UNIVERSITI TEKNOLOGI MARA

STANDARDISED SYRINGIN-RICH *Tinospora crispa* AQUEOUS EXTRACT WITH ANTIOXIDANT, ANTICHOLESTEROL AND ANTIATHEROSCLEROTIC PROPERTIES

ZAMREE BIN MD SHAH

PhD

February 2019

ABSTRACT

Tinospora crispa or known as Patawali is one of medicinal plants which are rich of bioresource for traditional systems of medicine, food supplements and pharmaceutical preparations. According to scientific researches, the stem of this plant is proven to possess many beneficial bioactivities and has great potential to be used in developing naturally occurring commercial products in market demand. However, very little research has been carried out with regards to the extraction procedure to obtain the optimum extraction yield with high content of its bioactive compound that can be further used in preparing standardized extract of this plant. Knowledge of its active compound and the mode of action that responsible to the desired effects is remains unfold. This study aimed to prepare the standardised *Tinospora crispa* aqueous extract (STCAE) and to investigate its anti-oxidant, anti-cholesterol and anti-atherosclerotic properties. Experiments were carried out to determine the effects of various operating parameters on the qualitative and quantitative aspects of *T. crispa* stems. Syringin was selected as the quality indicator in this study because this compound played a major role in bioactivities of *Tinospora crispa* including anti-hypertension, free radicals scavenging, anti-diabetic and anti-inflammatory. The optimum extraction conditions of *T. crispa* stems were determined as 60 °C, 1 hour of extraction time and the ratio of water to solid is 15:1 (ml:g). STCAE was produced on the basis of the extract containing at least 0.4 wt % of syringin. STCAE was found to possess high antioxidant activities through DPPH, FRAP and TBA bioassays. Cytotoxic study performed revealed that STCAE was not toxic to the cell. Through cell culture experiment, STCAE and syringin showed strong cholesterol reducing effects demonstrated by a significant increase in molecules levels involved in reverse cholesterol transport (Apo A1, LCAT, LDL-R, SRB-1 and HL). The efficacy of these activities is appreciably good when compared with standard drug simvastatin. However, STCAE and syringin showed moderate effect in controlling mevalonate pathway. The anti-cholesterol and anti-atherosclerotic properties of STCAE were further investigated *in vivo* using rabbits. The hypercholesterolemic rabbits were induced and the rabbit were given different concentration of STCAE (200, 450 and 600 mg/kg) for 10 weeks. Results from lipid analysis showed that the levels of total cholesterol (TC), triglyceride (TG) and LDL of the hypercholesterolemic rabbits were significantly reduced with the treatment of STCAE at all concentrations while HDL levels was elevated compared to hypercholesterolemic group. Through plasma analysis, the activity of gamma glutamyl transferase (GGT) and alkaline phosphates (ALP) were also reduced significantly with the treatment of STCAE at all concentration compared to hypercholesterolemic group. All group of rabbits tested with STCAE showed significantly high total antioxidant status (TAS), glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities. Among the concentrations of STCAE tested, medium dose (450 mg/kg) showed more potent effect in reducing blood serum TC, TG and LDL levels and increasing HDL levels compared to low and high dosages counterparts. No foam cell formation was visible in aorta of rabbits treated with STCAE in dose dependent manner. The compound that donates to the desired effects possibly contributed by syringin. The results obtained suggested that STCAE could be used as an easily accessible source of natural antioxidants and possible supplement by pharmaceutical industry for the management of hypercholesterolemia.

ACKNOWLEDGEMENT

In the name of Allah, the most gracious and most merciful.

My utmost grateful to Him for giving me the opportunity to embark on this tedious and challenging PhD journey and for completing it successfully. Without His mercy, I can never complete this thesis of mine.

My indebted gratitude and thanks to my extraordinary supervisor Prof Dr Zulkhairi bin Hj. Amom, former Co-supervisor Dr Rasadah binti Mat Ali and Co-supervisor Dr Zunoliza binti Abdullah for giving me the opportunity to work with their research group. The experience, skills and knowledge I received will always be the most valuable gift that can never be repeated. Their guidance and enthusiasm will keep inspiring me throughout my life.

My special appreciation also goes to FRIM management especially to YBhg. Dato' Dr Latif bin Mohmod and Puan Norhayati binti Nordin who have put their confidence in my research and provided the facilities and assistance. Also, a special thanks is dedicated to my wonderful colleagues from Herbal Technology Centre and Patawali Technofund research project team members for assisting and helping me throughout completing this research.

To my dearest mother and father, thank you for your unconditional love and support for there will be no one who could replace both of you. To my siblings, thank you for your care all these years.

Last but not least, to my beloved wife, thank you for always being there for me, care and spread positive vibes during my ups and downs in completing my thesis. Without your help and love, I will never be able to complete the thesis. To the love of my life, my 4 cheerful and amazing children, thank you so much for your patience and never-ending support during my struggle. Your cooperation, laughter, joy and happiness have given me the strength and motivation to move on and finally end this journey.

This piece of victory is dedicated to all of you. This master piece would never have been possible without all of you.

Alhamdu lillah.

TABLE OF CONTENT

CHAPTER ONE
INTRODUCTION

1.1 Research Background

A growing concern about the side effects and limitations of modern scientific medicine has led to a renewed curiosity about 'natural' products. Consumers are now interested in 'natural' products, as part of an increased focus on health, fitness, and wellbeing. They perceive natural ingredients as 'good' and 'pure' and botanical medicines as more 'natural' and better for the patient and the environment than synthetic drugs (Ito et al., 1985). The Global Industry Analysts Inc. based in USA estimated the herbal market to reach USD115 billion by the year 2020 (Ahmad et al., 2015). The industry is led by Europe and followed closely by fast growing Asia-Pacific region with 9.1% Compound Annual Growth Rate (CAGR). In Malaysia, according to the report of Malaysian Herbal Corporation, the value of industry in Malaysia is estimated about RM17 billion in 2013. Meanwhile, Ministry of the Natural Resources and Environment estimated the market value of the herbal industry in Malaysia is projected to reach RM29 billion in 2020, with the annual growth rate between 8% - 15%. % (Zakaria, 2015). The trend indicates the huge potential of herbal business to expand and flourish in near future.

Malaysia rainforest has a rich variety of plant species that are largely unexplored. The total area of the rainforest is estimated to be 19.12 million hectares, which covers 58.1 % of the country's land area. This area consists of more than 20,000 plant species, out of which 2,000 plant species have been reported to have medicinal values (Jaganath & Ng. 1999). The shift in healthcare, denoted by global growth in nutraceuticals, towards a preference for natural products with therapeutic value provides opportunities for Malaysia to become a significant global player, given our rich biodiversity.

According to World Health Organization (WHO), the non-communicable disease (NCD), defined as diseases of long duration and slow progression, are classified as the biggest global killers today. More than 60 % of adult mortality and morbidity worldwide are caused by NCDs. Among of all disorders, cardiovascular disease (CVD) is the most dominant contributor which causes 30 % of all deaths worldwide and almost