. The specific objectives of this study are;

- i. to extract, isolate and purify chemical compounds from various crude extracts of *Hibiscus sabdariffa* Linn.) which are hexane, dichloromethane and methanol crude extracts using chromatographic techniques

- ii. to elucidate the structure of isolated compounds from the calyces of roselle (*Hibiscus sabdariffa* Linn.)
- iii. to investigate the α -glucosidase inhibitory activity of *Hibiscus sabdariffa* Linn. crude extracts and the isolated compounds

1.3 Significant of Research

Hibiscus sabdariffa Linn. or roselle is a medicinal potential plant which is distributed throughout the tropics and subtropics countries. At Malaysia, it widely planted at Terengganu, Kelantan and Johor. The calyces of roselle had been used among Malaysian as juice, jam, jelly, herbal tea and also used in medical purpose such as for treatment of diabetes (Seema, 2014). According to study carried out by Rosemary, Rosidah and Ginda in 2014, ethanol extract from the calyces of roselle proven to reduce blood glucose levels in diabetic mice.

The cases of diabetes are increasing worldwide at an alarming rate due to changes in lifestyle. According to World Health Organization in 2016, 2380 cases of deaths due to diabetes were reported among Malaysian people in age range 30 – 69. There are two types of diabetes which are juvenile-onset diabetes (type 1) and non-insulin dependent diabetes (type 2) (International Diabetes Federation, 2018). More than 90% of diabetic population has type 2 diabetes. The maintenance of healthy blood glucose levels is very important for treating this type of diabetes (Tsujita et al., 2008). Therefore, in order to control the blood glucose levels, the patients need to undergo diet, weight control and do physical activities. If blood glucose level remains high, then

drug such as α -glucosidase inhibitors are usually advised. α -glucosidase inhibitors slow down digestion by blocking enzymes in the small intestine that break down carbohydrates. So that, it can slow down the digestion of carbohydrate, thus reducing the rise in blood glucose levels after eating.

Previous studies on this plant only focusing on the polar crude extracts which are methanol, ethanol and water crude extracts and were adding acid during the extraction process. Thus, this study was carried out to isolate chemical compounds from the calyces of *Hibiscus sabdariffa* Linn. by using non-polar and polar solvent without using an acid during the extraction process to compare any similar structures with previous studies and hopefully, having potential for α -glucosidase inhibitory activities as for discoverable potential new drugs in pharmaceutical.

1.4 Family of *Malvaceae*

1.4.1 Taxonomy

Malvaceae is a major group in Angiosperm or flowering plant. *Malvaceae* is estimated has 243 genera and 4225 species widely distributed throughout the world and particularly abundant in tropics (Berry, 2013). *Malvaceae* has three members which are herbs, shrubs and trees. It can be categorized based on the characteristics of the flower, leaf and woodiness. There are many genus under *Malvaceae* family for example, *Althaea*, *Brownlowia*, *Corchorus*, *Durio*, *Gossypium*, *Heritiera*, *Mansonia*, *Ochroma*,