



UNIVERSITI PUTRA MALAYSIA

***ANTIOXIDANT AND ANTIDIABETIC ACTIVITY OF PHENOLIC
COMPOUNDS EXTRACTED FROM *Orthosiphon stamineus*
EXTRACTS***

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COMPOUNDS EXTRACTED FROM *Orthosiphon stamineus* EXTRACTS**

By

NURAZRIN EZRINA ABDUL MUBDI

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

May 2019

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Master of Science

**ANTIOXIDANT AND ANTIDIABETIC ACTIVITY OF PHENOLIC
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May 2019

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Orthosiphon stamineus Benth (Lamiaceae), locally called Misai Kucing, is a local herb that grows widely in Malaysia. It is gaining popularity and well-known plant in herbal medicine. The objective of this study is to identified and quantified the antioxidant activity and phenolic compounds using HPLC, HPLC-PDA-MS² and HPLC with post-column on-line antioxidant detection based on 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging assay. The results had shown the phytochemicals in water extracts contained salvinalic acid, chlorogenic acid, caffeic acid and rosmarinic acid. Salvinalic acid and caffeic acid in *O. stamineus* extracts possess high Trolox Equivalent Antioxidant Ratio (TEAR) values by 0.74 and 0.50 respectively. *O. stamineus* has been scientifically proven to have hypoglycaemic and antihyperglycaemic activity. In this study, hypoglycaemic and antihyperglycaemic activity of *O. stamineus* water extracts in normal and diabetic rats (streptozotocin induced) was evaluated. In evaluation of hypoglycaemic activity of *O. stamineus* extracts in normal rats, medium doses (15 mg/kg) and high doses (30 mg/kg) significantly reduced blood glucose level to 4.5 mmol/L and 4.14 mmol/L after 6 (p < 0.05) hours extracts administration. Metformin, 500 mg/kg also shows hypoglycaemic activity after 6 hours administration. The evaluation of antihyperglycaemic activity, the results showed that *O. stamineus* extracts significantly reduced fasting and postprandial hyperglycaemic particularly after 2 and 4 hours extracts administration at high doses (30 mg/kg). In conclusion, the usage of analytical instruments such as HPLC, HPLC-PDA-MS² and antioxidant assays has prompted to identification of phenolic compounds and antioxidant activities in Malaysian traditional herbs. This study showed that *O. stamineus* water extract can be employed and suitable as dietary subordinate to deal with hyperglycemia in diabetes patients.

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

ANTIOKSIDAN DAN AKTIVITI ANTIDIABETIK SEBANYAK FENOLIK YANG DIEKSTRAK DARIPADA EKSTRAK *Orthosiphon stamineus*

Oleh

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Orthosiphon stamineus Benth (Lamiaceae), secara setempat dipanggil Misai Kucing, adalah herba tempatan yang tumbuh di Malaysia. Ia semakin popular dan tumbuhan terkenal dalam perubatan herba. Objektif kajian ini adalah untuk mengenalpasti dan mengkuantitikan aktiviti bahan antioksidan dan sebatian fenolik menggunakan HPLC, HPLC-PDA-MS² dan HPLC dengan tiang-turus pengesanan bahan antioksidan dalam talian berasaskan 2,2'-azinobis-(3-etilbenzotiazolin-6-asid sulfonik (ABTS) radikal cerakin skaveng. Keputusan telah menunjukkan fitokimia di dalam ekstrak air itu terkandung asid salvinalik, asid klorogenik, asid kafeik dan asid rosmarinik. Asid salvinalik dan asid kafeik pada ekstrak *O. stamineus* mempunyai tinggi nilai nisbah bahan antioksidan setara Trolox (TEAR) oleh masing-masing 0.74 dan 0.50. *O. stamineus* telah terbukti secara saintifik untuk mempunyai aktiviti hipoglisemia dan antihiperghlisemia. Dalam kajian ini, aktiviti hipoglisemia dan antihiperghlisemia *O. stamineus* ekstrak air pada normal dan tikus diabetik sederhana (aruhan streptozotosin) telah ternilai. Dalam penilaian aktiviti hipoglisemia ekstrak *O. stamineus* pada tikus normal, dos medium (15 mg/kg) dan dos tinggi (30 mg/kg) itu nyata sekali paras glukosa darah dikurangkan kepada 4.5 mmol/L dan 4.14 mmol/L selepas 6 jam ($p < 0.05$) pemberian ekstrak. Metformin, 500 mg/kg juga menunjukkan aktiviti hipoglisemia selepas 6 jam ($p < 0.05$) pemberian ekstrak. Penilaian aktiviti antihiperghlisemia, keputusan menunjukkan ekstrak *O. stamineus* itu nyata sekali mengurangkan hiperghlisemia berpuasa dan selepas makan terutamanya selepas 2 dan 4 jam pemberian ekstrak pada dos tinggi (30 mg/kg). Kesimpulannya, penggunaan alat analisis seperti HPLC, HPLC-PDA-MS² dan cerakinan bahan antioksidan telah digesa untuk pengenalan sebatian fenolik dan aktiviti bahan antioksidan dalam herba tradisional Malaysia. Kajian ini menunjukkan *O. stamineus* ekstrak air itu boleh digunakan dan sesuai sebagai subordinat dietari untuk berurusan dengan hiperghlisemia di dalam pesakit diabetes.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

%	Percentage
µg	Microgram
µl	Microliter
µm	Micrometer
ABTS	2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt
ATP	Adenosine triphosphate
cm ³	Centimeter cubic
CO ₂	Carbon dioxide
DNA	Deoxyribonucleic acid
ESI	Electrospray ionization
FBG	Fasting blood glucose
GC	Gas chromatography
H ₂ O	Water
HCL	Hydrochloric acid
HPLC	High performance liquid chromatography
IDDM	Insulin dependent diabetes mellitus
kg	Kilogram
L	Liter
M	Molar
MARDI	Malaysian Agricultural Research and Development Institute
mg	Milligram
ml	Milliliter
mM	Millimolar
mm	Millimeter

MS	Mass spectrometry
[M-H] ⁻	Negatively charged molecular ion
<i>m/z</i>	Mass to charge ratio
NaCl	Sodium chloride
NIDDM	Non-insulin dependent diabetes mellitus
°C	Degree Celsius
OGTT	Oral glucose tolerance test
PBS	Phosphate buffer saline
PDA	Photodiode array
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
STZ	Streptozotocin
SU _s	Sulfonylurea
t _R	Retention time
TZD _s	Thiazolidinediones
UPM	Universiti Putra Malaysia
USA	United State of America
UV	Ultraviolet
TEAR	Trolox equivalent antioxidant ratio
v/v	Volume per volume
w/v	Weight per volume
WHO	World Health Organization
α	Alpha
β	Beta

CHAPTER 1

INTRODUCTION

1.1 Background of study

Herbal products have been generally usefulness as therapeutically specialists and nutritional enhancements in both Eastern and Western societies. Around 15,000 blooming flowering species develop in Malaysia and more than 3000 varieties have remained distinguished as conceivable therapeutic plants (Ramlan, 2009; Akowuah et al., 2012). Some of the Malaysian locality herbs, regularly identified as *Orthosiphon stamineus* Benth (Lamiaceae) otherwise privately named 'Misai Kucing', gives widely application for study intend.

Numerous terms intended for *O. stamineus* incorporate *Orthosiphon aristatus*, *Orthosiphon blaetter*, and Java tea, 'Misai kucing', 'Kumis kucing', "Indischer Nierentee", "Feuilles de Barbiflore" also "de Java". *Orthosiphon stamineus* is likewise found in different nations, for example, Thailand, Indonesia and Europe (Almatar et al., 2014). It is a folklore Asian herbal medicine which is widely interest for treatment of variety of disease, particularly to treat inflammation, bacterial infections, urinary tract infections, rheumatism, jaundice and angiogenesis-related problems like cancer (Al-Suede et al., 2014; Ucuzian et al., 2010; Geng et al., 2013). In Malaysia, *O. stamineus* has two variants based on flowery appearance which was not very clear if not wisely detected. Some of the assortments generate white blooms while another different show corolla with existence purplish color at feather-edge of the petal lobes, subsequently called as the white and purple assortments, separately (Lee, 2004).

Health benefits of the traditional application of *O. stamineus* were verified by the separation and identification of a few chemical constituents, including flavonoids (Malterud et al., 1989; Sumaryono et al., 1991), terpenoids (Masuda et al., 1992; Tezuka et al., 2000), saponins, chromene, hexoses, myo-inositols, caffeic acid derivatives and organic acid (Masuda et al., 1992; Tezuka et al., 2000; Olah et al., 2003). According to Olah et al., 2003, the most important parts of *O. stamineus* leaves consist of polyphenols and caffeic acid derivatives (rosmarinic acid, cichoric acid and caffeic acid); polymethoxylated flavonoids (sinensetin and eupatorine).

1.2 Problem statement

Nowadays, *O. stamineus* is gaining popularity as an herbal supplement as it gives a lot of benefits to overcome the disease. It is because most people start

to realize the important of healthy awareness. A wide-range of research has been carried out on *O. stamineus*, including identification of bioactive compounds and bioassays of solvent extracts. However, not many studies reported about the study of free phenolic acids (rosmarinic acid and caffeic acid) within water extract for the advancement as new anti-diabetic agent.

O. stamineus is commonly used by soaking leaves in hot water. The most recent study was undertaken by using methanol and acetone to develop standardized extract which may not be safe and not applicable for food industries. Therefore, the aim of the present study on the use of non-solvent materials to extract and optimize activity of phenolic compounds in *O. stamineus* need to be carried out. Extraction and optimization of phenolic compounds can be done by using high temperature or pressure.

The benefits of plant phenolics, which contribute to health, especially antidiabetic, rely upon their bioavailability. In human, plant phenolics appear to be ingested in high amounts in the stomach and small digestive system. Most of the plant phenolics are metabolized in colon by action of colonic microflora into phenolic acids. Interaction with food matrices (i.e. lipid and protein) also decreased the absorption of plant phenolics. In this research, high free phenolic acids of *O. stamineus* extract with antidiabetic properties, highly and rapidly absorbed in human, need to be developed into producing and commercialize as a new product.

1.3 Significance of study

According to Mafauzy, (2004), plant such as *Averrhoa bilimbi*, *Tinospora crispa*, *Andrographis paniculata*, *Moringa oleifera* and *Gynura procumbens* have been accounted through scientific studies showing antidiabetic activities and have been widely utilized traditionally in the society as medicines for diabetes mellitus in Malaysia. Besides these plants, there is common plant which has been claimed to possess antidiabetic property, namely *Orthosiphon stamineus* or locally known as Misai kucing. *O. stamineus* is establish with its therapeutical and dietary supplements in both Eastern and Western societies (Chan, 2003).

Generally, the active chemical compounds such as oleanolic acid, polyphenols, flavonoids and terpenoids is rich in *O. stamienus*. The most dominant compound in *O. stamineus* leaves prevents formation of lipid peroxidation and has a considerable role in reducing oxidative stress (Shekarchi et al., 2012; Lanez et al., 2016; Lanez et al., 2015). Almost 20 phenolic compounds, for example, 9 caffeic acid derivatives (rosmarinic acid), 9 lipophilic flavones and 2 flavonol glycosides are presence which can be related to its antioxidant property (Tezuka et al., 2000). As indicated by Akowuah et al., 2005, *O. stamineus* leaf has the highest antioxidant properties as a result of its better phenolic fractionation compared to the other parts of plant.

1.4 Objectives of study

General objective

To analysis phenolic acid and antidiabetic activities of *O. stamineus* water extracts.

Specific objectives

1. To quantify phenolic compounds and its antioxidant activity of *O. stamineus* water extracts using HPLC and HPLC-PDA-MS².
2. To determine the hypoglycaemic and antihyperglycaemic activity of *O. stamineus* water extracts on Sprague-Dawley rat models.

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