



UNIVERSITI PUTRA MALAYSIA

***EVALUATION OF DIFFERENT POST-HARVEST TREATMENTS ON
MALAYSIAN MISTLETOE (*Scurrula ferruginea* (Jack) Dans.) LEAVES
EXTRACTS YIELD, ANTIOXIDANT AND ANTIMICROBIAL
PERFORMANCE***

VANIELIE TERRENCE JUSTINE

FS 2016 80



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By

VANIELIE TERRENCE JUSTINE

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in fulfilment of the Requirements for the Degree of Master of
Science**

November 2016

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
Fulfillment of the requirement for the degree of Master of Science

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November 2016

Chairman : Professor Rusea Go, PhD
Faculty : Science

Scurrula ferruginea is hemiparasitic mistletoes from the family Loranthaceae that widely distributed in tropical regions and has been used traditionally as medicinal plants in Southeast Asian countries. In earliest studies, the plant is known to have antioxidant and antimicrobial activities. However, the best drying methods towards the preservation of phenolic compounds of the plant as well as a suitable solvent for extracting the antioxidants and antimicrobial activity have not been optimized. In this study, the determination of the best drying methods (air and oven) and extraction solvents (aqueous, organic and aqueous organic) on phenolic compounds, antioxidant and antimicrobial activity were carried out. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were used to determine the phenolic compound, while antioxidant activity was evaluated by measuring the scavenging effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and Ferric Reducing Antioxidant Power (FRAP). Antimicrobial activity of the extracts was tested against 2 pathogenic bacteria (*Staphylococcus aureus* S261 and *Escherichia coli* E57) and 2 pathogenic fungi species (*Candida albicans* C205 and *Trichophyton rubrum* T62) using Disc Diffusion Method, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) respectively. The optimal conditions for *S. ferruginea* were oven drying, using 80% methanol solvent with values of 2.72 mg/10g DW (extract yield), 168.18 mg GAE/1g DW (TPC), 25.03 mg QE/1g DW (TFC), 27.86 mg TE/1g DW (FRAP), 94.0% (DPPH) and 19.32 µg/mL (IC₅₀). The result of this research shows a potent antioxidant activity in *S. ferruginea* leaves extracts as compared to standard antioxidants (Ascorbic acid and Trolox). A correlation study of the extracts revealed significant positive correlations ($P < 0.05$) between extraction yields, phenolic compounds and antioxidant properties. The extracts exhibited optimum antimicrobial activities on both *Staphylococcus aureus* S261 and *Escherichia coli* E57 with a zone of inhibition ranging from 7.98 to 9.71 mm and 450 to 900 µg/mL (MIC and MBC). However, the extracts show no inhibitory effects on *C. albicans* C205 and *T. rubrum* T62. Present results suggested that post-harvest preparations such as drying method (oven drying) and extractions solvents (80% methanol) variability play a crucial role in enhancing the efficiency and efficacy of

plant samples. The findings obtained in this study shows that *S. ferruginea* leaves are highly potential source of antioxidant and antimicrobial discovery that leads for new nutraceutical.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

**PENILAIAN PROSES LEPAS TUAJ YANG BERBEZA TERHADAP PRESTASI
HASIL EKSTRAK DAUN, ANTIOKSIDAN DAN ANTIMIKROB POKOK
DEDALU (*Scurrula ferruginea* (Jack) Dans.)**

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Scurrula ferruginea adalah pokok dedalu separa parasit daripada famili Loranthaceae yang tersebar meluas di rantau tropika dan digunakan secara tradisional sebagai tumbuhan ubatan di negara Asia Tenggara. Dalam kajian-kajian yang lepas, tumbuhan ini diketahui mempunyai aktiviti antioksidan dan antimikrob. Walaubagaimanapun, kaedah pengeringan terbaik ke arah pengekalan sebatian fenolik tumbuhan serta pelarut yang sesuai untuk mengekstrak aktiviti antioksidan dan antimikrob masih belum dioptimumkan. Dalam kajian ini, penentuan kaedah terbaik pengeringan (samada udara atau ketuhar) dan pelarut pengekstrakan (air, organik dan organik berair) pada sebatian fenolik, antioksidan dan antimikrob aktiviti telah dijalankan. Jumlah Kandungan Fenolik (JFK) dan Jumlah Kandungan Flavonoid (JKF) telah digunakan untuk menentukan sebatian fenolik, manakala aktiviti antioksidan telah dinilai dengan mengukur kesan pada radikal 2,2-difenil-1-picrylhydrazyl (DPPH) dan Ferik Mengurangkan Kuasa Antioksidan (FMKA). Aktiviti antimikrob daripada ekstrak telah diuji terhadap 2 bakteria patogenik (*Staphylococcus aureus* S261 dan *Eschericia coli* E57) dan 2 spesies kulat patogenik (*Candida albicans* C205 dan *Trichophyton rubrum* T62) menggunakan Kaedah Cakera Resapan, Minimum Kepekatan Bantut (MKB), Minimum Kepekatan Bunuh Bakteria (MKBB) dan Minimum Kepekatan Bunuh Kulat (MKBK) masing-masing. Hasil yang optimum untuk *S. ferruginea* adalah melalui pengeringan ketuhar, penggunaan pelarut 80% metanol dengan nilai sebanyak 2.72 mg/10g DW (hasil ekstrak), 168.18 mg GAE/1g DW (TFK), 25.03 mg QE/1g DW (TFK), 27.86 mg TE/1g DW (FMKA), 94.0% (DPPH) and 19.32 µg/mL (IC₅₀). Hasil kajian ini menunjukkan aktiviti antioksidan di dalam ekstrak daun *S. ferruginea* adalah tinggi jika dibandingkan dengan antioksidan piawai (Asid askorbik dan Trolox). Kajian korelasi daripada ekstrak tersebut menunjukkan korelasi signifikan yang positif ($P < 0.05$) di antara hasil ekstrak, sebatian fenolik dan aktiviti antioksidan. Ekstrak juga menunjukkan aktiviti yang optimum terhadap antimikrob di kedua-dua *Staphylococcus aureus* S261 dan *Eschericia coli* E57 dengan zon perencatan antara 7.98 ke 9.71 mm dan 450 ke 900 µg/mL (MKB dan MKBB). Akan tetapi, ekstrak tidak menunjukkan kesan pembantutan terhadap *C. albicans* C205 dan *T. rubrum* T62. Hasil kajian ini, mencadangkan proses lepas tuaian seperti kepelbagaian kaedah pengeringan (ketuhar) dan pelarut pengekstrakan (80% metanol) memainkan peranan penting di dalam meningkatkan kecekapan dan

keberkesanan sampel kajian. Hasil kajian yang diperolehi menunjukkan bahawa daun *S. ferruginea* amat berpotensi tinggi kepada penemuan sumber antioksidan dan antimikrob yang baru untuk nutraseutikal.



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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
AD	Anno Domini
BC	Before Christ
CFU/ml	Colony forming unit per milliliter
°C	Degree Celsius
DPPH	2,2'-diphenyl – 1 – picrylhydrazyl
DW	Dry weight
FRAP	Ferric Reducing Antioxidant Power
GAE	Gallic Acid Equivalent
g	Gram
h	Hour
IC ₅₀	Inhibition concentration 50%
μl	Micro liter
MBC	Minimum Bactericidal Concentration
MFC	Minimum Fungicidal Concentration
MIC	Minimum Inhibitory Concentration
mg	Milligram
ml	Milliliter
mM	Millimole
min	Minute
MHA	Mueller-Hinton Agar
MHB	Mueller-Hinton Broth
nm	Nanometer
NA	Nutrient agar
NB	Nutrient broth
%	Percentage
rpm	Revolutions per minute
SDA	Sabouraud Dextrose Agar
SDB	Sabouraud Dextrose Broth
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
TE	Trolox Equivalent
w/v	Weight over volume

CHAPTER 1

INTRODUCTION

1.1 Overview of study

World health issues have gained a global attention nowadays. The rise in the population of the world has led to the rampant increase in the chronic and infectious diseases. The attempt to combat this human healthcare problem is one of the greatest challenges confronting mankind due to the side effects, lack of affordable drugs, costlier nature and development of microbial resistances to modern medicines (Sen & Chakraborty, 2015). Thus, there is a resurgence in the use of herbal medicine that have been used traditionally which are more affordable, ease available, little side effects and very effective for curing various ailments.

The prospect of herbal medicine as an alternative to other methods in treating ailment is gaining worldwide acceptance and Malaysia is not an exception (Pan *et al.*, 2013). Malaysia is one of the 17th mega biodiversity countries that are gifted with diverse medicinal plant resources. Several popular medicinal plants in Malaysia such as *Eurycoma longifolia* (Tongkat ali), *Labisia pumila* (Kacip fatimah), *Andrographis paniculata* (Hempedu bumi), *Orthosiphon stamineus* (Misai kucing), *Centella asiatica* (Pegaga), *Phyllanthus niruri* (Dukung anak) and *Momordica charantia* (Peria) have been extensively studied scientifically, however, there are a lot more to be explored (Jamal, 2006).

1.2 Problem statements

One of the potential herbal medicines in Malaysia that has not been explored sufficiently through scientific experiments for its therapeutic properties is mistletoe species. Malaysian mistletoes, *Scurrula ferruginea* (Roxb. ex Jack) Danser a hemiparasitic plant from Loranthaceae family is chosen due to its high medicinal values in ethnobotany and promising therapeutic properties in several studies that have been conducted (Ameer *et al.*, 2009; Ameer, Salman, Quek, & Asmawi, 2015; Marvibaigi *et al.*, 2014a; Marvibaigi, Supriyanto, Amini, Abdul Majid, & Jaganathan, 2014b). However, from the limited researches conducted, the focus has been on the scientific experimentation of therapeutics properties and effects of the plant without proper consideration for plant sample preparations on post-harvest processes which is of great significance towards understanding the therapeutics potential of *S. ferruginea*.

Thus, post-harvest treatment variability namely drying methods and extraction solvents are applied in this study to enhance the efficiency and efficacy of bioavailability in *S.*

ferruginea crude extracts. Drying is an important method for reduction of the moisture content variability as well as preservation of plant phytochemical to attain superior quality of crude extract. However, the diversity of plants, morphological characteristics and chemical constituents may determine the effects of different drying methods on such plants. Extraction solvents were used for extracting the varying polarities mixture of secondary metabolites in plant cellular matrix. The choice of solvents with varying polarity is recommended to select the optimum curative activity and extract yield of the active constituents.

In view of the above and to the best of our knowledge on available literatures, there is no study on the preservation of phenolic compounds, antioxidant properties and antimicrobial activities of *S. ferruginea* based on sample preparation of drying methods and extraction solvents. Hence, this study aim is to evaluate the effect of different post-harvest treatment on extract yield, phenolic compounds, antioxidant properties and antimicrobial activity of *Scurrula ferruginea* leaves crude extracts. Such information is beneficial for scientific experiment on sample preparation of medicinal plant which could enhance the quality of crude extract as well as reduced the cost factor in production. Moreover, the efforts been put into drug discovery in the world especially from plants with high potential medicinal value such as Malaysian mistletoe could be revealed, hence leading to its utilization commercially that can affect human healthcare.

1.3 Objectives of study

Thus, the objectives of this study are:

1. To determine the best drying method based on extract yield and phenolic compounds of *S. ferruginea* leaves.
2. To determine the best extraction solvent based on the antioxidant activity of *S. ferruginea* leaves.
3. To determine the antimicrobial activity based on the optimum antioxidant activity of *S. ferruginea* leaves.

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