UNIVERSITI PUTRA MALAYSIA

ANTIBACTERIAL ACTIVITIES OF Carica papaya L. SEED AS FOOD PRESERVATIVE

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Chairman : Professor Jamilah Bakar, PhD
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Presently, no known commercial application of *Carica papaya* seeds has been recorded. It is mainly discarded; therefore, the aim of this study is to evaluate the potential of these seeds as a source of antibacterial compounds for possible application as food preservatives. The phytochemical components and extraction efficiency were first carried out using ten different solvents based on different polarities. The best solvent extraction was further carried out and improved by evaluating the effect of sonication (SAE), contact time (CT) and solvent-to-solid ratio (SSR). The effective of the initial extraction and the improved method were evaluated by determining the total phenolic and flavonoid contents and antibacterial activity against *Salmonella enteritidis*, *Bacillus cereus*, *Vibrio vulnificus* and *Proteus mirabilis* as indicator microorganisms. The composition of the *Carica papaya* seed crude extract, hexane: ethyl acetate (1:1) fraction and sub-fraction C were identified using gas chromatography mass spectrometer (GC/MS). The antibacterial activity and toxicity of these extracts were also studied. The most potent with least toxic extract was evaluated for antibacterial activity and stability as antibacterial agent in food model systems and finally as an antibacterial agent in yellow noodle. Based on disk diffusion (DDT) and minimum inhibitory concentration (MIC) tests, the hierarchy of extract potency can be ranked as methanol > acetone > acetonitrile > chloroform > hexane > diethyl ether = petroleum ether > ethanol > dichloromethane. The *S. enteritidis*, *V. vulnificus, P. mirabilis* and *B. cereus* were selected as indicator microorganisms in this study due to the lowest MIC (5.63 mg/mL). Improved extraction of *Carica papaya* seed antibacterial compounds was best performed without SAE at 8 h CT with 10:1 SSR. These SAE, CT and SSR extraction treatments produced the yield of 21.59 - 81.15 mg/g, 20.10 - 62.78 mg GAE/g DW of TPCs, the lowest MIC (5.63 mg/mL), MIC_{50} (1.87 - 3.67 mg/mL) and the lowest MIC_0 (< 0.02 mg/mL). The antibacterial activity of crude and partially purified *Carica papaya* seed extracted on *S. enteritidis*, *V. vulnificus*, *B. cereus* and *P. mirabilis* were evaluated using bioautography of thin layer chromatography (TLC) and MIC test which had found that

hexane: ethyl acetate (1:1) fraction and sub-fraction C (R_f= 0.94 ± 0.03) showed highest bioautographic potency against tested microorganisms; however, having equal MIC (5.63 mg/mL) but higher toxicity (LC_{50} = 1.797 mg/mL and 0.332 mg/mL, respectively) as compared to crude extract (LC_{50} = 5.505 mg/mL). Thus, indicating the latter was adopted for further antibacterial application study. Twenty-one groups of components were identified in derivatized and non-derivatized crude extracts using GC/MS where fatty acids and fatty acid methyl esters (80.23%) were the major components. Cis-vaccenic acid, which was the dominant component in sub-fraction C, in pure form had demonstrated the lowest MIC against *B. cereus* (1.41 mg/mL), *P. mirabilis* (1.41 mg/mL) and *S. enteritidis* (0.70 mg/mL); thus, signifying its potency as antibacterial component in the extract. The extract had shown potency in acidic condition (pH 4), water activity > 0.909 and temperature < 80°C at 5.63 mg/mL (MIC). The extract was capable of inhibiting the tested microorganisms in 5%, 10%, 20% and 30% of carbohydrate and 2%, 5%, 10% and 15% oil media and 5% protein medium. The shelf life of yellow noodle treated with *Carica papaya* seed extract was extended from 3 d - 12 d for 1.41, 5.63, 11.25 and 22.5 mg/g extracts and from 3 d - 10 d for 0.70 mg/g extract. The principle component (PCA), cluster (CA) and discriminant (DA) analyses had indicated the effective time of antibacterial activity of *Carica papaya* seed and the safe consumption of the treated yellow noodle was to be at least by day sixth of storage. In conclusion, *Carica papaya* seed extract is a potential antibacterial extract that able to act as a food preservative.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah.

AKTIVITI-AKTIVITI ANTIBAKTERIA BIJI *Carica papaya* L. SEBAGAI PENGAWET MAKANAN

Oleh

MUHAMAD SHIRWAN ABDULLAH SANI

Januari 2018

Pengerusi : Profesor Jamilah Bakar, PhD
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Pada masa kini, tiada aplikasi komersil biji *Carica papaya* telah direkodkan. Biji betik pada kebiasaannya dibuang. Oleh sebab itu, tujuan kajian ini dijalankan adalah untuk menilai potensi biji ini sebagai sumber agen antibakteria bagi potensi aplikasi sebagai pengawet makanan. Komponen fitokimia dan kecekapan pengekstrakan pada mulanya dijalankan menggunakan sepuluh pelarut yang berbeza berdasarkan kepolaran yang berbeza. Kaedah pengekstrakan terbaik dijalankan dan dipertingkatkan dengan menilai kesan sonikasi (SAE), masa pengekstrakan (CT) dan nisbah pelarut kepada pepejal (SSR). Pengekstrakan permulaan yang berkesan dan kaedah pengekstrakan yang telah dipertingkatkan telah dinilai dengan menentukan jumlah kandungan fenol dan flavonoid dan aktiviti antibakteria terhadap *Salmonella enteritidis*, *Bacillus cereus*, *Vibrio vulnificus* dan *Proteus mirabilis* sebagai penanda mikroorganisma. Komposisi ekstrak *Carica papaya* mentah, fraksi heksana: etil asetat (1: 1) dan subfraksi C telah dikenalpasti menggunakan kromatografi gas spektrometer jisim (GC/MS). Aktiviti antibakteria dan ketoksikan ekstrak ini juga telah dikaji. Ekstrak yang paling poten dan berketoksikan terendah dinilai bagi aktiviti antibakteria dan kestabilannya sebagai agen antibakteria dalam sistem model makanan dan akhirnya sebagai agen antibakteria di dalam mee kuning. Berdasarkan ujian penyebaran cakera (DDT) dan ujian perencatan kepekatan minimum (MIC), hierarki keupayaan ekstrak dapat disenaraikan sebagai metanol > aseton > asetonitril > kloroform > heksana > dietil eter = petroleum eter > etanol > diklorometana. *S. enteritidis*, *V. vulnificus*, *P. mirabilis* dan *B. cereus* dipilih sebagai mikroorganisma-mikroorganisma penanda dalam kajian ini kerana mempunyai MIC terendah (5.63 mg/mL). Pengektrakan biji *Carica papaya* yang terbaik dilakukan tanpa SAE pada 8 h CT dengan 10: 1 SSR. Pengekstrakan SAE, CT dan SSR memberikan hasil antara 21.59 - 81.15 mg/g, 20.10 - 62.78 mg GAE/g DW total kandungan fenol, MIC terendah (5.63 mg/mL), MIC_{50} (1.87 - 3.67 mg/mL) dan MIC_0 terendah (< 0.02 mg/mL). Aktiviti antibakteria biji *Carica papaya* mentah dan separa penulenan terhadap *S. enteritidis*, *V. vulnificus*, *B. cereus* dan *P. mirabilis* telah dinilai

menggunakan bioautografi kromatografi lapisan nipis (TLC) dan ujian MIC yang telah mendapati bahawa fraksi heksana: etil asetat (1: 1) dan subfraksi C (R_f = 0.94 ± 0.03) menunjukkan potensi bioautografi tertinggi terhadap mikroorganisma-mikroorganisma yang diuji dengan memberikan MIC (5.63 mg/mL) yang sama tetapi berketoksikan yang lebih tinggi (LC_{50} = 1.797 mg/mL dan 0.332 mg/mL) berbanding ekstrak mentah (LC_{50} = 5.505 mg/mL). Oleh sebab itu, ekstrak biji *Carica papaya* mentah digunakan untuk kajian antibakteria lanjut. Dua puluh satu kumpulan komponen telah dikenalpasti dalam ekstrak mentah melalui kaedah terbitan dan bukan terbitan menggunakan GC/MS di mana asid lemak dan asid lemak metil ester (80.23%) menjadi komponen utama. Asid cis-vasenik, yang merupakan komponen utama di dalam subfraksi C, di dalam keadaan tulen telah menunjukkan MIC terendah terhadap *B. cereus* (1.41 mg/mL), *P. mirabilis* (1.41 mg/mL) dan *S. enteritidis* (0.70 mg/mL) lalu menunjukkan ia sebagai komponen antibakteria yang poten di dalam ekstrak mentah. Ekstrak mentah juga telah menunjukkan potensi dalam keadaan berasid (pH 4), aktiviti air > 0.909 dan suhu < 80°C pada kepekatan 5.63 mg/mL (MIC). Ekstrak ini mampu merencatkan pertumbuhan mikroorganisma yang diuji dalam kepekatan 5%, 10%, 20% dan 30% media karbohidrat dan minyak dan 5% medium protein. Jangka hayat mee kuning yang dirawat dengan ekstrak biji *Carica papaya* boleh dilanjutkan dari 3 hari hingga 12 hari dengan menggunakan kepekatan ekstrak 1.41, 5.63, 11.25 dan 22.5 mg/g, dan 10 hari menggunakan kepekatan ektrak 0.70 mg/g. Analisis komponen asas (PCA), analisis kluster (CA) dan analisis diskriminasi (DA) telah menentukan masa berkesan bagi biji *Carica papaya* menunjukkan aktiviti antibakterianya dan penggunaan selamat mee yang dirawat adalah sekurang-kurangnya sehingga hari keenam penyimpanan. Sebagai kesimpulan, ekstrak biji adalah berpotensi untuk menjadi esktrak antibakteria yang mampu bertindak sebagai pengawet makanan.

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Finally, Thank you Allah for this priceless journey.

I certify that a Thesis Examination Committee has met on 26 January 2018 to conduct the final examination of Muhamad Shirwan bin Abdullah Sani on his thesis entitled "Antibacterial Activities of *Carica papaya* L. Seed as Food Preservative" 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 Doctor of Philosophy.

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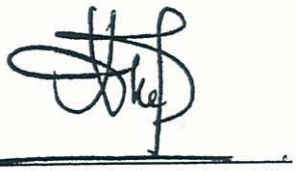

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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LIST OF ABBREVIATIONS

PE	Petroleum ether
DE	Diethyl ether
CHCL3	Chloroform
DCM	Dichloromethane
EtOH	Ethanol
MeOH	Methanol
ACN	Acetonitrile
GAE	Gallic acid equivalent
QE	Quercetin equivalent
Rt	Retention time
SAE	Sonication-assisted extraction
CT	Contact time
SSR	Solvent-to-solid ratio
TPC	Total phenolic content
TFC	Total flavonoid content
MIC	Minimum inhibitory concentration
R_f	Retention factor
TLC	Thin layer chromatography
UV-VIS	Ultraviolet-visible light
NIST	National Institute of Standard and Technology
LTP	Linear-temperature programming
NLTP	Non-linear-temperature programming
TMS	Trimethylsilyl derivatives
TMCS	Trimethylchlorosilane
BSTFA	*N, O*-bis-trimethylsilyl-trifluoroacetamide
GC/MS	Gas chromatography mass spectrometer
RI	Retention index
LRI	Linear retention index
SIM	Selected ion monitoring
R^2	Coefficient determination
PI	Percentage inhibition
FL	Factor loading
PCA	Principle component analysis
CA	Cluster analysis
DA	Discriminant analysis

CHAPTER 1

INTRODUCTION

1.1 Background

One of the most significant attempt to curb the foodborne illness is by introducing synthetic chemicals such as antibacterial agents or preservatives during food processing and preservation. Despite its efficacy, synthetic antimicrobial agents have several major drawbacks such as hypersensitivity, allergic reaction, immunity suppression and toxic to human, which has become an immense anxiety to consumers. The emergence of antibiotic resistance microorganisms such as methicillin-resistant *Staphylococcus aureus* indicates the developing microorganism's resistance towards these chemicals, thus the need to use natural antibacterial agent as a healthier alternative.

Natural source of antibacterial agent originated from plants including discarded plant parts such as peel, seed, leaves and barks. Papaya (*Carica papaya)* is one of the most widely grown plant which has plenty discarded parts during the processing. Papaya is popular consumed as a dessert fruits all over the world because it is delicious, has abundant nutrition, and high economic value. Papaya is known differently in different parts of the world, for example, 'pawpaw' in Sri Langka, 'betik' in Malaysia, 'papali' in India, and 'lechosa' in Venezuela. Furthermore, papaya consists of different varieties such as *Maradol* (Southen America), *Coorg Honey* (India), Mexican Yellow and Mexican Red (Mexico), *Solo* (Hawaii) and *Sekaki* (Malaysia).

In 2014, India and Brazil were the major producers of 5.6 and 1.6 million metric tonnes papaya, respectively. Malaysia also produced 55000 metric tonnes of papaya within the same period. The fruits are normally eaten ripe or unripe or are processed into jam and pickles; however, the seeds which is accounted for 16% of the whole papaya fruit are frequently discarded. This discarding of papaya seed takes an approximate of 2.1 million metric tonnes that goes to waste. The seeds have been reported to be used as a salad dressing due to its spicy taste. It is also reported as an adulterant to black pepper. To date, the study on of papaya seeds as antibacterial agent is very scanty.

1.2 Problem statements

The processing of papaya involves separating the valuable fruit part from its by-products such as seeds and peels. Disposal of these by-products poses an environmental use and loss of potential revenue if the seeds and peels contain valuable components for food processing or other industrial applications. In most cases, the discarded by-products can present similar or even higher contents of bioactive compounds than the final produce

does. Even though the protein precipitated from papaya seeds has been reported to give significant antibacterial activity, the antibacterial study of papaya seed from selected variety, extraction method and quantification of potent concentration are still very scanty. Moreover, the exhaustive investigation of papaya seed composition and further application of antibacterial agent from papaya seed on food has never been recorded.

1.3 Significance of the study

To date, there is negligible information of the antibacterial activity of papaya seed. Through the finding from this study, the papaya seed usage can contribute to the utilization of renewable resources as antibacterial agents not only in food, but other applications as well such as cosmetics, pharmaceuticals etc. In addition, since the papaya seed are discarded during papaya processing, the finding in this study also can generate income for the papaya producers. Furthermore, by utilizing the seed, the papaya producers can reduce the amount agriculture-based waste and thus mitigate environmental issues related to the disposal of discarded or underutilized papaya by-products. Moreover, the papaya seed as antibacterial agent can fulfil the requirement of making Halal and toyyiban product since it is plant origin and can be used as an alternative to replace toxic antibacterial agent such as boric acid in the market.

1.4 Objectives

The main objective of this study was to investigate the antibacterial properties of papaya seed extract as food preservative, where the study covered specific objectives as follows:

a) To determine antibacterial properties and phytochemicals from papaya seed crude extract obtained from potential effective solvents.
b) To identify the effect of solvent mixture, contact time, solvent to solid ratio on antibacterial capacities.
c) To determine the composition, antibacterial activities, stability of crude and partially purified *Carica papaya* seed extracts and their effective range in food model systems.
d) To determine the antibacterial efficiency in extending the shelf life of fresh yellow noodle.

1.5 Expected outcomes

The expected outcomes from this study were:

a) Objective 1: The antibacterial capacity of papaya seed, the best extracts solvents for the most sensitive microorganisms against the extracts.
b) Objective 2: The optimum extraction procedure can be achieved which possessed antibacterial properties

c) Objective 3: Identification of papaya seed composition, the stability and the antibacterial capability of crude and partially purified extract
d) Objective 4: Validation of the antibacterial efficacy of the extract in actual food system.

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