



**CHEMICAL CHARACTERIZATION AND ANTIOXIDANT POTENTIAL OF
Stevia rebaudiana BERTONI ESSENTIAL OIL OBTAINED FROM
SUBCRITICAL CARBON DIOXIDE EXTRACTION**

MUHAMMAD FAHMI BIN MAT YUSOF

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By

MUHAMMAD FAHMI BIN MAT YUSOF

**Thesis Submitted to the School of Graduate, University Putra Malaysia, in
Fulfillment of the Requirements for the Master of Science**

August 2018

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfillment of the requirement for the Degree of Master of Science

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Chairman: Uswatun Hasanah Zaidan, PhD
Faculty : Biotechnology and Biomolecular Sciences

Many aromatic and medicinal plants are available in Malaysia from which the essential oil (EO) can be extracted and used for multipurpose. The increasing number of cases of noncommunicable diseases (NCDs) such as diabetes, cancer, respiratory diseases, and cardiovascular diseases is becoming a growing concern, thus through the extraction of EO from *Stevia rebaudiana*, it can become an agent to cure these diseases. *S. rebaudiana* EO was extracted using subcritical carbon dioxide (SC-CO₂) method for the first time and their chemical compounds were characterized by gas chromatography-mass spectrometry (GC-MS) and Fourier transform-infrared (FT-IR) spectroscopy. The highest productivity, yield of the extracted EO was found in the sample to an ethanol ratio of 1:0.5 with 5.6%, followed by the ratio of 1:1 with 3.6% and 1:1.5 with 2.4%. About 33 up to 50 compounds were identified in EO through GC-MS method which representing 89.32%, 92.45%, and 81.42% of identified compound out of the total oil at different extraction ratio of 1:0.5, 1:1, and 1:1.5, respectively. The principal components in the EO were lupeol acetate, epiputranjivol, α -linoleic acid, hexadecanoic acid, and spathulenol. Interestingly, some new compounds were identified, such as globulol, betulinic aldehyde, ionone, and γ -sitosterol which had not previously been reported in *S. rebaudiana* EO. In addition, FT-IR analysis showed a greater abundance of alkynes, aldehydes, alkene, and alkanes were present in the EO. Furthermore, *S. rebaudiana* EO presented an interesting radical scavenging activity (RSA) against 1,1-diphenyl-2-picryl hydrazyl (DPPH) with IC₅₀ values of 0.91 ± 0.25 μ g/mL. Results obtained indicated the efficiency of SC-CO₂ employed for extracting *S. rebaudiana* essential oil, which could be a good source of natural antioxidant and may be beneficial as a functional biomaterial with possible applications in food and pharmaceutical which can be used to cure the NCDs.

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

PENCIRIAN KIMIA DAN POTENSI ANTIOKSIDA MINYAK PATI *Stevia rebaudiana* BERTONI DIPEROLEHI DARI PENGEKSTRAKAN SUBKRITIKAL KARBON DIOKSIDA

Oleh

MUHAMMAD FAHMI BIN MAT YUSOF

Ogos 2018

Pengerusi: Uswatun Hasanah Zaidan, PhD
Fakulti : Bioteknologi dan Sains Biomolekul

Tumbuh-tumbuhan aromatik dan ubat-ubatan terdapat banyak di Malaysia di mana minyak pati (EO) boleh diekstrak dan digunakan untuk pelbagai kegunaan. Bilangan kes-kes 'non communicable diseases' (NCDs) seperti kegemukan, kanser, penyakit gangguan sistem pernafasan, dan penyakit jantung yang semakin bertambah menjadikan penyakit ini sesuatu yang membimbangkan, maka dengan pengekstrakan EO daripada *Stevia rebaudiana* ini, ianya boleh menjadi agen untuk mengubati penyakit-penyakit ini. EO *S. rebaudiana* telah diekstrak dengan menggunakan kaedah subkritikal karbon dioksida (SC-CO₂) buat pertama kalinya dan sebatian kimia minyak pati ini telah dicirikan dengan menggunakan spektroskopi jisim kromatografi gas (GC-MS) dan Fourier spektroskopi inframerah (FT-IR). Produktiviti tertinggi ekstrak EO yang telah didapati dalam nisbah sampel kepada etanol pada 1:0.5 sebanyak 5.6%, diikuti dengan nisbah 1:1 sebanyak 3.6%, dan 1:1.5 sebanyak 2.4%. Kira-kira 33 sehingga 50 sebatian telah dikenalpasti melalui kaedah GC-MS yang mewakili 89.32%, 92.45%, dan 81.42% daripada jumlah minyak yang telah diekstrak masing-masing pada nisbah 1:0.5, 1:1, dan 1:1.5. Komponen utama di dalam EO adalah lupeol asetat, epiputranjivol, asid linoleik, asid heksadekanoik, dan spatulenol. Menariknya, beberapa sebatian baru telah dikenalpasti seperti globulol, betulinik aldehyd, ionone, γ -sitosterol di mana sebelum ini belum pernah dilaporkan di dalam EO *S. rebaudiana*. Di samping itu, analisis FT-IR telah menunjukkan bilangan yang banyak bagi alkena, alkina, aldehid, dan alkana yang hadir di dalam EO. Selain itu, EO *S. rebaudiana* menunjukkan aktiviti pemerangkapan radikal terhadap hidrazil 1,1-difenil-2-pikril (DPPH) dengan nilai IC₅₀ 0.91 \pm 0.25 μ g/mL. Keputusan yang diperolehi menunjukkan kecekapan SC-CO₂ untuk mengekstrak EO *S. rebaudiana* boleh menjadi sumber antioksidan yang boleh memberi manfaat seperti biobahan berfungsi bersama dengan aplikasi di dalam makanan dan farmaseutikal yang mungkin boleh digunakan untuk merawat NCDs.

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

Uswatun Hasanah binti Zaidan, PhD

Senior Lecturer

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Chairman)

Syahida binti Ahmad, PhD

Senior Lecturer

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Member)

Azizah binti Abdul Hamid, PhD

Associate Professor

Faculty of Food Science and Technology

Universiti Putra Malaysia

(Member)

ROBIAH BINTI YUNUS, PhD

Professor and Dean

School of Graduate Studies

Universiti Putra Malaysia

Date:

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Signature: _____
Name of Chairman of
Supervisory
Committee: Dr. Uswatun Hasanah binti Zaidan

Signature: _____
Name of Member of
Supervisory
Committee: Dr. Syahida binti Ahmad

Signature: _____
Name of Member of
Supervisory
Committee: Professor Dr. Azizah binti Abdul Hamid

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LIST OF ABBREVIATIONS

BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
CO ₂	Carbon dioxide
cm	Centimeter
DPPH	2,2-diphenyl-1-picryl-hydrazyl
DNA	Deoxyribose nucleic acid
DMSO	Dimethyl sulfoxide
EO	Essential oil
FT-IR	Fourier transform infrared spectroscopy
GAE	Gallic acid equivalent
GC-MS	Gas chromatography mass spectrometry
GRAS	Generally recognized as safe
GHz	Gigahertz
g	Grams
IC ₅₀	Inhibitory concentration
IR	Infrared
IU	International unit
kg	Kilograms
L	Litre
mm	Millimeters
mg	Milligrams
MPa	Megapascal

m	Minutes
NA	Nutrient agar
nm	Nanometer
NCD	Noncommunicable disease
NSPNCD	National Strategic Plan for Noncommunicable Disease
PEPCK	Phosphoenolpyruvate carboxykinase
ROS	Reactive oxygen species
SC-CO ₂	Subcritical carbon dioxide
SC	Scavenging capacity
THz	Terahertz
VCE	Vitamin equivalent
WHO	World health organization
w/v	Weight per volume
%	Percentage
°C	Celsius
μL	Microlitres
μg	Micrograms



CHAPTER 1

INTRODUCTION

1.1 Research background

The increasing number of cases on noncommunicable diseases (NCDs) have alarmed the global community. NCDs such as cardiovascular diseases, cancer, respiratory diseases, and diabetes have been reported to become the victim of NCDs. The NCDs can lead to death which 40 million were killed every year by these diseases. Presently in Malaysia, NCDs are becoming a growing concern with 73% (146,000) of total deaths were reported in 2014 especially in cardiovascular diseases (36%), cancers (15%), respiratory diseases (7%), and diabetes (3%). According to the National Statagic Plan for Noncommunicable Disease (NSPNCD), cardiovascular disease and diabetes were taken seriously by the government by introducing prevention method and policies in order to prevent and reduce the number of death that caused by these diseases. There were estimated that three million out of 33 million of its population were diagnosed with these diseases (Sharma *et al.*, 2012).

In particular, the increasing cases of NCDs have creating a significant opportunities in developing healthier lifestyle people in Malaysia. The present of free radicals will damage cells by stealing electron *via* a process of oxidation, so in recent years, the prevention and treatment of major diseases using traditional medicines has attracted interest through the action of antioxidant. Regarding to the above mentioned problems, natural remedy from plant bases have attracted attention among researcher to explore its potential, which can be a solution to the above problem. Thus, *Stevia rebaudiana* is one of the example natural remedy of plant that can become an agent to cure these diseases.

The genus *Stevia* is one of the most distinctive genera within the tribe Eupatorieae. Its origin from Paraguay, Brazil, and South America region (Hossain *et al.*, 2010). *Stevia* can adapt and behaves under some environmental conditions and management situations since it can behave as an annual or mixture of plants of both types. The sweetness properties come from the leaf of the plant, thus the proportion of leaf and weight ratio is important to determine the level of the sweetness. *S. rebaudiana* is one of the species that was claimed for the potential of sweetening properties.

Essential oils (EO) have been widely used all over the world. The demand of EO is increasing because of the promising outcome from its usage (Do *et al.*, 2015). It has aroused the attention of researchers and industrial person with its naturally occurring bioactive agents. The EO is a mixture of volatile compounds produced by the plants as secondary metabolites, as a protective mechanism against predator or weather adversities (Andrade *et al.*, 2014).

The mixture of compounds in *S. rebaudiana* EO has give many benefits. It have been a great interest for the potential in using the EO as alternative remedies for the treatment of infectious disease and pharmaceutical alternative medicine. EO clearly defined as a pure natural ingredients of plant origin, obtained by the extraction process such as hydrodistillation, soxhlet extraction, mechanical process, and water extraction (Atti-Santos *et al.*, 2005). Commonly, the conventional methods are used to extract the EO from the origin plant, but recently the researcher has found safer and environmentally friendly methods to extract the EO from the plants. The method which has been claimed as a green technology is subcritical carbon dioxide extraction (SC-CO₂). The conventional method is using chemical reagent such as hexane to extract the EO, but in SC-CO₂ uses a non chemical reagent which is carbon dioxide. The compound in the EO can be remained as carbon dioxide not is strong enough to diminish bioactive compound during extraction process. Thus, the EO from this extraction technique will extracted a good and active bioactive compound which increase the bioactivities of the EO.

In addition, EO of *S. rebaudiana* contains many active compounds such as caryophyllene oxide, carvacrol, luteol acetate, and other antioxidant compounds with antimicrobial and antioxidant properties. These compounds have been suggested to give beneficial effect to human health, including hypertensive, antihyperglycemic and antirotavirus activities (Tongnuanchan & Benjakul, 2014). The method used to extract EO also influences the biological activities of the compounds in EO (Charles & Simon, 1990).

1.2 Problem statement

The common conventional method used to extract the EO are hydrodistillation and soxhlet extraction. These methods use a chemical solvent as agent to extract the compound in *S. rebaudiana* leaves. The lack of awareness in using conventional method is the compound in the sample can be diminished by the solvent used and the yield produced will become low and less quality of extract. Thus, the method of SC-CO₂ will improve and increase the yield and also maintain the quality by reducing compound diminished during the process. The chemical properties of *S. rebaudiana* are still not well researched, especially using SC-CO₂ extraction method. In Malaysia, EO extracted from *S. rebaudiana* has not been explored on its chemical compositions, antioxidant activity properties, yet the knowledge regarding to the EO extraction by means of SC-CO₂ is still limited and unexplored.

1.3 Hypothesis

- 1) The extraction method of SC-CO₂ will produce a better extraction of EO at different sample of *S. rebaudiana* EO to an ethanol ratio.
- 2) The chemical composition and functional group profile of EO will be identified by using gas chromatography mass spectrometry (GC-MS) and Fourier transform-infrared (FT-IR) spectroscopy.
- 3) The EO will show biological activity of antioxidant properties.

1.4 Objectives

- 1) To determine the extraction efficiency of EO at different sample to ethanol ratio using subcritical carbon dioxide (SC-CO₂) extraction method.
- 2) To identify the chemical composition and functional group profile of EO by using gas chromatography mass spectrometry (GC-MS) and Fourier transform-infrared (FT-IR) spectroscopy.
- 3) To investigate the antioxidant activity of EO by using 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method.

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