



**ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES, PHYTOCHEMICAL
AND TOXICITY ANALYSES OF ETHANOLIC *JAMBU BOL* [*Syzygium
malaccense* (L.) Merr. and Perry] LEAVES EXTRACT AND ITS
APPLICATION ON FOOD**

By

AL-ZABT ABDALRAHMAN MOHAMMAD KHAMEES

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Philosophy**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES, PHYTOCHEMICAL AND TOXICITY ANALYSES OF ETHANOLIC *JAMBU BOL* [*Syzygium malaccense* (L.) Merr. and Perry] LEAVES EXTRACT AND ITS APPLICATION ON FOOD

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December 2021

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Illnesses caused by food consumption are considered serious issues and must be solved. Synthetic food preservatives and sanitizers were used to overcome this issue, but many reports proved that these chemicals have side effects and could threaten human health. In contrast, plant-based preservatives and sanitizers are more recommended because plants are generally recognized as safe (GRAS), such as using *Syzygium malaccense* (L.) Merr. and Perry, one of the known plants used in folk medication. Thus, this research aimed to determine the antibacterial and stability of the ethanolic leaves extract of *S. malaccense* L. against *Bacillus* sp., *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella* Typhimurium, *Staphylococcus aureus*, and *Vibrio parahaemolyticus*, to determine the extract's total phenolic content (TPC) and antioxidants activity, to analyze toxicity and mode of action, to analyze its phytochemical constituents, and to evaluate the effect of the extract on microbial population in food samples. The results showed that the extract had an antibacterial activity with inhibition zone ranging 7.83 – 16.00 mm, MIC values ranging 0.31 – 5.00 mg/mL, MBC values went 0.62 – 5.00 mg/mL, and the analysis of the time-kill curves showed that the extract could reduce the bacterial population $\geq 3\text{Log}_{10}$ at a concentration of $4\times\text{MIC}$ within 4.0 hours. Generally, the antibacterial activity of the extract was stable/no significant change after being treated at different pHs and temperatures. The total phenolic content of the extract was determined to be 44.10 ± 0.06 mg GAE/g and the antioxidants reducing power was 1013.50 ± 0.07 mM Fe^{2+} /g. The IC_{50} scavenging activity of DPPH and ABTS• were 0.0334 mg Ascorbic acid E/g and 0.1352 mg TE/g, respectively. Bacterial cell's constituent release recorded remarkable increases in the OD values of the absorbed cell's constituents (0.788) at 260nm at $4\times\text{MIC}$, which is the highest recorded value after the positive control chlorohexidine (0.813). Similarly, crystal violet assay results found that increasing the extract concentration increased the crystal violate uptake up to 91%. The GC-MS

analyses of the extract detected seven active compounds, and they possibly are pyrogallol, D-allose, palmitic acid, squalene, anacardic acid, α -tocopherol, and β -sitosterol. In comparison, LC-MS analysis detected eight active compounds, possibly be asiatic acid, astragalol, anacardic acid, ursolic acid, quercetin, myricetin, and catechin. These detected phytochemicals had reported having antimicrobial and antioxidant activities. The toxicity of the ethanolic extract was determined using hatched brine-shrimp larvae with LC_{50} of 7.402 mg/mL. In food application, the extract could kill and inhibit bacterial growth. The optimum antibacterial activity of the extract was recorded when food samples were treated for 30 minutes at concentrations 0.25%, 0.50%, and 1.00%, and storage temperature at 4°C and -18°C. In conclusion, ethanolic *S. malaccense* L. leaves extract had antibacterial and antioxidant activities, was stable under different pHs and temperatures, non-toxic at active concentration, and might be developed as a natural food preservative or food sanitizer before cooking.

Keywords: Antibacterial activity, antioxidant activity, natural food preservatives, *Syzygium malaccense* (L.) Merr. and Perry, toxicity.

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

**AKTIVITI ANTIBAKTERIA DAN ANTIOKSIDAN, ANALISIS FITOKIMIA DAN
KETOKSIKAN EKSTRAK ETANOL DAUN JAMBU BOL [*Syzygium
malaccense* (L.) Merr. dan Perry] DAN PENGGUNAANNYA PADA
MAKANAN**

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Penyakit yang disebabkan oleh pengambilan makanan dikira sebagai isu serius dan perlu diselesaikan. Pengawet makanan sintetik dan pensanitasi telah digunakan untuk menyelesaikan isu ini, tetapi terdapat banyak laporan yang telah membuktikan bahan kimia ini mempunyai kesan sampingan dan boleh mengancam kesihatan manusia. Sebaliknya, bahan pengawet dan pensanitasi berasaskan tumbuhan adalah lebih digalakkan kerana tumbuhan secara umumnya dikenalpasti adalah selamat (GRAS), seperti menggunakan *Syzygium malaccense* (L.) Merr. and Perry, salah satu tanaman dikenali dalam perubatan tradisional. Oleh itu, kajian ini bertujuan untuk menentukan ciri antibakteria dan kestabilan ekstrak etanol daun *S. malaccense* L. terhadap *Bacillus* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella* Typhimurium, *Staphylococcus aureus*, dan *Vibrio parahaemolyticus*, untuk menentukan jumlah kandungan fenolik (TPC) ekstrak dan sifat antioksidan, untuk menganalisis ketoksikan dan cara tindakan, untuk menganalisis juzuk fitokimianya, dan untuk menilai kesan ekstrak ke atas populasi mikrob dalam sampel makanan. Keputusan menunjukkan bahawa ekstrak mempunyai aktiviti antibakteria dengan zon perencatan antara 7.83 – 16.00 mm, nilai MIC sekitar 0.31 – 5.00 mg/mL, nilai MBC sekitar 0.62 – 5.00 mg/mL, dan keluk masa-membunuh telah menunjukkan ekstrak tersebut dapat mengurangkan populasi bakteria $\geq 3\text{Log}_{10}$ pada kepekatan $4\times\text{MIC}$ dalam masa 4.0 jam. Secara amnya, aktiviti antibakteria ekstrak adalah stabil/tiada perubahan yang ketara selepas dirawat pada pH dan suhu yang berbeza. Jumlah kandungan fenolik ekstrak telah dikenalpasti pada 44.10 ± 0.06 mg GAE/g dan kuasa pengurangan antioksidan adalah pada 1013.50 ± 0.07 mM Fe^{2+} /g. Nilai IC_{50} aktiviti pemerangkapan radikal DPPH dan ABTS• masing-masing pada 0.0334 mg Asid Askorbik E/g dan 0.1352 mg TE/g. Pembebasan konstituen sel bakteria merekodkan peningkatan yang luar biasa dalam nilai OD bagi konstituen sel yang diserap (0.788) pada 260nm dengan $4\times\text{MIC}$ yang merupakan nilai tertinggi

yang direkodkan selepas kawalan positif kloroheksidin (0.813). Begitu juga, keputusan ujian kristal violet mendapati bahawa peningkatan kepekatan ekstrak telah meningkatkan penyerapan kristal violet sehingga 91%. Analisis GC-MS terhadap ekstrak telah mengenalpasti tujuh bahan aktif, dan bahan tersebut berkemungkinan adalah pyrogallol, D-allose, asid palmitik, squalene, asid anakardik, α -tokoferol dan β -sitosterol. Sebaliknya, analisis LC-MS telah mengesan lapan sebatian aktif, mungkin asid asiatik, astragalin, asid anakardik, asid ursolik, kuersetin, miricetin, dan katekin. Fitokimia yang dikenalpasti ini dilaporkan mempunyai aktiviti antimikrob dan antioksidan. Ketoksikan ekstrak etanol telah dikenalpasti menggunakan larva air garam-udang yang menetas dengan LC_{50} sebanyak 7.402 mg/mL. Dalam aplikasi makanan, ekstrak ini dapat membunuh dan menghalang pertumbuhan bakteria. Optimum aktiviti antibakteria ekstrak direkodkan apabila sampel makanan dirawat selama 30 minit pada kepekatan 0.25%, 0.50%, dan 1.00% dan suhu penyimpanan pada 4°C dan -18°C. Kesimpulannya, ekstrak etanol daun *S. malaccense* L. mempunyai aktiviti antibakteria dan antioksidan, stabil pada pHs dan suhu yang berbeza, tidak toksik pada kepekatan yang aktif, dan boleh dibangunkan sebagai pengawet makanan semula jadi atau pensanitasi makanan sebelum dimasak.

Kata Kunci: aktiviti antibakteria, aktiviti antioksidan, ketoksikan, pengawet makanan semula jadi, *Syzygium malaccense* (L.) Merr. dan Perry.

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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 were as follows:

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- the research conducted and the writing of this thesis was under our supervision;
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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ATCC	American Type Culture Collection
CDC	Centers for Disease Control and Prevention
CFU	Colony forming unit
CHX	Chlorhexidine
CLSI	Clinical and Laboratory Standards Institute
DMSO	Dimethyl sulfoxide
GC-MS	Gas Chromatography – Mass Spectrometry
GRAS	Generally Recognized as Safe
LC	Lethality concentration
LC-MS	Liquid Chromatography – Mass Spectrometry
m/z	Mass/charge ratio
MBC	Minimum Bactericidal Concentration
MHB	Mueller Hinton broth
MIC	Minimum Inhibitory Concentration
MOH	Ministry of Health
OD	Optical density
PBS	Phosphate buffered saline
PCA	Plate Count Agar
PDB	Potato Dextrose Broth
ppm	Parts per million
rpm	Revolutions per minute
sp.	Species

sp.	Species among specific group
TPC	Total Plate Count
UPM	Universiti Putra Malaysia
WHO	World Health organization



CHAPTER 1

INTRODUCTION

1.1 Background

Raw food materials contamination, water supply contamination, poor handling practices, hygienic issues, improper treatment or storage, and incorrect declarations or expiration dates are significant factors leading to food spoilage and illness. Other factors that have increased the cases of foodborne diseases are food trading and food distribution worldwide, allowing cross-contamination among various food materials or ingredients, leading to changes in the microbial quality of contaminated food (Hussain and Dawson, 2013). However, food illness is a food disease that consumers consume contaminated food with foodborne pathogens. The most common symptoms associated with food disease that may appear within half to six hours of consuming contaminated food are vomiting, nausea, gut disturbance, and diarrhea. Food disease may lead to death (Hoffmann and Scallan, 2017). According to Faour-Klingbeil and Todd (2020), around six hundred people (almost 1 to 10 people in the world) become ill. They have food illness symptoms because of consuming contaminated food with foodborne pathogens. The most common pathogenic bacteria that cause food illnesses worldwide are *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium botulinum*, *C. perfringens*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* sp., *Shigella* sp., *Staphylococcus aureus*, *Vibrio* sp. and *Yersinia enterocolitica* (Bintsis, 2017).

Food poisoning outbreaks mainly occur in places where food is prepared and distributed to a group of people, such as wedding ceremonies, hotels, restaurants that provide all-you-can-eat buffet services, schools, prisons, and older adults' homes (Tewari and Abdullah, 2015). In 2016, Maharashtra, one of the Indian states, reported an outbreak at Men's Working Hostel related to food poisoning from improperly prepared meals that left 170 people ill (Grewal and Khera, 2017). The outbreak report of the hospital suggested that cooked chicken meals were the main reason for the outbreak. At the same time, the patent related to this case was believed to be *C. perfringens* or *B. cereus* based on the symptoms observed on illness people, which were diarrhea, abdominal cramps, and nausea. Another outbreak was reported in Maharashtra religious event that left 4000 people sick from consuming mung bean dal in 2015 (Bajaj and Dudeja, 2019). Food poisoning symptoms on poisoned people were diarrhea, abdominal cramps, fever with chills, and vomiting. At the same time, the expected pathogen that caused this outbreak was *E. coli* or *Salmonella* sp. based on the symptoms. In 2020, 84 patients with acute gastroenteritis were reported to be victims of an epidemic in a company cafeteria in Chungcheongnam-do Province, Korea (Lee et al., 2020). The investigations found that *E. coli* contaminated food and *C. perfringens*. The source of contamination was three employees who handled rice where the employees found to be positive of *E. coli* and *C. perfringens*. In Malaysia, the incidence rate of reported food poisoning in 2013 was 47.79 per

100,000 populations, with a mortality rate of 0.04% (MOH, 2014). Moreover, in the year 2016, a total of 21 food poisoning cases involving schoolchildren were reported in Terengganu, Malaysia (Abdullah and Ismail, 2021). According to the reports, poultry was the most common food vehicle, and *Salmonella* sp. was the most common microbial etiological agent. Food poisoning incidents affect people's health and affect the country's reputation, leading to costs, especially those related to the tourism sector.

Food additives have uncountable benefits such as maintaining the quality of food, preventing the texture from loss or separating, and preservatives for extending the shelf life of food to keep it available for consumption any time in the year. As part of food additives, food preservatives have advantages, but they also have some disadvantages related to consumer health. For example, benzoate as an antimicrobial agent is a chemical preservative used in small amounts. Still, it has been reported that consuming benzoate causes skin allergen and brain damage (Abdulmumeen et al., 2014). Chemical preservatives are generally recognized as safe (GRAS) in small and controlled amounts. However, they are still a source of concern where consumers are looking for a safer alternative that may be derived from green sources. Hence, industries tend to use plant-based preservatives and sanitizers to preserve food and increase its shelf-life and quality maintenance (Bondi et al., 2017).

Plants are a valuable source of bioactive compounds with antibacterial and antioxidant activities. Plant bioactive compounds have different chemical properties. Many plants possess bioactive compounds that have antibacterial effects on foodborne pathogens and scavenging free radicals. Herbs have been used in food since ancient times for flavoring and saving fare for a long time without being spoiled. Plant extracts, essential oils, and peptides have a wide range of bioactivities. The antibacterial and antioxidant properties of plants are mainly attributed to secondary metabolites such as polyphenols, phenylpropanoid, flavonoids, terpenes, and anthocyanins (Dhiman and Aggarwal, 2019). One of the plant genera known as the medical plant is *Syzygium*. *Syzygium* is a kind of flora belonging to the Myrtaceae myrtle family. The genus contains around 1200 species and is endemic to the Pacific from Africa to South Asia. Its maximum diversity is in Malaysia and northeast Australia (Ahmad et al., 2016; Bouman et al., 2021). Essential oils of *S. aromaticum* are known for their antibacterial activity against several pathogenic bacteria such as *S. aureus*, *E. coli*, *L. monocytogenes*, and *S. Typhimurium* (Radünz et al., 2019). Furthermore, *S. cumini* leaves crude extract and essential oil have been reported to have antioxidant activity and antibacterial activity against foodborne pathogens, including *Bacillus subtilis*, *E. coli*, *Enterococcus faecalis*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, and *S. aureus* (Mohamed et al., 2013). Moreover, *S. aquarium* and *S. samarangense* Leaves extracts have been found to exhibit antioxidant activity, in addition, to be effective as an antimicrobial agent against most foodborne pathogens, including *E. coli*, *Klebsiella pneumoniae*, *L. monocytogenes*, *P. aeruginosa*, *S. aureus*, and *Salmonella* Typhi and against food spoilage pathogens including *Candida* sp, *Cochliobolus* sp, *Penicillium* sp., and *Fusarium* sp. (Khandaker et al., 2015; Habisukan et al., 2021; Raj et al., 2021). Hence, the *Syzygium* genus contains various plants whose extracts

possess antibacterial and antioxidant activities and may be used in food as preservatives or washing sanitizers.

Syzygium malaccense (L.) Merr. and Perry, also known as the Malay apple, is a flowering tree believed to be a native of Malaysia. It is commonly cultivated from Indonesia to the Philippines and Vietnam in Bengal and South India (Morton, 1987). Different studies have been done on Malay apple species. The results suggest that these species are rich in phytochemicals such as alkaloids, flavonoids, quinones, phenols, steroids, triterpenoids, saponins, and tannins (Fauziah et al., 2019). In folk medicine, Malay apple bark powder was used to treat throat thrush caused by *C. albicans* growth through the mouth and throat. Seed, fruit, bark, stem, and leaves extracts to exhibit variable antibiotic activity levels against *Micrococcus pyrogenus* Var. *aureus*. The fruit extract shows antibacterial activity against *E. coli*, while the bark and leaves excerpts are efficient against *Shigella*. The root extract treats itching and a diuretic and edema reduction. The powdered leaves effectively cure tongue cracks, and the crushed leaves are used as skin lotion (Pullaiah, 2006). Furthermore, leaves were reported to have cytotoxic (Itam and Anna, 2020), antibiotic (Morton 1987), antimicrobial (Savi et al., 2020), antioxidant, and antihyperglycemic activities (Arumugam et al., 2014). Due to the variety of bioactivities of this plant, this study has been focused on the possibility of using *S. malaccense* L. leaves extract in the food matrix as a food preservative or food washing sanitizer.

1.2 Problem Statements

Food can be easily contaminated at any stage, from farm to fork, during harvesting, storing, handling, pre-processing, or even after processing. The causes of the contamination are many, but cross-contamination among food staff and contaminants from food handlers are the most common causes (Bencardino et al., 2021; Kirchner et al., 2021). Without proper decontamination techniques, pathogenic bacteria that live naturally on food or are transferred to the food from contaminant sources will keep growing and multiplying and produce toxins that raising the possibility of the occurrence of outbreaks (Coskun et al., 2021). To overcome this issue, types of food sanitizer and food preservatives were used to prevent bacterial growth on food or to reduce their numbers, such as chlorine, organic acids, hydrogen peroxide, sorbic acid, sodium sorbate, benzoic acid, sodium benzoate, nitrites, nitrates, and sulfur dioxide (Martins et al., 2021). Using these chemicals has solved the issue of preventing bacterial growth in a food or increased the shelf-life of food. Still, on the other hand, many health issues have been reported and associated with using chemical preservatives or food sanitizers in food, such as food allergies, food intolerance, cancer, multiple sclerosis, attention deficit, hyperactivity, brain damage, nausea, and cardiac diseases (Gupta and Yadav, 2021). Thus, applying plant-based extracts or essential oils was the suggested effective alternative of using chemicals as food sanitizers or preservatives. Antimicrobials, antioxidants, and color or texture enhancers (Pateiro et al., 2021). Hence, this study was undertaken to discover the antibacterial activity of ethanolic *S. malaccense* L. leaves extract as a food

washing sanitizer and food preservative against natural bacteria found in raw food and evaluate its toxicity.

S. malaccense L. has been reported to have a diversity of phytochemicals that possess different bioactivities. Thus, there were reports on the antimicrobial activity of extracts extracted from other plant parts and various solvents. According to Varghese (2015), the methanolic leaves extract of *S. malaccense* L. had antimicrobial activity against *Proteus* and *Candida albicans*. Furthermore, the ethanolic leaves extract of *S. malaccense* L. was effective as an antibacterial substance against *B. subtilis*, *E. coli*, *P. aeruginosa*, and *S. aureus* (Yuniarni et al., 2020). Moreover, the leaves and seeds extracts of *S. malaccense* L. have antibacterial activity against *B. subtilis*, *E. coli*, and *S. aureus*, as reported by Madhushika and Udukala (2021). However, more investigations on the antibacterial activity of *S. malaccense* L. leaves extract need to be studied especially related to foodborne pathogens because a wide range of pathogens can be found even on the same type of food and cause food poisoning. Hence, this research has extensively studied the ethanolic leaves extract of *S. malaccense* L. and focused on its antibacterial activity, mode of action, and antioxidant activity.

1.3 Objectives

1. To determine the antibacterial activity of ethanolic *S. malaccense* L. leaves extract against selected foodborne pathogens.
2. To determine the total phenolic content (TPC) and the antioxidant activity of ethanolic *S. malaccense* L. leaves extract using DPPH and ABTS• radicals scavenging activity assays and ferric reducing power (FRAP).
3. To determine the toxicity of ethanolic *S. malaccense* L. leaves extract on brine-shrimp (*Artemia salina*) in lethality assay and to determine the antibacterial mode of action of the extract through cell's constituent release and crystal violet assays.
4. To analyse the phytochemical compounds in ethanolic *S. malaccense* L. leaves extract
5. To evaluate the effect of ethanolic *S. malaccense* L. leaves extract on the microbial population on raw food samples.

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