UNIVERSITI PUTRA MALAYSIA

ANTIBACTERIAL ACTIVITY OF *Parkia speciosa Hassk* POD CRUDE EXTRACT AGAINST *Pseudomonas fuscovaginae*

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By

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A PROJECT REPORT SUBMITTED TO FACULTY OF AGRICULTURE, UNIVERSITI PUTRA MALAYSIA, IN FULFILLMENT OF THE REQUIREMENT OF PRT 4999 (FINAL YEAR PROJECT) FOR THE AWARD OF THE DEGREE OF BACHELOR OF AGRICULTURAL SCIENCE.

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This project report entitled antibacterial activity of *Parkia speciosa* Hassk pod crude extract against *Pseudomonas fuscovaginae* is prepared by Kah Oi Yuan and submitted to the Faculty of Agriculture in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science.

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LIST OF ABBREVIATION

cm	Centimeter
eg	Example
sp	Species
%	Percentage
ha	Hectare
°C	Degree Celsius
g	Gram
L	Liter
ø	Diameter
mm	Millimeter
rpm	Revolutions per minute
mL	Milliliter
nm	Nanometer
A	Area
H	Height
Mol.weight	Molecular weight
mg	Milligram

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ABSTRACT

Rice is one of the most important food crops that feed almost over 3 billion of people worldwide. However, the outbreak of pest and diseases affected the world rice production. One of the serious infection on paddy plant is Bacterial Sheath Brown Rot caused by *Pseudomonas fuscovaginae. Pseudomonas fuscovaginae* is a florescent gram negative pathogenic bacterium. Several control methods had been carried out and found to be effective toward *Pseudomonas fuscovaginae* yet does not completely eliminate the pathogen from the plant. *Parkia speciosa* Hassk has been used as folk medicine to treat various human diseases and sickness. Alkaloid, Saponin, Terpenoids, Phenolic, Flavonoid, and Tannin were the phytochemical substances that can be found in all parts of *Parkia speciosa* plant. Several research had found that methanolic extract of *Parkia speciosa* pod can inhibit the growth of bacteria *Staphylococcus aureus* and *Escherichia coli*. However, there is no research on antibacterial activities of this plant extract toward plant pathogenic bacteria. This study is to identify the antibacterial activities of *Parkia speciosa* pod extract toward *Pseudomonas fuscovaginae* and to determine the active chemical compounds in the *Parkia speciosa* pod. Methanolic pod extract of *Parkia speciosa* with concentration of 25, 50, 100, 150, 200,250, 300, 350, and 400mg/mL were tested on the pathogenic bacteria for antibacterial activities using agar well diffusion assay. Streptomycin sulfate was used as positive control while methanol used as negative control. Phytochemical analysis was done by using standard method. The inhibition zone of the extract show positive results such as 1.90cm, 2.00cm, 2.00cm, 2.00cm, 2.00cm, and 1.92cm on concentration 100, 150, 200, 250, 300 and 350mg/ml respectively. Flavonoid, saponin, terpenoid, and tannin were present in *Parkia speciosa* pod extract. Pyrogallol which is known as antimicrobial chemical compound was identified in *Parkia speciosa* crude extract using Gas chromatography–Mass spectrum analysis.

Keywords: *Parkia speciosa*; Bacteria brown sheat rot; pod extract; *Pseudomonas fuscovaginae*; agar well diffusion assay; phytochemical analysis; inhibition zone; Gas chromatography–Mass spectrum analysis.

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ABSTRAK

Nasi merupakan salah satu sumber makanan yang penting bagi hamper 3 billion penduduk bumi di dunia ini. Walau bagaimanapun,wabak perosak dan penyakit telah menjejaskan pengeluaran padi di dunia ini. Salah satu jangkitan serius yang melanda padi adalah penyakit Brown Sheath Rot yang disebabkan oleh bacteria *Pseudomonas fuscovaginae. Pseudomonas fuscovaginae* merupakan bacteria floresen yang bergram negative. Beberapa kaedah pengawalan penyakit telah dijalankan, namun kaedah tersebut didapati tidak memberi kesan kepada *Pseudomonas fuscovaginae*. Petai atau dikenali sebagai *Parkia speci*osa Hassk digunakan sebagai ubat tradisional untuk merawat pelbagai penyakit manusia. Alkaloid, Saponin, Terpenoid, Fenolik, Flavonoid, dan Tannin ialah bahan fitokimia yang boleh didapati dalam semua bahagian tumbuhan *Parkia speciosa*. Beberapa penyelidikan telah membuktikan bahawa ekstrak metanolic daripada kulit *Parkia speciosa* mampu membantutkan tumbesaran bakteria *Staphylococcus aureus* dan *Escherichia coli*. Walau bagaimanapun, tidak ada kajian berkaitan dengan aktiviti anti-bakteria ekstrak tumbuhan ini ke atas bacteria patogenik tumbuhan. Kajian ini bertujuan untuk mengenalpasti aktiviti antibakteria ekstrak kulit *Parkia speciosa* dalam mengawal bakteria *Pseudomonas fuscovaginae* dan untuk menentukan bahan kimia aktif yang terkandung dalam kulit *P.speciosa*. Ekstrak kulit *Parkia speciosa* dengan kepekatan 25, 50, 100, 150, 200, 250, 300, 350, dan 400 mg/mL telah diuji pada bakteria patogenik untuk mengenalpasti reaksi aktiviti anti-bakteria dengan menggunakan agar well diffusion assay. Streptomisin sulfat telah digunakan sebagai kawalan positif manakala metanol digunakan sebagai kawalan negatif. Analisis fitokimia telah dilakukan dengan menggunakan kaedah yang ditentukan. Zon perencatan seluas 1.90cm, 2.00cm, 2.00cm, 2.00cm, 2.00cm dan 1.92cm pada kepekatan 100, 150, 200, 250, 300 dan 350mg/ml menunjukkan keputusan positif pada kajian ini. Flavonoid, saponin, terpenoid, dan tannin hadir dalam ekstrak kulit *Parkia speciosa*. Pyrogallol yang dikenali sebagai sebatian kimia antimikrob telah dikenalpasti dalam ekstrak kulit *Parkia speciosa* menggunakan kaedah Gas kromatografi-Mass analisis spectrum.

Kata kunci: *Parkia speciosa*; Bakteria Brown Sheath Rot; ekstrak kulit; *Pseudomonas fuscovaginae*; agar well diffusion assay; analisis fitokimia; zon perencatan; Gas kromatografi-Mass analisis spektrum.

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CHAPTER 1 INTRODUCTION

1.1 Overview

Just like fungi, virus, and nematodes, plant pathogenic bacteria also caused diseases to plant. Bacteria usually caused diseases with visible symptoms such as, bacterial spot & blights, bacterial wilt, bacterial soft rot, bacteria gall, bacterial cankers and bacterial scab. Most of bacterial diseases show similar symptom which is water-soaked lesion and foul odour. Some bacterial diseases are hard to recognize as the bacteria reproduce at rapid rate, carried systemically to plant cells through xylem and phloem, caused infection without show any visible symptoms and lead to chronic death of entire plant. Bacteria diseases are huge problems when the environments are warm, humid and rainy weather with poor air circulation . Bacteria attacked wide range of agricultural crops, eg: tomatoes, potatoes, carrot, paddy, tobacco, cotton, cucumbers, solanaceous plants and others. Since bacteria infection favour conducive environment and it can caused chronic disease in a short period, it is hard to control bacterial disease.

Pseudomonas fuscovaginae is a plant pathogenic bacterium and also the causal agent of bacterial brown sheath rot of rice. It is a non-spore forming, fluorescent, gram negative bacteria with rod shaped (Tanii *et al.* 1976). Brown Sheath Rot was detected and reported as the most important bacteria disease of rice at year 1976 in Hokkaido, Japan (Tanii *et al.* 1976). Even though brown sheath rot is considered as newly detected disease, yet, it is found and reported at several countries such as Latin America (Zeigler and Alvarez, 1987a; Zeigler and Alvarez, 1987b), Philippines (Cottyn *et al*. 1996),

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Central Africa (Autrique and Maraite, 1983) and China (Xie, 2003). The symptoms of brown sheath rot disease can be seen at early stage of plant growth, yet, it will only infect the plant at a later growth stage. Normally, infected paddy plants will be discolouration and become yellowish. Sheath lesion can be observed clearly either colour turned light or dark brown and slowly become necrotic after infection. Duveiller (1990) recorded, 10-20cm length angular and blackish brown lesion can be observed on the leaf sheath at the booting stage. It also caused paddy grain empty, malformed and grain sterility. The disease had distributed widely throughout the world and highly affected the economic yield loss of rice production. Method of using tolerant or resistant varieties to control and manage the disease was recommended as effective method by some researcher (Klement *et al.* 1990).

Phytochemicals are from the Greek word "phyto" which means "plant". Phytochemicals are non-nutritive, and natural compound that found in the plant which act for plant protection or preventive. These phytochemicals are found in vegetables, grains, legume, fruits, spices, and seeds. be used for antibacterial activities. Other that having the protective properties, these compounds are the reason of foods have their smell, taste, and colour. Phytochemicals also appear to have positive effect on human body. Food that being consumed in daily diet such as fruit and vegetable not only rich in fibre, vitamins and mineral, it also containing phytochemicals which gave support in human body action, like, antioxidant, stimulation of enzymes, interfering with DNA replication of cancel cells, antimicrobial activities, and physical action . Isoflavones, carotenoids, and flavonoid are examples of well-known phytochemical. Isoflavones can inhibit cell

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replication in gastrointestinal tract, in the same time may reduce the risk of breast cancer and colon caner which is estrogen-sensitive based cancer. Isoflavones are normally found in soybean and legumes. Carotenoids usually act as antioxidant and may enhance the immune system response, it can be found in carrots, sweet potatoes, and pumpkins; flavonoid act as antioxidnat, anti inflammatory, anti cancer, and may prevent heart disease. These compound can be found in onion, whole grains, tea and broccoli.

Kalemba and Kunicka (2003) reported that aromatic and medicinal plant contain essential oils that can act as a bio-control agent against bacteria, fungi, protozoa, viruses, plant and insect. In addition, essential oils from *Origanum vulgare* plant had tested and show positive result for in vitro inhibitory effect on several plant pathogenic bacteria, *X.vesicatoria* , *Pseudomonas syringae* pv *syringae*, *P. marginalis pv marginalis Bacillus sp.* Furthermore, flavonoid show anticancer, antimicrobial and anti-inflammatory activities (Aiyelaagbe, 2009); anti-invasive, anti-eicosanoid, and pro-apoptotic actions in breast and prostate cancer cells (Kawaii and Lansky, 2004). Presence of Tannin in methanolic extract of fruit rind *Punica granatum* show antibacterial activity (Chaturvedula *et al*. 2011)

Parkia speciosa Hassk, or normally known as petai is commonly popular as culinary ingredient in several country such as Malaysia, Indonesia, Singapore, Laos, and Thailand. People often consumed it either raw or cooked. It is known that Petai bean can be use in folk medicine to treat various diseases and symptoms such as diabetes, kidney disorder, hypertension and headache (Samuel *et al.* 2010; 2011; 2012). Petai seeds shown antibacterial activities (Gmelin *et al.* 1981; Fathaiya *et al.* 1995)

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because of the presence of antibacterial cyclic polysulfides. Flavonoids which act as antioxidant were found in ethanolic extract of petai seeds (Maisuthisakul *et al.* 2008). Petai is also recommended for blood-cleansing (Jocelyn Ling, 2013) and body detoxifying effect. Plant phenolic compounds extracts from empty petai pod and the petai beans were reported contain high amount of total polyphenol, phytosterol and flavonoids. Pitriyatin (2009) stated that strong and foul smell from urine is due to the amino acid contain in the Petai. In addition, this contain together with sulfur can inhibit pest growth. Nowadays, petai beans are the one that being consumed by human and the petai pods are considered as agricultural waste.

1.2 Problem Statement

The aim of this study was to investigate the antibacterial activity of *Parkia speciosa* pod extract against *Pseudomonas fuscovaginae*, and to determine the phytochemical compound present in the *Parkia speciosa* pod extract. Presently, there is no report and study about *Parkia speciosa* pod extract against plant pathogenic bacteria. Therefore, this will be the first report on the antimicrobial activity of *Parkia speciosa* pod extract against *Pseudomonas fuscovaginae*.

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1.3 Objectives:

a. To determine the efficiency of *Parkia speciosa's* crude extract in controlling *Pseudomonas fuscovaginae*

b. To determine the phytochemical compound in *Parkia speciosa* pod extract.

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