EFFECTS OF PROCESSING CONDITIONS ON EXTRACTION OF TOTAL SAPONINS FROM BITTER GOURD (Momordica charantia L.) AND ITS ANTIMICROBIAL ACTIVITY AGAINST FOODBORNE PATHOGENS

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HANAA ABDELKARIM SALMAN ABOAZRA

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Chairman: Hjh. Rabiha Hj. Sulaiman, PhD
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A tropical plant, *Momordica charantia* L., bitter gourd (BG), subcontinent variety, is widely found in Asian countries. The World Health Organization of the United Nations estimates that every year one in three people worldwide get food poisoning leading to food illness or food diseases from consuming contaminated food. Many researches have reported that saponins in BG fruit have biological potency. Therefore, the need to explore a natural source of antimicrobial for food safety and using it as a natural sanitizer was important. The aim of this study is to examine the antimicrobial effect of hot air dried, spray dried, and extrudates of BG crude extracts against selected foodborne pathogens. Optimization of extraction condition using Response Surface Methodology (RSM) was conducted using solvent type, time, and solid to solvent ratio as independent variables while total saponins measured as a dependent response. Different processing methods includes hot air drying at 45°C, spray drying (90, 120, 147.5, 175 and 200°C), and extrusion (80, 100, and 120°C) were applied to produce BG fruit powder. Optimal crude extracts of samples which contain different amount of total saponins were evaluated for antimicrobial activity against eight foodborne pathogens. The extracts that could inhibit the growth of tested pathogens were used in sanitizing test at different exposure times (5, 10, and 15 min). The results of RSM showed that 0.02 g/ml of solid to solvent ratio, time (75 - 120 min), and solvent type (ethanol) was the optimum extraction condition. Hot air dried and extrudate at temperature of 80°C showed a good retention of total saponins with 103.0, 108.8 mg DE/g DM, respectively, compared to initial content of fresh fruit sample 172.7 mg/g DM. BG fruit powder which contain high total saponins had potential bactericidal activity against the tested food pathogens with less than 10 mg/ml in 24 h. Ethanolic extracts of extrudate at temperature of 80°C and hot air dried samples had inhibited the growth of *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, *Bacillus cereus*,

Streptococcus mutans, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* with a minimum inhibitory concentration (MIC) of 0.625 - 5.625 mg/ml. The sample extracts possessed bactericidal and bacteriostatic activities against foodborne bacteria. Based on time-kill curve, it showed time needed to destroy the bacteria was within 4 h, except for *P. aeruginosa*. In conclusions, different processing method possess different total saponin yields in the dried BG fruit powder could have important bactericidal activity and this study will enhance the attempts of using total saponins compound as a potential natural antibacterial agent in food applications.

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

KESAN KAEDAH PEMPROSESAN PADA PENGEKSTRAKAN JUMLAH SAPONIN DARIPADA PERIA DAN AKTIVITI ANTIMIKROB TERHADAP PATOGEN MAKANAN

Oleh

HANAA ABDELKARIM SALMAN ABOAZRA

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Pengerusi: Hjh. Rabiha Hj. Sulaiman, PhD
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Momordica charantia L., buah peria (BG) merupakan tumbuhan tropika yang terdapat secara meluas di negara-negara Asia. Pertubuhan Kesihatan Sedunia Pertubuhan Bangsa-Bangsa Bersatu menganggarkan bahawa setiap tahun masih ada satu dalam tiga orang di seluruh dunia mendapat keracunan makanan dari memakan makanan yang tercemar. Banyak kajian telah melaporkan bahawa saponin dalam buah BG mempunyai potensi biologi. Menyelidik sumber semula jadi antimikrob dari tumbuhan sebagai sanitizer semula jadi adalah perlu. Tujuan kajian ini adalah untuk mengkaji kesan antimikrobial serbuk buah BG yang telah dikeringkan melalui udara panas, semburan kering, dan proses penyemperitan. Pengoptimuman cara pengekstrakan menggunakan Metodologi Response Surface (RSM) telah dijalankan menggunakan jenis pelarut, masa, dan nisbah sampel kepada pelarut sebagai pembolehubah bebas, manakala jumlah saponin adalah respon. Kaedah pemprosesan yang berbeza iaitu pengeringan udara panas pada suhu 45°C, semburan kering (90, 120, 147.5, 175 dan 200°C), dan proses penyemperitan (80, 100, dan 120°C) telah digunakan untuk menghasilkan serbuk buah BG. Ekstrak mentah daripada sampel udara panas, semburan kering dan proses penyemperitan mengandungi kandungan saponin yang berbeza dan kesemua sampel telah dinilai untuk aktiviti antimikrob terhadap lapan patogen makanan. Ekstrak yang boleh menghalang pertumbuhan patogen diuji dengan menggunakan ujian sanitasi pada masa pendedahan yang berlainan (5, 10, dan 15 min). Keputusan RSM menunjukkan bahawa 0.02 g/ml nisbah sampel kepada pelarut, masa (75 - 120 min), dan jenis pelarut (etanol) adalah keadaan optimum pengektrakan saponin. Sampel daripada pengeringan udara panas dan proses penyemperitan pada suhu 80 ℃ menunjukkan kandungan saponin 103.0 dan 108.8 mg DE/g DM, masing-masing, berbanding kandungan awal sampel buah BG segar iaitu 172.7 mg/g DM. Serbuk buah

BG yang mengandungi jumlah saponin yang tinggi mempunyai potensi aktiviti bakteria terhadap patogen makanan dengan kurang daripada 10 mg/ml dalam masa 24 jam. Ekstrak etanol daripada serbuk dihasilkan melalui proses penyemperitan pada suhu 80℃ dan sampel udara panas telah menghalang pertumbuhan *Klebsiella pneumoniae, Staphylococcus epidermidis, Bacillus cereus, Streptococcus mutans, Escherichia coli, Staphylococcus aureus, dan Pseudomonas aeruginosa* dengan kepekatan perencatan minimum (MIC) 0.625 - 5.625 mg/ml. Manakala, masa yang diperlukan untuk memusnahkan bakteria adalah dalam 4 jam, kecuali P. aeruginosa. Hasil kajian menunjukkan bahawa ekstrak mentah buah peria memiliki sifat bacteriostatic terhadap bakteria makanan. Pada kesimpulannya, kaedah pemprosesan yang berbeza memberi kesan terhadap jumlah hasil saponin dalam serbuk buah BG dan saponin berpotensi sebagai agen anti-bakteria semulajadi dalam aplikasi makanan.

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I certify that a Thesis Examination Committee has met on 9th November 2016 to conduct the final examination of Hanaa Abdelkarim Salman Aboazra on her thesis entitled ″Effects of Processing Conditions on Extraction of Total Saponins from Bitter Gourd (*Momordica Charantia* L.) and its Antimicrobial Activity Against Foodborne Pathogens″ in accordance with the Universities and University Colleges Act 1971 and the Constitution of Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Science.

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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

BBD	Box-Behnken Design
BG	Bitter gourd
CFU	Colony forming unit
CLSI	Clinical and Laboratory Standards Institute
D	Diameter
DE	Diosgenin Equivalent
DM	Dry Material
DMSO	Dimethyl sulfoxide
DOE	Design of Experiments
Eq.	Equation
g	Gram
h	Hour
MBC	Minimum Bactericidal Concentration
mg	Milligram
MHA	Mueller Hinton agar
min	Minute
ml	Milliliter
mm	Millimeters
PBS	Phosphate buffered saline
R_f	Retention factor
rpm	Revolutions per minute
RSM	Response Surface Methodology
SEM	Scanning Electron Microscopy
spp.	Species
USDA	US Department of Agriculture
UV	Ultraviolet
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background

Momordica charantia L., bitter gourd (BG) is widely distributed, especially in India, China, Malaysia and other tropical countries. It is often neglected in terms of overall consumption due to its bitter taste. However, BG is among the candidates in recent research for its antidiabetic, anticancer, anti-inflammatory, and anti-triglycerides properties (Harinantenaina *et al.*, 2006; Zulbadli *et al*., 2011; Joseph and Jini, 2013). Malaysia published a country report on the state of plant genetic resources for food and agriculture (1997-2007) stating that the government encourages the development of value-added processing of 'diversity-rich' products for commercial purposes. Its recommendations include the processing of BG into dietary supplements (Nordin *et al*., 2007). BG fruit is rich with phytochemical compounds including saponins, which could introduce the fruit to the food industry sector (Reyes-Jáquez *et al*., 2011). The food market is strongly moving towards products with beneficial nutraceutical values.

BG fruit powders could be used as an ingredient in product formulation and as a source of saponins. Investigation on optimal extraction conditions of total saponins, including factors such as solvent type, extraction time, and solid to solvent ratio of hot air-dried BG powder will provide comprehensive information. For quantification study, this can give an implication of extraction solvent capability in obtaining significant total saponins yields. The finding will be for food and pharmaceutical industries. Additionally, saponins have no noted harmful effect on the kidney or liver at reasonable levels of consumption (Virdi *et al*., 2003).

Optimized extract that rich in total saponins content from processed bitter gourd fruit may have strong antimicrobial effects against foodborne pathogens, *in vitro*. Use of bitter gourd optimized extract that rich in total saponins content from processed bitter gourd fruit may protect fresh meat from spoilage by reducing its microbial population significantly. This study focus on effect of processing conditions on the extraction of total saponins from BG fruit and determine the antibacterial effect of hot air dried, spray dried, and extrudates of BG powders extracts against *Candida albicans, B. cereus, E. coli, P. aeruginosa, S. aureus, K. pneumoniae, S. mutans* and *S. epidermidis.*

1.2 Objectives

Overall, the research is undertaken with the aim to study effect of processing condition on total saponins yields and examine the antimicrobial effects of dried BG fruit powder. In particular, this study attempts

1. To optimize the extraction conditions for total saponins present in hot air dried BG fruit using response surface methodology (RSM).

2. To determine effect of processing conditions (hot air drying, spray drying, and extrusion process) on physicochemical properties of dried BG fruit powder.

3. To determine the antimicrobial activity of bitter gourd crude extract obtained through RSM model in term of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and time-kill curve against selected foodborne pathogens.

4. To evaluate antimicrobial activities of bitter gourd crude extract on food application (raw chicken meat) as a natural sanitizer.

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