# EXTRACTION OPTIMIZATION FOR *MURRAYA KOENIGII* LEAVES WITH HIGH ANTIOXIDANT AND ANTIBACTERIAL PROPERTIES

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## ABSTRACT

*Murraya koenigii* or commonly known as curry tree is an aromatic shrub in the family Rutaceae. The tree can be found in a tropical to sub-tropical regions and people use it for centuries in cooking, and ethnomedicinal purposes in traditional remedy. This study was focused to investigate the effects of ethanol concentration and solid to liquid ratio on the *M. koenigii* leaf extraction to obtain high total phenolic content (TPC) with high antioxidant and antibacterial properties. The extraction was carried out in a reflux system at three different solid to liquid ratios, namely 1:5, 1:10 and 1:20, in two different ethanol concentrations, which were 75% and 99% ethanol. The other fixed extraction conditions were set at 50°C for one hour. The antioxidant activity of *M. koenigii* leaf extracts were determined using the colorimetric DPPH radical scavenging assay, and recorded the highest antioxidant activity with the $IC_{50}$ 0.517mg/ml from the 1:5 solid to liquid ratio in 75% ethanol. This ratio also produced the highest TPC 51.04 mg GAE/g dried extract. The optimized leaf extract was then analysed for its antibacterial properties against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* with significant inhibitory actions at the minimum inhibition concentration, 2.708 mg/ml, 0.338 mg/ml, and 5.415 mg/ml, respectively. This study showed that ethanol 75% with solid to liquid ratio of 1:5 had the highest antioxidant and TPC content respectively. The data obtained implicated that an increased phenolic content in M.koenigii leaves extracts proportionally increases their antioxidant efficiency.

## ABSTRAK

*Murraya koenigii* atau biasa dikenali sebagai pokok kari ialah pokok renek aromatik dalam keluarga Rutaceae. Pokok ini boleh ditemui di kawasan tropika hingga sub-tropika dan orang ramai menggunakannya selama berabad-abad dalam masakan, dan tujuan etnoperubatan dalam ubat tradisional. Kajian ini difokuskan untuk mengkaji kesan kepekatan etanol dan nisbah pepejal kepada cecair ke atas pengekstrakan daun *M. koenigii* untuk mendapatkan jumlah kandungan fenolik (TPC) yang tinggi dengan sifat antioksidan dan antibakteria yang tinggi. Pengekstrakan dilakukan dalam sistem refluks pada tiga nisbah pepejal kepada cecair yang berbeza, iaitu 1:5, 1:10 dan 1:20, dalam dua kepekatan etanol yang berbeza, iaitu 75% dan 99% etanol. Keadaan pengekstrakan tetap yang lain ditetapkan pada 50°C selama satu jam. Aktiviti antioksidan ekstrak daun *M. koenigii* telah ditentukan dengan menggunakan ujian reagen DPPH kolorimetrik, dan merekodkan aktiviti antioksidan tertinggi dengan $IC_{50}$ 0.517mg/ml daripada nisbah pepejal kepada cecair 1:5 dalam 75% etanol. Nisbah ini juga menghasilkan TPC tertinggi 51.04 mg GAE/g ekstrak kering. Ekstrak daun yang dioptimumkan kemudiannya dianalisis untuk sifat antibakterianya terhadap *Pseudomonas aeruginosa*, *Staphylococcus aureus*, dan *Escherichia coli* dengan tindakan perencatan yang ketara pada kepekatan perencatan minimum, MIC 2.708 mg/ml, 0.338 mg/ml, dan 5.415 mg/ml, masing-masing. Kajian ini menunjukkan etanol 75% dengan nisbah pepejal kepada cecair 1:5 masing-masing mempunyai kandungan antioksidan dan TPC yang paling tinggi. Data yang diperolehi menunjukkan bahawa kandungan fenolik yang meningkat dalam ekstrak daun M.koenigii secara berkadar meningkatkan kecekapan antioksidannya

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## LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| *M. koenigii* | - | *Murraya koenigii* |
| FDA | - | Food and Drug Administration |
| IOM | - | Institute of Medicine |
| *S. aureus* | - | *Staphylococcus aureus* |
| *P. aeruginosa* | - | *Pseudomonas aeruginosa* |
| E. coli | - | *Escherichia coli* |
| UTM | - | Universiti Teknologi Malaysia |
| DPPH | - | 2,2-diphenyl-1-picrylhydrazyl |
| MTT | - | 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide |
| TPC | - | Total Phenolic Content |
| CFU | - | Colony-forming unit |

## LIST OF SYMBOLS

| | | |
|---|---|---|
| μg | - | microgram |
| $R^2$ | - | Coefficient of Determination |
| $IC_{50}$ | - | Half maximal inhibitory concentration |
| GAE | - | Gallic Acid Equivalent |

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of the Study

Antioxidants are man-made or natural substances that act to stop oxidation, which can have detrimental effect to the body. Reactive oxygen species (ROS) are elements that cause damage to DNA, protein, lipids and their uncontrolled growth during cell life cycle gives rise to the development of diseases that develop as we age such as cancer, arthritis and so on. Under stress, our bodies produce ROS that causes cell injury (Zahin et al., 2013).

Antioxidants sourced from plants sources have been observed as a potential to prevent and treat these diaseases, which includes diseases of the cardiovascular, neurodegenerative disorders, various type of cancers and more. A spectrum of bioactive compounds, which includes mahanine (Figure 2.4), isomahanine (Figure 2.6), mahanimbine (Figure 2.5), girinimbine isolongifolene, koenoline, koenimbine (Figure 2.7), and O-methylmurrayamine which were present in *M. koenigii* exhibited astounding antioxidant properties (Balakrishnan et al, 2016).

Solvent is one of the important factors in extraction. Their type and polarity can affect the extraction quality and quantity. Besides that, toxicity, extraction velocity and biosafety of the extract also relies on the solvent. (Zhang et al, 2018). Solvent and plants parts used for extraction determines the secondary metabolites extracted, as well as their antioxidant capacity. (Rafińska et al., 2019). Hence, selection of solvent for extraction plays a vital part in determining the quality and the quantity of the extract.

Independent studies and national surveillance data exhibit that drug-resistant, disease-causing bacteria have multiplied and spread at alarming rates in recent decades. The Institute of Medicine (IOM), Centre for Disease Control and Prevention

(CDC) and National Institutes of Health (NIH) has warned that antibiotic-resistant bacteria may cause consequential public health threat. (Ventola, C.L., 2015).

A bacterial human pathogen, *Staphylococcus aureus* (*S. aureus*) causes harmful infections. It is present both in community and hospital settings and treatment has become exigent due to multi-drug resistant strains such as MRSA (Methicillin-Resistant *S. aureus*) that are emerging rapidly. Several first and second line antibiotics are expeditiously becoming ineffective for treatment due to this acquired resistance. In recent times, Vancomycin is among the few antibiotics which has shown to be ineffective against MRSA (Zaki, W. K., & Hager, R.,2018).

*Escherichia coli,* a common inhabitant of the human gastrointestinal tract as well as other warm-blooded animals, comes from the Enterobacteriaceae family (Alocati et al, 2013). They are a commensal, and lives unanimously in beneficial association with the hosts, and seldom causes any diseases. But as it's a common pathogen, it may also cause broad spectrum of diseases. Capable to accumulate resistant genes, it is also resistant to therapeutic levels of penicillin G (Poirel L, et al, 2018).

Eradicating *Pseudomonas aeruginosa* has become more difficult over time due to its extraordinary magnitude to resist currently available antibiotics. The strains of *P. aeruginosa* counter most antibiotics by using their high levels of acquired resistance mechanisms. Recently, biofilm-mediated resistance and development of multidrug-tolerant persister cells were identified as mechanisms that have been employed by the bacteria to develop antibiotic resistance (Pang et al, 2019).

*M. koenigii* leaves or better known as curry leaves, have been used around the world for various purposes, including in cooking and treating certain medical conditions. Studies have been done on the *M. koenigii*, and findings include antibacterial, antioxidant, and anticancer activity. Eleven alkaloids and four flavonoids from this plant material have been shown to possess anticancer activity (Samanta et al., 2018). Various factors influence the extraction of phytochemicals from plant material. Several studies have demonstrated that leaves of *M. koenigii* has antioxidant

and antibacterial activity. Optimizing factors and parameters that affect extraction is important to maximize yield make the most of the benefits of the plant.

## 1.2 Problem statement

There are several studies done on the varying concentrations of ethanol in extraction of *M koenigii* leaves, but very few has experimented on varying the material to solid ratio, and how the slight aqueous content in the solvent affects the yield of phenolics and on the antioxidant activity of the extract. In this study, ethanol has been chosen in two different concentrations as they have shown to be a promising solvent, as well as for having a better safety profile. Different solid-to-liquid ratio was applied to extract the local variant of *M. koenigii* leaves, in order to study the differences in their phenolic content, as well as antioxidant activity, and how the solvent and sample concentration impact them. Even though studies have been done of *M koenigii* from few states in Malaysia, there has also not been any studies specifically targeting *M. koenigii* from Seremban.

Discovery of new antimicrobial substance from plant materials is necessary to combat the issue of increase in antimicrobial resistance. This study was also done to investigate if this commonly available *M. koenigii* species in Malaysia has antibacterial activity against strains of bacteria, namely *S. aureus, E. coli* and *P. aeruginosa* that have developed resistance towards commonly used antibiotics in the recent years, and at which concentration it is able to inhibit the microbes. By proving its antibacterial property, *M. koenigii* leaves extract may serve as a source of antimicrobial substance.

## 1.3 Objective of the Study

The objectives of this study are:

a) To optimize the effects of solid-to-solvent ratio and ethanol concentration on the reflux extraction of *Murayya Koenigii* leaves.

b) To characterize the *Murraya Koenigii* leaf extract based on total phenolic content (TPC), free radical scavenging assay by 2,2-diphenyl-1-picrylhydrazyl **(**DPPH) method, and antibacterial properties.

## 1.4 Scope of study

The optimization was carried out by varying the ratio of solid-to-solvent from 1:5, 1:10 and 1:20 in two different solvent systems consisting of 99% and 75% ethanol. The prepared *M. koenigii* extract was then characterised based on its total phenolic content (Folin-Ciocâlteu assay), antioxidant (DPPH assay) and antibacterial activities (*E. coli, P. aeruginosa,* and *S. aureus*)

## 1.5 Significance of Study

The findings of this study may benefit future formulation of products using *M. koenigii* leaf extract. The solvent with the highest antioxidant activity and TPC may assist in deciding a suitable solvent to yield high quality of *M. koenigii* leaf extract. It may also pave way for future studies dealing with *M. koenigii* leaves on their antioxidant potentials.

Other than that, the antibacterial potential of locally sourced *M. koenigii* leaves could have comparable effect with existing antibiotics that are effective against the same selected bacterial strains.

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