UNIVERSITI PUTRA MALAYSIA

ANTIMICROBIAL ACTIVITY OF Boesenbergia rotunda (L.) MANSF. A. EXTRACT AGAINST MULTI-ANTIBIOTICS RESISTANT FOODBORNE PATHOGENS

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FSTM 2015 17

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By

NURUL SYAZWANI BINTI MOHD ZAININ

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

May 2015

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

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Chairman: Assoc. Prof. Yaya Rukayadi, PhD
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Evolution of resistance in foodborne pathogens to the currently available antibiotics in the market demonstrate the importance of identifying novel antimicrobial agent. Therefore, in this study, *Boesenbergia rotunda* (fingerroot) from Zingiberaceae family was chosen to be further assessed as antimicrobial agent against eight species of reference foodborne pathogens including its multi-antibiotics resistant isolates, namely *Escherichia coli*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, *Vibrio parahaemolyticus* and *Candida albicans*. *B. rotunda* methanolic extract showed antimicrobial activity against the microorganisms tested. The ranges of MIC and MBC obtained showed that *B. rotunda* extract had strong antimicrobial activity against *V. parahaemolyticus*, *B. cereus* and *S. aureus* KCCM 12255 with 9.77 – 312.5 µg/ml and 9.77 – 625 µg/ml, respectively. Meanwhile, the *B. rotunda* extract was least active against *L. monocytogenes*, *Salmonella* spp. and *C. albicans* with MIC values range were 1250 – 5000 µg/ml and MBC/MFC values range were 2500 - >5000 µg/ml. *E. coli* and *K. pneumoniae* showed wide range of inhibition and bactericidal concentrations which were 19.53 – 2500 µg/ml and 39.06 – 5000 µg/ml, respectively. The time-kill curves demonstrated that *B. rotunda* extract can kill the microorganisms tested with more than 3 $\log_{10}$ (99.99%) within 0.5 to 4 hours of incubation with bactericidal endpoints for *B. cereus*, *V. parahaemolyticus*, *E. coli* ATCC 25922 and *S. aureus* KCCM 12255 were from 78.13 – 1250 µg/ml (2× MIC and 4× MIC), whereas the bactericidal/fungicidal endpoints for isolated *E. coli* O157:H7, *K. pneumoniae*, *L. monocytogenes*, *Salmonella* spp. including *C. albicans* ATCC 14503 were from 2500 – 5000 µg/ml (2× MIC, 4× MIC, and 8× MIC). Generally, the antimicrobial activities of *B. rotunda* extract were not significantly affected by pH (3, 7, and 11) and temperatures (30˚C, 50˚C, 80˚C and 121˚C). Natural microflora in fruit and vegetable samples also can be reduced at least by 2 $\log_{10}$ CFU/g after treatment with 0.05% (0.5 mg/ml), 0.5% (5 mg/ml) and 5% (50 mg/ml) of *B. rotunda* extract. Among these treatment solutions, the relative best combination between antimicrobial ability and sensory acceptability can be achieved with 0.05% (0.5 mg/ml) *B. rotunda* extract, where it showed a significant bacterial population reduction as well as high mean score for the tested sensory attributes. The *B. rotunda* extract was found not to be toxic with LC_{50} more than 1.0 mg/ml. GC-MS analysis showed that the antimicrobial properties of the

extract possibly attributed by major bioactive compounds, namely camphor, geraniol, methyl cinnamate, pinocembrin and pinostrobin chalcone. It can be concluded that *B. rotunda* extract has a potential as antimicrobial agent against multi-antibiotics resistant foodborne pathogens which deserves further investigation for development of natural food sanitizer.

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

AKTIVITI ANTIMIKROBIAL EKSTRAK *Boesenbergia rotunda* (L.) MANSF. A. MELAWAN PATOGEN BAWAAN MAKANAN RINTANG PELBAGAI ANTIBIOTIK

Oleh

NURUL SYAZWANI BINTI MOHD ZAININ

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Pengerusi: Prof. Madya Yaya Rukayadi, PhD
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Evolusi rintangan patogen bawaaan makanan terhadap antibiotik yang berada di pasaran menunjukkan kepentingan pengenalpastian ejen antimikrob yang baru. Oleh itu, dalam kajian ini, *Boesenbergia rotunda* (temukunci) dari keluarga Zingiberaceae telah dipilih untuk dikaji sebagai agen antimikrob terhadap lapan spesies rujukan patogen bawaan makanan termasuk pencilan bakteria yang rintang kepada pelbagai antibiotik, iaitu *Escherichia coli*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, *Vibrio parahaemolyticus* dan *Candida albicans*. *B. rotunda* yang diekstrak menggunakan metanol menunjukkan aktiviti antimikrob terhadap mikroorganisma yang diuji. Kepekatan Minimum Perencatan (MIC) dan Kepekatan Minimum Bakterisidal (MBC) yang diperolehi menunjukkan ekstrak *B. rotunda* mempunyai aktiviti antimikrob yang kuat terhadap *B. Cereus*, S. *aureus* KCCM 12255 dan *V. parahaemolyticus*, dengan nilai 9.77 - 625 µg/ml dan 9.77- 312.5 µg/ml, masing-masing. Sementara itu, ekstrak *B. rotunda* kurang aktif terhadap *L. monocytogenes*, *Salmonella* spp. dan *C. albicans* dengan julat nilai MIC adalah 1250 - 5000 µg/ml dan julat nilai MBC/MFC adalah 2500 - >5000 µg/ml. *E. coli* dan *K. pneumoniae* menunjukkan kepekatan perencatan and bakterisidal yang pelbagai iaitu 19.53 - 2500 µg/ml dan 39.06 - 5000 µg/ml, masing-masing. Lekuk masa pembunuhan menunjukkan bahawa ekstrak *B. rotunda* boleh membunuh mikroorganisma yang diuji dengan lebih daripada 3 $\log_{10}$ (99.99%) dalam 0.5 - 4 jam masa pengeraman dengan titik akhir bakterisidal untuk *B. cereus*, *V. parahaemolyticus*, *E. coli* ATCC 25922 dan *S. aureus* KCCM 12255 adalah 78.13 - 1250 µg/ml (2× MIC dan 4× MIC), manakala titik akhir bacterisidal/titik akhir fungisidal untuk *E. coli* O157:H7, *K. pneumoniae*, *L. monocytogenes*, *Salmonella* spp. termasuk *C. albicans* ATCC 14503 adalah 2500 - 5000 µg/ml (2× MIC, 4× MIC, dan 8× MIC). Secara keseluruhannya, aktiviti antimikrobial ekstrak *B. rotunda* tidak terjejas dengan ketara oleh pH (3, 7, dan 11) dan suhu (30˚C, 50˚C, 80˚C dan 121˚C). Di samping itu, mikroflora semula jadi dalam sampel buah-buahan dan sayur-sayuran juga boleh dikurangkan sebanyak 2 $\log_{10}$ CFU/g selepas dirawat dengan 0.05% (0.5 mg/ml), 0.5% (5 mg/ml) dan 5% (50 mg/ml) ekstrak *B. rotunda*. Di antara semua cecair rawatan, kombinasi terbaik diantara keupayaan antimikrob dan penerimaan deria boleh dicapai dengan 0.05% (0.5 mg/ml) ekstrak *B. rotunda*, di mana ia menunjukkan pengurangan ketara populasi bakteria dan skor min

yang tinggi untuk pengujian deria. Ekstrak *B. rotunda* didapati tidak toksik dengan LC_{50} lebih daripada 1.0 mg/ml. Analisis GC-MS menunjukkan bahawa ciri-ciri antimikrob ekstrak mungkin disebabkan oleh sebatian bioaktif utama iaitu kamfor, geraniol, metil sinamat, pinosembrin dan kalkon pinostrobin. Ia boleh disimpulkan bahawa ekstrak *B. rotunda* mempunyai potensi sebagai ejen antimikrob melawan patogen bawaan makanan yang rintang terhadap pelbagai antibiotik dan layak dikaji lebih lanjut untuk penghasilan pembersih makanan semula jadi.

ACKNOWLEDGEMENTS

Bismillahirrahmanirrahim,

Alhamdulillah. Thanks to Allah SWT, whom with His willing giving me the opportunity and strength to complete this research project entitled "**Antimicrobial Activity of *Boesenbergia rotunda* (L.) Mansf. A. Extract Against Multi-Antibiotics Resistant Foodborne Pathogens**".

I would like to take this opportunity to express my gratitude and appreciation to my supervisor, Assoc. Prof. Dr. Yaya Rukayadi for his supervision, guidance, constructive comments and continuous supports throughout my study. Without the precious instruction, it is impossible for me to complete my project.

My deepest appreciation also express to my co-supervisors, Prof. Dr. Son Radu and Dr. Ahmad Faizal Abdull Razis, faculty's lecturers and all staff of Biochemistry Laboratory and Microbiology Laboratory in Faculty of Food Science and Technology, Universiti Putra Malaysia for their kindness and cooperation during completion the research project.

Sincere thanks to my laboratory and post graduate colleagues who provide continuous support; Lau Kah Yan, Maya Puteri Malina Zakaria, Nor Asma Husna Yusoff, Lew Kok Fang, Nurul Husna Mahazar, Sylvester William Silan, NurulHanis Sanusi and Shirwan Sani. Thank you for the endless help, assistance and guidance given to me. All the sweet memories will be remembered forever. Special thanks to Lye Ying Ling, Elexson Nillian, Soopna Puspanadan and Najwa Mohd Shahril for their contributions, suggestions and exchange of opinion in this study.

My heartfelt and special gratitude to my beloved parents, siblings and loved ones, who supported me spiritually throughout the entire process, for the sacrifices, understanding and endless love for me. Last but not least, I would like to thank my fellow best friends for their unconditional support and encouragement throughout my life.

I certify that a Thesis Examination Committee has met on 18 May 2015 to conduct the final examination of Nurul Syazwani Binti Mohd Zainin on her thesis entitled "**Antimicrobial Activity of *Boesenbergia rotunda* (L.) Mansf. A. Extract Against Multi-antibiotics Resistant Foodborne Pathogens**" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the 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

ATCC	American Type Culture Collection
B. cereus	*Bacillus cereus*
B. rotunda	*Boesenbergia rotunda*
C. albicans	*Candida albicans*
CDC	Centres for Diesease Control and Prevention
CFU	Colony forming unit
DMSO	Dimethylsulfoxide
E. coli	*Escherichia coli*
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
HCl	Hidrochloric acid
HUS	Hemolytic-uremic syndrome
IBS	Institute of Bioscience
KCCM	Korean Culture Centre of Microorganism
K. pneumoniae	*Klebsiella pneumonie*
L. monocytogenes	*Listeria monocytogenes*
MBC	Minimum bactericidal concentration
MFC	Minimum fungicidal concentration
MHA	Mueller Hinton agar
MIC	Minimum inhibitory concentration
NaCl	Sodium chloride
NaOH	Sodium hydroxide
Salmonella spp.	*Salmonella* species
SDA	Sabouraud dextrose agar
S. Typhimurium	*Salmonella* Typhimurium
S. aureus	*Staphylococcus aureus*
TSA	Tryptic Soy agar
UPM	Universiti Putra Malaysia
V. parahaemolyticus	*Vibrio parahaemolyticus*
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Introduction

Foodborne illness becomes a major problem in public health around the world with more than 76 million cases per year (WHO, 2003). Most of the foodborne illness cause by variety of bacteria, virus and parasite. The common symptoms of foodborne illness are nausea, vomiting, abdominal cramps and diarrhea. Although many diseases resulted in diarrhoea as the final stage, immunocompromised individual can suffer severe and prolonged illness which may require antimicrobial treatments. However, development of antimicrobial resistant in foodborne pathogens can potentially lead to the limitation in human drug treatments (White *et al*., 2002).

The extensive use of antimicrobials in human and veterinary medicine, agriculture and aquaculture has led to the survival and emergence of resistant foodborne pathogens. Huge amounts of antimicrobials are given to food producing animals in feed and water as control and treatment of disease as well as for growth promotion purposes resulted in high levels of resistance trait in foodborne bacteria. Currently, there is concern in food safety to counter the rising of resistant pathogens (Koluman and Dikici, 2013).

The studies on natural antimicrobial agents from plants in recent years turn out to be prominent because some of the available antimicrobials in the market are no longer functioning. Being natural foodstuffs, they appeal to consumers who tend to question the safety of synthetic chemical antimicrobial agents (Tornuk *et al*., 2011). Many studies have reported that most of the medicinal plants, including spices and herbs have strong antimicrobial compounds (Mahesh and Satish, 2008). Any part of the plant may contain active components. For example, the roots of ginseng plants contain the active saponins and essential oils, while eucalyptus leaves are harvested for their essential oils and tannins (Cowan, 1999). Additionally, the phenolic compounds also have significantly contributed to their antioxidant properties (Wu *et al*., 2006; Shan *et al*., 2005). Hence, the present study was conducted to evaluate the antimicrobial ability of medicinal plants, *B. rotunda* against multi-antibiotic resistant foodborne pathogens.

1.2 Research problems

1. Variety of antimicrobials, including antibiotics, antifungals, sanitizers, and food preservatives that are applied during food production and manufacturing can promote microbial resistance because microorganisms have the ability to evolve according to environmental stressors.

2. There is an increasing interest in using natural products in food sanitation and preservation.

1.3 Objectives

1.3.1 To determine the antimicrobial activity of *B. rotunda* extract, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration/Minimum Fungicidal Concentration (MBC/MFC), and time-killing curve against multi-antibiotics resistant *E. coli*, *B. cereus*, *K. pneumoniae*, *L. monocytogenes*, *Salmonella* spp., *S. aureus*, *V. parahaemolyticus*, and *C. albicans*.

1.3.2 To examine the effect of different concentration *B. rotunda* extract on natural microflora in fruits and vegetables and its sensory acceptability.

1.3.3 To analyze the bioactive compounds in *B. rotunda* extract that might be responsible to its antimicrobial activity.

1.3.4 To assess the toxicity effect of *B. rotunda* extract on brine shrimp.

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