EXTRACTION AND EFFECT OF SAPONIN EXTRACTED FROM PEEL OF *Musa acuminata* TOWARDS *Sf*9 CELLS

AFRINA BINTI ZAINAL

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
BACHELOR OF BIOTECHNOLOGY (HONOURS)

FACULTY OF HEALTH AND LIFE SCIENCES
INTI INTERNATIONAL UNIVERSITY
PUTRA NILAI, MALAYSIA

2017

NON-PLAGIARISM DECLARATION

By this letter I declare that I have written this thesis completely by myself, and that I have used no other sources or resources than the ones mentioned.

I have indicated all quotes and citations that were literally taken from publications, or that were in close accordance with the meaning of those publications, as such. All sources and other resources used are stated in the references.

Moreover I have not handed in a thesis similar in contents elsewhere.

In case of proof that the thesis has not been constructed in accordance with this declaration, the Faculty of Health and Life Sciences has the right to consider the research thesis as a deliberate act that has been aimed at making correct judgment of the candidate's expertise, insights and skills impossible.

I acknowledge that the assessor of this item may, for the purpose of assessing this item,

- reproduce this assessment item and provide a copy to another member of the University; and/or,
- communicate a copy of this assessment item to a plagiarism checking service (which may then retain a copy of the assessment item on its database for the purpose of future plagiarism checking).

In case of plagiarism the examiner has the right to fail me and take action as prescribed by the rules regarding Academic Misconduct practiced by INTI International University.

Afrina Binti Zainal	
Name	Signature
I14006944	18th December 2017
I.D. Number	Date

DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged, and completed under the supervision of Ms. Emily Quek Ming Poh.

Afrina Binti Zainal	Ms. Emily Quek Ming Poh
Student ID: I14006944	(SUPERVISOR)

ACKNOWLEDGEMENT

First of all, I would like to thank MARA for the financial support throughout my three-year studies at Inti International University. Next, my greatest appreciation to all biotechnology lecturers for the knowledge, guidance, and support throughout my learning in biotechnology course. Special thanks to laboratory assistants for the guidance and help provided during the research period. Furthermore, I would like to express my gratitude towards my family and friends for the endless moral support and strength given to me to finish my undergraduate study. Last but not least, I would like to express my deepest gratitude to my supervisor, Ms. Emily Quek Ming Poh for her patience, guidance, and support provided to ensure that I can successfully complete my final year project.

ABSTRACT

Saponins are phytochemicals found in many plants. Their structural diversity make them to have various biological activities. Saponin is produced in various parts of plants such as fruits and fruit peels. Since saponin has been used in many industrial applications, it will be excellent if the source of saponin comes from the fruit waste as this source is more economical and it can be converted into a useful bioproduct. One of the properties of saponin currently being studied is the insecticidal properties. The aims of this research were to extract saponin from the peel of banana *Musa acuminata* by using three different sample preparation methods, namely dried, fresh, and macerated peels, to determine the saponin content from the peel of banana, and to preliminary screen the insecticidal properties of saponin extracted on the growth of an insect cell line, *Spodoptera frugiperda* (*Sf9*). Fresh and macerated peels were prepared by blending and dried peels were oven-dried at 60°C before blended into powder. Both dried and fresh peels were exposed to solvent for 24 hours while the macerated peels was subjected to 72 hours. The extraction of saponin from the peels was done using two solvents namely, 50% (v/v) ethanol and 85% (w/v) hexane. All extracts were profiled at wavelength ranging from 300 nm to 700 nm. These profiles were compared with an absorbance profile containing 5 μg/mL of quercetin (Qu) which was used as flavonoid standard. Total saponin (TS) content was quantified by treating the extracts with vanillin and sulfuric acid, and the absorbance were recorded at 544 nm. The *Sf9* cells in suspension were treated with fresh-ethanol crude peel extract at a volume-to-volume ratio of 1:1 for 24 hours and the cell growth was measured its absorbance at 630 nm. From the profiling of crude peel extracts, dried extracts in ethanol showed higher absorbance profile than both fresh and macerated extracts in ethanol in which these two extracts shared similar absorbance profiles. In addition, hexane was able to extract saponin in fresh peel better than ethanol by giving higher absorbance profile. This observation of fresh peel extract was in contrast to the macerated peel extract. Based on the total saponin assay, dried peel extracts in ethanol showed the highest saponin content compared to both fresh and macerated peel extracts in ethanol. Total saponin contents in both fresh and macerated extracts were higher when hexane was used as the extraction solvent compared to ethanol. Fresh-hexane extract was found to contain more flavonoids compared to fresh-ethanol, macerated-ethanol and macerated-hexane extracts based on the comparison of peaks at 380 nm of quercetin absorbance profile . The percentages of growth inhibition for *Sf9* cells treated with fresh-ethanol extract and 50% (v/v) ethanol was 8.04% and 7.59% respectively. In conclusion,

dried peel extract in ethanol has higher saponin content compared to both fresh and macerated extracts based on the absorbance profiling and TS assay. Hexane was suggested as the suitable solvent to extract saponin compared to ethanol. The inhibition of *Sf*9 cells' growth with fresh-ethanol extract did not show any significant difference with the solvent used.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	percent
°C	degree Celsius
A_{380}	absorbance at 380 nanometer
A_{544}	absorbance at 544 nanometer
A_{630}	absorbance at 630 nanometer
CABI	CAB International
cells/mL	cells per mL
Eds.	editors
et al.	et alia
FAO	Food and Agriculture Organization of the United Nation
g	gram
g/mL	gram per millilitre
HCT-116	human colorectal carcinoma cell line
HIV	human immunodeficiency virus
HT-29	human colorectal adenocarcinoma cell line
JPNPP	Jabatan Pertanian Pulau Pinang
mg	milligram
mg/g	milligram per gram
mg/mL	milligram per millilitre
mL	millilitre
mm	millimeter
MOA	Ministry of Agriculture & Agro-Based Industry Malaysia

nm	nanometer
rpm	revolutions per minute
QS-21	*Quilliaja* saponin
Qu	Quercetin
*Sf*9	*Spodoptera frugiperda*
SF-900 II SFM	*Sf*-900 II Serum-Free Media
sp.	species
subsp.	subspecies
TS	total saponin
UV	ultraviolet
v/v	volume per volume
w/v	weight per volume
µg/mL	microgram per millilitre
µL	microlitre

CHAPTER 1

INTRODUCTION

Fruits, along with vegetables, are one of the important components in food pyramid for a healthy human diet. They are the major source of minerals, vitamins, and fibres that are important for maintaining human health (Mañiyan, John & Mathew, 2015). The goodness of fruits has become a market interest in food industry to produce many fruit-based food products. Most of the food processing industry and also regular fruit consumption by consumers only use the edible parts of the fruits (Deng et al., 2012). A very little number of the fruit wastes such as the cores and the peels are processed to make other types of edible food or for other agricultural purposes. Most of the time, the fruit wastes are usually thrown away as municipal waste. According to Deng et al. (2012), fruit wastes are among the major sources of municipal wastes, raising an environmental issue on waste management. Based on the report published by Food and Agriculture Organization (FAO) of the United Nation in 2011, approximately 20% from total of 50% fruit and vegetables are lost or wasted due to food processing in South and Southeast Asia (FAO, 2011). Due to this, many researches have been done in identifying potential compounds from fruit wastes for industrial applications, converting the waste into a possible value-added bioproducts.

Besides food products, fruits are also used to make nutraceutical products such as health supplements and cosmetics. This is because fruits are also rich in phytochemicals, or bioactive compounds which provide colour, flavour, and as natural protection to the fruits (Maniyan et al., 2015). These compounds possess various promising health benefits such as antioxidants, anticancer, antifungal, and other benefits (Maniyan et al., 2015). An example of a phytochemical is saponin, which is widely distributed in plants (Moses, Papadopoulou & Osbourn, 2014), accumulating in organ and/or tissue-specific manner (Moses et al., 2014). Many plants synthesized and stored saponin in underground organ such as roots (Moses et al., 2014). Besides roots, different types of saponin have been isolated from stems, barks, leaves, seeds, fruits and flowers (Moses et al., 2014). Saponin has been commercially used as foaming agent in food industry, and as dietary supplements in pharmaceutical industry (Moses et al., 2014). Ginseng, soybean, and soapbark are commonly known plants for their saponin content (Choon, Hanaa & Rabiha, 2014).