BANANA (*Musa* spp.) BY-PRODUCTS AS POTENTIAL ANTIMICROBIAL BIOPRESERVATIVE

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FACULTY OF FOOD SCIENCE AND NUTRITION
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DECLARATION

I hereby declare that the materials in this thesis is my own except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

27 January 2015

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CERTIFICATION

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ABSTRACT

Banana by-products were investigated for its antibacterial components and selected bioactive fractions have been applied as bio-preservative in broth and food model. Banana plant parts were extracted using various solvent infusions and tested for antibacterial activity using agar-well diffusion assay. The by-product with the strongest antibacterial activity was further studied for the influences of geographical origin, drying methods and extraction methods in succession. Selected extraction parameters (time, temperature and solvent percentage) were then optimized using Face-Center Central Composite Design (FFCCD) in Response Surface Methodology (RSM). Purification and identification of bioactive components were performed using chromatographic approach based on the spectroscopic data. Potent antibacterial fraction was applied in broth model to evaluate the effects of food compositions on the antibacterial efficacy. Subsequently, carrot cubes were used as food model in assessing the bioactive properties of BWF-3 and its' mechanism of inhibition was also elucidated. Results showed the methanolic extract obtained from banana inflorescence (buds) of *Musa balbisiana* cv. Saba having the highest antibacterial activity against *Staphylococcus aureus* (SA)*, Bacillus cereus* (BC)*, Listeria monocytogenes* (LM) and *Vibrio parahaemolyticus* (VP).The geographical origin of banana inflorescence does not seem to affect the antibacterial properties. However, oven dried samples at 50°C was found to preserve the antibacterial activity similarly to those freeze dried samples, but significantly ($p<0.05$) better than sun dried samples. Optimized extraction parameters by RSM (extraction time: 6.0 h, extraction temperature: 35ºC, methanol to water percentage: 94% v/v) achieved higher antibacterial activity against the four tested pathogenic bacteria. The optimized methanolic extract obtained from the inflorescence buds was then partitioned into chloroform, ethyl acetate, butanol and water fractions. Water partition was further undergone SPE purification yield 3 fractions and tested for bioactivity. The bioactive fraction 3 (BWF-3) contained epigallocatechin and its derivatives and tryptophan identified using LC-ESI-MS/MS. Meanwhile, three antibacterial compounds namely 31-norcyclolaudenone, cycloartenol and (24R)-4a,24-trimethyl-5a-cholesta-8,25(27)-dien-3b-ol were identified from the chloroform partition. Methanolic-water fraction (H_2O Fr.) and SPE-fraction 3 (BWF-3) showed the most prominent antibacterial activity (MIC H_2O Fr.: 8.0 mg/ml – 25.0 mg/ml, MIC BWF-3: 0.6 mg/ml – 2.5 mg/ml) against SA, BC, LM and VP, as compared to other fractions. Among the food components, only protein and oil at 1% significantly ($p<0.05$) reduced the antibacterial efficacy of BWF-3 against SA and LM. Food model based decontamination of carrot cubes using BWF-3 effectively suppressed the growth of LM for seven consecutive days in chilled (4°C) storage, which is comparable to sodium hypochlorite at the concentration of 100 ppm. Microscopic examination revealed cell membrane LM was altered after exposed to the bioactive BWF-3. Additionally, survival of LM increased with the fortification of ferum (II) and (III) at concentration as low as 1 mM but not for calcium, magnesium, manganese and glucose. Compatibility of BWF-3 as food bio-preservatives was proven as their efficacy and applicability was found comparable to those industrial synthetic

preservatives. In conclusion, banana inflorescence fractions are potential ingredient that could serves as an alternative to the current synthetic antibacterial as a decontaminating solution as well as preserving the minimally processed foods. Nevertheless, more studies associated with toxicity and safety evaluation should be carried out before the antibacterial fraction could be used as biopreservative in foods.

ABSTRAK

BAHAN HASILAN SAMPINGAN PISANG (Musa spp.) SEBAGAI BIO-PENGAWET ANTIMIKROORGANISMA BERPOTENSI

Bahan hasilan sampingan pisang telah dikaji bagi mengasingkan dan mengenalpasti komponen antibakteria bagi kegunaan bio-pengawet dalam model kaldu mikrobiologi dan makanan. Bahagian-bahagian daripada hasilan sampingan pisang telah diekstrak menggunakan pelbagai pelarut diuji untuk aktiviti antibakteria menggunakan kaedah penyerapan agar. Kesan kedudukan geografi, cara pengeringan dan faktor pengektrakan terhadap aktiviti antibakteria turut diuji. Pendekatan sistematik digunakan untuk mengkaji factor pengekstrakan (cnth. masa, suhu dan campuran pelarut) dan pengoptimuman kaedah pengekstrakan menggunakan Face-Center Central Composite Design (FFCCD) design "response surface methodology" (RSM) diaplikasikan untuk memperoleh kaedah pengekstrakan efektif serta mempunyai ekstrak bersifat antibakteria yang tinggi. Pengasingan dan pengenalpastian kompoun antibakteria dilakukan dengan menggunakan pendekatan kromatografi berdasarkan prinsip spektrometri. Kesan komposisi makanan terhadap potenti fraksi antibakteria dilakukan dalam kaldu mikrobiologi. Seterusnya, kiub lobak merah diaplikasikan sebagai model makanan dalam ujian penilaian potensi antibakteria dan mekanisma antibakteria turut diselidik. Ujian menunjukan ekstrak metanol daripada bunga jantung pisang *Musa balbisiana* cv. Saba menunjukan sifat antibakteria terhadap *Staphylococcus aureus* (SA), *Bacillus cereus* (BC), *Listeria monocytogenes* (LM) dan *Vibrio parahaemolyticus* (VP). Kedudukan geografi tidak menunjukan kesan yang signifikan terhadap aktiviti antibakteria. Akan tetapi, pengeringan ketuhar pada suhu 50°C didapati mengekalkan aktiviti antibakteria serupa dengan pengeringan sejukbeku dan signifikan lebih baik dapipada pengeringan matahari. Kaedah RSM telah membolehkan pengekstrakan komponen antibakteria pada tahap optimum dengan efisyen (masa pengekstrakan: 6.0 jam, suhu pengekstakan: 35ºC, peratus metanol kepada air: 94%). Ekstrak metanolik optimum turut disesekat kepada klorofom, etil asetat, butanol dan air. Sesekat air terus ditulenkan dengan menggunaan ektraksi fasa pejal dan 3 fraksi diperoleh. Fraksi bio-aktif tersebut dikenalpasti mengandungi kompoun antibakteria seperti epigalokatekin and derivatif dan triptofan dengan menggunakan kromatografi cecair bepandukan jisim ion molekul. Selain itu, tiga kompaun bersifat anktibakteria turut diasingkan datipada sesekat klorofom iaitu 31-norsiklolaudenon, sikloartenol dan 24R)-4a,24-trimetil-5a-kolesta-8,25(27)-dien-3b-ol. Sesekat metanolic air (H_2O Fr.) dan fraksi SPE 3 (BWF-3) menunjukkan kelebihan dalam aktiviti antibakteria (MIC H_2O Fr.: 8.0 mg/ml – 25.0 mg/ml, MIC BWF-3: 0.6 mg/ml – 2.5 mg/ml) terhadap SA, BC, LM and VP. Di kalangan komposisi makanan, 1% penambahan protein dan lemak menurunkan aktiviti BWF-3 terhadap SA dan LM secara signifikan ($p<0.05$). Proses penyahkontaminasi permukaan lobak merah merencat pertumbuhan LM 7 hari berturut pada penyimpanan sejuk (4°C), setanding dengan piawai komersial natrium klorida pada kepekatan 100ppm. Pemeriksaan mikroskop menunjukkan perubahan pada sel membran sel selepas didedahkan kepada BWF-3. Tambahan

pula, pertumbuhan LM meningkat selepas penambahan ion besi (II) and (III) pada kepekatan sebanyak 1 mM, tetapi peningkatan ini tidak didapati pada penambahan kalsium, magnesium, mangan dan glukosa. Keberkesanan BWF-3 sebagai bio-pengawet telah dibuktikan setanding dengan pengawet sintetik komersial. Oleh yang demikian, fraksi jantung pisang berpotensi untuk digunakan sebagai alternatif kepada pengawet sintetik sebagai cecair penyahkontaminasi bagi makan proses minimum. Namun, pengajian terhadap toksikologi dan keselamatan fraksi sebelum digunakan sebagai bio-pengawet dalam makanan perlu dijalankan.

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ATP	adenosine triphosphate
α-	alpha
ATCC	American Type Culture Collection
AUD	Australian Dollar
BC	*Bacillus cereus*
β-	beta
BWF-3	bioactive water fraction
Ca	Calsium
C	carbon
CO_2	carbon dioxide
CDC	Centre for Disease Control, Unites States of America
$CHCL_3$	chloroform
C.V.	coefficient of variance
CFU	colony forming unit
COSY	Correlation spectroscopy
cv.	cultivar
Da	Dalton
°C	degree Celsius
DI H_2O	deionized water
DOA	Department of Agriculture, Malaysia
Diff.	differences
DAD	diode array detector
EGC	(-)-epigallocatechin
eV	electron volt
ESI	electrospray ionization
et al.	*et alii* (and others, individuals)
EtOH	ethanol
EtoAC	ethyl acetate
e.g.	*exemplii gratia* (such as)

$FeCl_3$	ferric chloride
Fe(III)	ferric ion
Fe^{3+}	ferric ion
$FeCl_2$	ferrous chloride
Fe(II)	ferrous ion
Fe^{2+}	ferrous ion
F-value	Fisher value
FD	freeze drying
GAEs	gallic acid equivalents
g	gram
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear single quantum coherence spectroscopy
h	hour
HCl	hydrochloric acid
H_2	hydrogen
H_2O_2	hydrogen peroxide
kg	kilogram
LC	liquid chromatography
LC/MS	liquid chromatography-mass spectrometry
LC/MS/MS	liquid chromatography-tandem mass spectrometry
LLE	liquid-liquid extraction
LM	*Listeria monocytogenes*
Mg	magnesium
Mn	manganase
m/z	mass to charge ratio
MeOH	methanol
MT	metric ton
µg	microgram
µg/ml	microgram per milliliter
µl	microlitre
µM	micromolar
µM	micromolar

mg	milligram
mg/L	milligram per liter
mg/ml	milligram per milliliter
ml	milliliter
mm	millimeter
mM	millimolar
MBC	minimum bactericidal concentration
MIC	minimum inhibition concentration
MOH	Ministry of Health, Malaysia
min	minute
mol/L	mol per liter
M	molar
n-BuOH	n-butanol
NMR	neuclear magnetic resonance
na	no inhibitory activity
ND	not determined
NOESY	Nuclear Overhauser effect spectroscopy
n	number
OD	optical density
OD	oven drying
%	percentage
p	pi
±	plus-minus
pH	power of hydrogen
H	proton
Qtof	quadrupole-time of flight
ROS	reactive oxygen species
R	regression coefficient
RSM	response surface methodology
rpm	revolution per minute
Na	sodium
NaCl	sodium chloride

SPE	solid phase extraction
SD	standard deviation
SA	*Staphylococcus aureus*
SD	sun drying
TLC	thin layer chromatography
TSA	Tryptone soy agar
TSB	Tryptone soy broth
UV	ultraviolet
USD	United States Dollar
US	United States of America
VP	*Vibrio parahaemolyticus*
VIS	visible light
v/v	volume by volume
H_2O	water
w/v	weight by volume
WTP	willingness to pay
WHO	World Health Organization
WTO	World Trade Organization

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