UNIVERSITI PUTRA MALAYSIA

ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF Garcinia mangostana L. LEAF EXTRACTS AGAINST Xanthomonas oryzae pv. oryzae AND Pseudomonas syringae pv. TOMATO

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By

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The immense diversity of plant pathogens, which include viruses, bacteria, fungi, nematodes, and insects, approximates 7100 species. Among these, roughly 150 are bacterial species that cause diseases to plants. Bacterial plant diseases are most frequent and severe in tropical and subtropical places, where warm and humid conditions like Malaysia are ideal for bacterial growth. Indeed, consistent annual crop losses are recorded in all countries.

The problem of plant diseases is depended on chemical control as antibiotic. This chemical control is very expensive and yet not very effective against for many bacterial diseases. In this study, the leaf of mangosteen was used to prepare extract to bio-control for two types of plant pathogens namely *Pseudomonas syringe pv. tomato* and *Xanthomonas oryzae pv. Oryzae*.

The potential of mangosteen (Garcinia mangostana L.) leaf extract as a biological control agent against plant pathogenic bacteria which are responsible to decrease the quality and volume of crop production worldwide was assessed. Extract was obtained by maceration of the leaves using chloroform, n-hexane, and methanol. Crude extracts of about 1.45 % were derived using chloroform, 1.25 % using n-hexane, and 1.65 % using methanol leaf crude. Compared to chloroform and n-hexane, effective extraction of readily soluble compounds was observed in case of methanol, as the highest yield was collected from it.

For the in-vitro antibacterial activity, two plant pathogenic bacteria, namely *Pseudomonas syringe pv. tomato* and *Xanthomonas oryzae pv. oryzae* were acquired. Four different concentrations of 12.5, 25, 50, and 100 mg/mL were used through the cup-plate agar diffusion technique. Streptomycin sulphate at 30 µg/mL concentration was set as a positive control, whereas every respective solvent used in the leaf extraction was set as negative controls. The highest diameter value of inhibition zone was observed in *P. syrange pv. tomato* at all range of concentrations, followed by *X. oryzae pv. oryzae*.

Since only the methanol extract demonstrated antibacterial activity, it was the only solvent subjected in the assay for minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) determination. The least methanol extract concentration utilised in MIC assay was at 1.56 mg/mL, inhibiting X. oryzae pv. oryzae followed by P. syrange pv. tomato at a concentration of 3.13 mg/mL. This assay indicated that methanol extract caused bactericidal impacts at concentrations of 1.56 mg/ml and 3.13 mg/ml for varying plant pathogenic bacteria species.

The least concentration of MBC noted was at 3.13 mg/mL against X. oryzae pv. oryzae and 6.25 mg/mL against P. syrange pv. tomato. Mangosteen methanol leaf extracts' primary phytochemical screening indicated the existence of flavonoids, alkaloids, saponins, tannins, anthraquinones, terpenoids, and phenols. When visualised in the thin layer chromatography (TLC) profiling of methanol extract using acetone and n-hexane in a ratio of 6:4 (v/v) gave 11 maximum colourful bands.

Retention factor (Rf) values proved the presence of various active secondary metabolites within the methanol extract. Methanol extract's antibacterial activity was screened through the direct bioautography procedure. The intention was to determine the location of active bands on chromatograms developed for TLC profiling in the same manner. The most active Rf values that inhibited every tested plant pathogenic bacteria at the same location of Rf values were noted at 0.93, 0.86, 0.66, 0.46, 0.33 and 0.16. A comparison of the Rf values acquired was made with earlier studies utilising the same solvent system. Antibacterial impacts of the most effectual extract of mangosteen crude were supported by the existence of chemical components identified by Gas Chromatography-Mass Spectrometry (GC-MS).

The G. mangostana leaf extract was exhibited good potential to be used to inhibit growth of Xanthomonas oryzae pv. oryzae and Pseudomonas syringae pv. tomato invitro. The results showed that methanol extract demonstrated antibacterial activity when tested on the plant pathogenic bacteria in-vitro. On the other hand, chloroform and n-hexane did not exhibit any antibacterial activity against plant pathogenic bacteria, as there was no inhibition zone noted under these treatments. Cycloartenol, caryophyllene, docosane, and 4, 4-methylenebis (2, 6-di-tert-butylphenol) were noted as key compounds in the mangosteen leaf extract.

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AKTIVITI ANTIBAKTERIA DAN ANALISIS PHYTOKIMIA *Garcinia mangostana* L. LEAF EKSTRAK TERHADAP *Xanthomonas oryzae* pv. *oryzae* dan *Pseudomonas syringae* pv. TOMATO

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Kepelbagaian besar patogen tumbuhan, termasuk virus, bakteria, kulat, nematod dan serangga, lebih kurang 7100 spesies. Antaranya, kira-kira 150 spesies bakteria yang menyebabkan penyakit kepada tumbuhan. Penyakit tumbuhan bakteria yang paling kerap dan teruk di tempat-tempat tropika dan subtropika, di mana keadaan panas dan lembap seperti Malaysia adalah sesuai untuk pertumbuhan bakteria. Sesungguhnya, selaras kerugian tanaman tahunan direkodkan di semua negara.

Masalah penyakit tumbuhan adalah bergantung kepada kawalan kimia sebagai antibiotik. Ini kawalan kimia adalah sangat mahal tetapi tidak sangat berkesan terhadap pelbagai penyakit bakteria. Dalam kajian ini, daun manggis telah digunakan untuk menyediakan ekstrak untuk bio-kawalan untuk dua jenis patogen tumbuhan iaitu Pseudomonas syringae pv. tomato dan Xanthomonas oryzae pv. Oryzae.Potensi Garcinia mangostana sebagai agen kawalan biologi terhadap bakteria penyakit tumbuhan atau mikrob memudaratkan yang mengurangkan kualiti dan jumlah pengeluaran tanaman di seluruh dunia telah dinilai. Daun manggis diekstrak secara maksima menggunakan chloroform, n-heksana dan metanol.

Ekstrak mentah kira-kira 1.45% telah diperolehi menggunakan kloroform , 1.25 % menggunakan n- heksana, dan 1.65 % menggunakan metanol daun mentah. Berbanding dengan kloroform dan heksana, pengekstrakan sebatian mudah larut adalah lebih berkesan di dalam metanol, dengan hasil tertinggi dikumpulkan daripadanya. Bagi aktiviti anti-bakteria secara in-vitro, dua spesies berbeza bakteria penyakit tumbuhan, iaitu Pseudomonas syringae pv. tomato dan Xanthomonas oryzae pv . oryzae diperolehi. Empat kepekatan berbeza, 12.5, 25, 50 dan 100 mg/ml yang diperolehi melalui teknik cawan-plat serapan agar. Streptomycin sulfate pada kepekatan 30 µg/ml telah disetkan sebagai kawalan positif, dan setiap pelarut yang digunakan di dalam pengekstrakan daun disetkan sebagai kawalan negatif. Zon perencatan paling

tinggi diperhatikan pada P. syrange pv. tomato pada pelbagai julat kepekatan, diikuti oleh X. oryzae pv. oryzae. Oleh kerana hanya ekstrak methanol mempamerkan aktiviti anti-bakteria, hanya pelarut ini sahaja digunakan untuk menentukan percubaan kepekatan bakterisidal minimum (MBC) dan kepekatan perencatan minimum (MIC). Kepekatan ekstrak methanol yang paling sedikit digunakan di dalam MIC adalah pada 1.56 mg/ml, merencatkan X. oryzae pv. oryzae, diikuti oleh P. syringae pv. tomato pada kepekatan 3.13 mg/ml. percubaan ini menunjukkan ekstrak methanol menyebabkan kesan bakteriostatik pada kepekatan 1.56 mg/ml dan 3.13 mg/ml untuk pelbagai spesies bakteria penyakit tumbuhan.

Kepekatan yang paling sedikit untuk MBC telah dicatatkan pada 3.13 mg/ml terhadap X. oryzae pv. oryzae dan 6.25 mg/ml terhadap P. syringae pv. tomato. Pemeriksaan fitokimia utama bagi ekstrat metanol daun manggis telah menyatakan kewujudan flavonoid, alkaloid, saponin, tannin, anthraquinone, terpenoid dan phenol. Apabila diperhatikan, pemprofilan kromatografi lapisan nipis (TLC) bagi ekstrak metanol menggunakan aseton dan heksana dalam nisbah 6:4 (v/v) telah memberikan maksimum 11 jalur warna warni. Nilai Rf mengesahkan kehadiran pelbagai metabolit sekunder yang aktif di dalam ekstrak metanol.

Aktiviti anti-bakteria bagi ekstrak metanol telah dijalankan melalui prosedur bio-autografi secara langsung. Ianya bertujuan untuk menentukan lokasi jalur yang aktif pada kromatogram yang telah dibuat bagi pemprofilan TLC dalam perkara yang sama. Nilai faktor pengekalan yang merencatkan setiap bakteria penyakit tumbuhan pada lokasi Rf yang sama telah dicatatkan pada 0.93, 0.86, 0.66, 0.46, 0.33 dan 0.16. Suatu perbandingan nilai Rf yang diperolehi telah dibuat melalui kajian awal menggunakan system pelarut yang sama. Kesan anti-bakteria bagi ekstrak mentah manggis yang paling berkesan telah disokong melalui kehadiran komponen kimia yang dikenalpasti melalui GC-MS.

G. ekstrak daun mangostana dipamerkan potensi yang baik untuk digunakan untuk menghalang pertumbuhan Xanthomonas oryzae pv. oryzae dan Pseudomonas syringae pv. tomato invitro. Hasil kajian menunjukkan bahawa ekstrak metanol menunjukkan aktiviti anti-bakteria apabila diuji pada tumbuhan bakteria patogenik in-vitro. Sebaliknya, kloroform dan n-heksana tidak menunjukkan apa-apa aktiviti antibakteria terhadap tumbuhan bakteria patogenik, kerana tidak ada zon perencatan dinyatakan di bawah rawatan ini. Cycloartenol, caryophyllene, docosane, dan 4, 4-methylenebis (2, 6-di-tert-butylphenol) telah diperhatikan sebagai sebatian utama dalam ekstrak daun manggis.

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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

ANOVA	Analysis of Variance
cfu/ml	Colony forming unit per milliliter
CRD	Completely Randomized Design
cm	Centimeter
GC-MS	Gas chromatography mass spectrometry
TLC	Thin layer chromatography
TTC	2,3,5- Triphenylterazolium chloride
GML	G. mangostana L
Kg	Kilogram
LCB	lacto phenol-Cotton blue
LSD	least significant difference
M	Molar
MeOH	Methanol
MBC	Minimum Bactericidal Concentration
MIC	Minimum inhibitory concentration
MHA	Muller Hinton Agar
MHB	Muller Hinton Broth
µl	Microliter
Mm	Mill molar
Ml	Millilitre
mL min-1	Millilitre per minute
µm	Micrometer
NA	Nutrient agar
NB	Nutrient broth
OD	Optical density
± SEM	Standard error of means
Temp	Temperature
v/v	Volume per volume
w/v	Weight per volume

CHAPTER 1

INTRODUCTION

1.1 Introduction

The rate of plant production worldwide is severely affected due to the presence of plant diseases. There are more than 200 types of diseases that affect plants, out of which 70% of the diseases are casued by fungal and bacterial infections (Janisiewicz et.al. 2001). The bacterial plant pathogens play havoc with the economy as they can destroy the economically relevant crops. They also affect the quality of the commercial crops. Hence, they need to be properly controlled. However, the major problem of effective bacterial pathogens controlled in the orchards is the unavailability of proper commercial antibacterial agents. The available antibiotics have a questionable efficacy and are banned in several countries, and they are also not very effective against many pathogenic agents.

Unfortunately, there are several reports that state the control of the fungal outbreaks is possible using chemicals compounds but, very few reports have stated the control of the bacterial diseases using nonchemicals. Hence, it is important to find alternative means for controlling the bacterial diseases, rather than using chemical means, and therefore, there is an increase in re-examining and improving several traditional practices and techniques and developing new practices for controlling bacterial crop diseases (Moazami, 2008).

The most popular botanical pesticides include the phenolic-rich plant extracts. The plant extracts which are rich in tannins have been widely used for the treatment of human infectious diseases (Tegegne, 2008). Moreover, they display antimicrobial activities against several bacterial species and the phytopathogenic fungi.

Mangosteen belonging to the Clusiaceae family is called as the “the queen of fruits”. This fruit is cultivated widely in the tropical rainforests of the Southeast Asian countries like Malaysia. The mangosteen pericarp has been used in traditional medicines for treating disorders like diarrhoea, dysentery, abdominal pain, suppuration, infected wounds, and chronic ulcers. There are reports in the literature which have stated that the leaf of mangosteen extracts contain antitumor, antiviral, antibacterial, anti-allergic, antioxidant, and anti-inflammatory activities (Pedraza-Chaverri et.al. 2008).

The xanthones can be extracted from the pericarp, heartwood, whole fruit, and leaves of GML. The mangosteen extracts were widely used in the medical field and pharmacology for the treatment of several animal and human diseases.
The *X. oryzae* pv. *oryzae*, is a very destructive and globally found bacterium, which mainly infects the rice crop and can lead to epidemic infections among

the high-yielding cultivars present in the temperate and the tropical regions of Asia (Mew et al; 1993; Young et al., 2008). Also, the *Pseudomonas syringae pv. tomato* (Okabe) causes the bacterial speck disease in tomatoes and this is a major disease afflicting the tomato plant worldwide (Milijašević, 2005).

Three major experiments have been done to achieve the objectives of this study. The *in-vitro* antibacterial activity assay was used through the cup-plate agar diffusion technique to determine the efficacy of the *G. mangostana* L plant extract. (MIC) Minimal Inhibitory Concentration and Minimum Bactericidal Concentration (MBC). Assay were used to determine the lowest concentrations of the most effective plant extracts.

Bioautography procedure enables recognition of known antimicrobial compounds in extracts. Gas Chromatography Mass Spectrometry (GC-MS) is the best method used to division, classification, and quantification of organic volatile and semi-volatile compounds.

1.2 Problem Statements

Chemical control is a very expensive process, and moreover, it is also not very effective against certain pathogens. Hence, developing biological control measures can help to manage the crop diseases. However, the biological controlling measures should also have additional economic attributes for making them more commercially viable. Since a very long time, the natural bioactive components, also called as herbal medicines or botanical pesticides have been used to contain the spread of plant diseases and killing the pathogens as well. However, the increase in the use of chemical pesticides in the agricultural sector has decreased the application of the botanical pesticides. Though the chemical pesticides are very effective, they have harmful side effects e.g., they damage the environment and are toxic to human and animal health.

1.3 Objectives

This study aim to prepare extracts from Mangostana leaves for antibacterial activity for two types of bacterial which are *Xanthomonas oryaza pv. oryaza* and *Pseudomonas Syringae pv. tomato.* and focusing on the following objectives:

1) To screen antibacterial activity of crude leaf of the *G. mangostana* L extracts against *Xanthomonas oryzae pv. oryzae* and *Pseudomonas syringae pv. tomato.* and determine the Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the active crude extract against two plant pathogen bacteria in vitro.

2) To determine the phytochemical in the active crude extracts of leaf *G. mangostana L.* using Thin Layer Chromatography (TLC) determination of phytcoumpound constituents by using Gass Chromatography Mass Spectrometry GC-MS.

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