ANALYSIS OF ESSENTIAL OIL, PHYTOCHEMICALS AND ANTIBACTERIAL ACTIVITY FROM *Piper rostratum* Roxb.

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UNIVERSITI PENDIDIKAN SULTAN IDRIS

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ABSTRACT

The purpose of this study was to investigate the essential oil composition, phytochemicals, and antibacterial activity from the leaves of *P. rostratum*. Essential oil was obtained from the leaves of *P. rostratum* by hydrodistillation, and its chemical compositions were determined using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Soxhlet extraction was performed successively on the leaves using *n*-hexane, ethyl acetate, and methanol. Next, the isolation of phytochemicals was carried out *via* silica gel column chromatography. The chemical structures of isolated compounds were determined using spectral analysis and comparison with literature data. Antibacterial activity was analysed using micro dilution method. The results showed that twenty components (94.2%) were identified in the leaf oil with γ-muurolene (14.1%), δ-cadinene (13.2%), allylpyrocatechol diacetate (11.5%), chavicol (8.2%), α-humulene (7.8%), and hydroxychavicol (6.9%) as major components. Meanwhile, eight compounds were isolated from the leaf extracts, including 4'-hydroxy-3',5',5,7-tetramethoxyflavone, 5,7-dimethoxyflavone, 4',5,7-trimethoxyflavone, 3',4',5,7-tetramethoxyflavone, 3′,4′,5′,5,7-pentamethoxyflavone, and 5-hydroxy-3',4',7-trimethoxyflavone, β-sitosterol, and stigmasterol. Besides, 5-hydroxy-3',4',7-trimethoxyflavone demonstrated the strongest antibacterial activity with MIC value of 25 µg/mL against *Staphylococcus aureus*, *Bacillus cereus*, and *Escherichia coli*. In conclusion, sesquiterpenes are the major components of the essential oil, and six flavonoids along with two terpenes were isolated with one compound displayed potential antibacterial activity, all of which are reported for the first time from *P. rostratum* leaves. These findings highlight the potential of *Piper* species as natural plant-based antibacterial agents and contribute for future drug research.

ANALISIS MINYAK PATI, FITOKIMIA DAN AKTIVITI ANTIBAKTERIA DARIPADA *Piper rostratum* Roxb.

ABSTRAK

Tujuan kajian ini adalah untuk menyiasat komposisi minyak pati, fitokimia, dan aktiviti antibakteria daripada daun *P. rostratum*. Minyak pati diperoleh daripada daun *P. rostratum* melalui penyulingan hidro dan komposisi kimianya dikenal pasti menggunakan kromatografi gas (GC) dan kromatografi gas-spektrometri jisim (GC-MS). Pengekstrakan soklet dijalankan berturutan ke atas daun menggunakan *n*-heksana, etil asetat, dan methanol. Seterusnya, pemencilan fitokimia menggunakan telah dijalankan melalui kromatografi turus silika gel. Struktur kimia yang dipencilkan ditentukan menggunakan analisis spektra dan perbandingan dengan data literatur. Aktiviti antibakteria telah dianalisis menggunakan kaedah pencairan mikro. Keputusan menunjukkan dua puluh komponen (94.2%) telah dikenal pasti dalam minyak daun dengan γ-muurolena (14.1%), δ-kadinena (13.2%), alilpirokatekol diasetat (11.5%), kavikol (8.2%), α-humulena (7.8%), dan hidroksikavikol (6.9%) sebagai komponen utama. Sementara itu, lapan sebatian telah dipencilkan daripada ekstrak daun, termasuk 4'-hidroksi-3',5',5,7-tetrametoksiflavon, 5,7-dimetoksiflavon, 4',5,7-trimetoksiflavon, 3',4',5,7-tetrametoksiflavon, 3′,4′,5′,5,7-pentametoksiflavon, dan 5-hidroksi-3',4',7-trimetoksiflavon, β-sitosterol, dan stigmasterol. Selain itu, 5-hidroksi-3',4',7-trimetoksiflavon mempamerkan aktiviti antibakteria terkuat dengan nilai MIC iaitu 25 µg/mL terhadap *Staphylococcus aureus*, *Bacillus cereus*, dan *Escherichia coli*. Kesimpulannya, seskuiterpena adalah komponen utama minyak pati, dan enam flavonoid serta dua terpena telah dipencilkan dengan satu sebatian menunjukkan potensi aktiviti antibakteria, yang kesemuanya dilaporkan buat pertama kali daripada daun *P. rostratum*. Dapatan ini menyerlahkan potensi spesies *Piper* sebagai agen antibakteria semula jadi berasaskan tumbuhan dan menyumbang kepada penyelidikan ubatan masa hadapan.

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LIST OF ABBREVIATIONS

α	Alpha
β	Beta
^{13}C	Carbon-13
CC	Column Chromatography
$CDCl_3$	Deuterated chloroform
$CHCl_3$	Chloroform
cm^{-1}	Per centimeter
COSY	Homonuclear correlation spectroscopy
1D	1 Dimension
2D	2 Dimension
δ	Chemical shift
d	doublet
DCM	Dichloromethane
dd	doublet of doublets
dt	doublet of triplets
DEPT	Distortionless Enchancement by Polarization Transfer
EIMS	Electron Impact Mass Spectrometry
Et_2O	Diethyl ether
EtOAc	Ethyl Acetate
γ	Gamma
GC	Gas Chromatography

GC-FID	Gas Chromatography-Flame Ionisation Detector
GC-MS	Gas Chromatography-Mass Spectrometry
^{1}H	Proton
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coherence
HPLC	High Performance Liquid Chromatography
HR-EIMS	High-Resolution Electron Impact Mass Spectrometry
Hz	Hertz
IR	Infrared
J	Coupling constant
KBr	Potassium bromide
KI	Kovats Index
L	Liter
m	multiplet
M^{+}	Molecular ion
MBC	Minimum Bactericidal Concentration
MeOH	Methanol
MHz	Megahertz
MIC	Minimum Inhibitory Concentration
min	Minute(s)
m/z	Mass to charge ion
mg	milligram
$MgSO_4$	Magnesium sulphate
mL	milliliter

mm	millimeter
MS	Mass Spectrometer
NA	Nutrient Agar
NaCl	Sodium Chloride
NB	Nutrient Broth
NMR	Nuclear Magnetic Resonance
nm	nanometer
PTLC	Preparative Thin Layer Chromatography
SiO_2	Silica gel
TLC	Thin Layer Chromatography
UV	Ultraviolet

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CHAPTER 1

INTRODUCTION

1. 1 General Introduction

Natural products have long been fundamental to human civilization, providing essential resources for survival, health, and innovation. These products, which are derived from living organisms, particularly microorganisms, plants, and marine species, encompass a wide variety of chemical compounds with significant biological activities (Pham et al., 2019). Their influence extends across various fields, including pharmaceuticals, cosmetics, and agriculture, where they have made crucial contributions to product development. Over time, natural products have inspired the creation of extensive bioactive compound libraries and semi-synthetic derivatives, which exhibit enhanced pharmacological properties. Moreover, their role in biodiversity conservation and sustainable development further underscores their value in modern scientific endeavors (Cordier et al., 2008).

On a global scale, natural products have not only served as the foundation for traditional medicine systems but have also become central to contemporary therapeutic practices. For centuries, various societies have relied on plant-based and other natural sources to address a wide range of health conditions (Bernardini et al., 2018). Two long-standing examples are Traditional Chinese Medicine and Ayurveda, whose pharmacopeias are historically grounded in preparations from natural sources. In recent decades, the pharmaceutical industry has turned its focus to these natural sources for drug discovery, resulting in the development of groundbreaking treatments such as paclitaxel and artemisinin (Li & Vederas, 2009). Furthermore, natural products are integral to the growing field of nutraceuticals, providing preventive health solutions that align with the worldwide shift toward wellness and holistic approaches to healthcare.

Natural products also play a crucial role in addressing pressing global health issues, such as antimicrobial resistance and chronic diseases. With the rise of drug-resistant pathogens, there has been a renewed interest in natural products as promising sources for the development of new antibiotics. Compounds like vancomycin and daptomycin, which are derived from microorganisms, have proven effective against resistant strains (Newman & Cragg, 2020). Additionally, natural products have proven valuable in the management of chronic conditions, such as cancer, diabetes, and cardiovascular diseases. Their ability to target multiple pathways, coupled with their lower side effect profiles, makes them appealing candidates for long-term therapeutic use (Wright, 2017).

In agriculture, natural products contribute significantly to sustainable practices, particularly in pest control and crop protection. Biopesticides derived from natural sources offer environmentally friendly alternatives to chemical pesticides. For example, neem oil is well-regarded for its insecticidal properties (Wright, 2017). These natural solutions not only reduce the environmental impact of farming but also promote soil health and improve crop yields. By integrating natural products into agricultural systems, Malaysia aligns itself with global efforts to combat climate change and ensure food security (Kumar et al., 2021).

The cosmetics and personal care industries have also seen a growing reliance on natural products for innovative formulations. Ingredients like aloe vera, argan oil, and shea butter have become staples due to their moisturizing and anti-aging properties. The increasing consumer demand for natural and organic products has driven research into plant-based extracts and essential oils (Prasad et al., 2014). This reflects a broader trend toward sustainability and ethical sourcing in the beauty industry. Incorporating natural products into cosmetic formulations not only improves efficacy but also reduces the environmental impact of the industry (Wright, 2017).

Asia's rich biodiversity and ancient medicinal traditions make it a valuable source for drug discovery. The varied ecosystems of Asia, ranging from arctic tundras to tropical rainforests, support a wealth of plant species with medicinal potential. In response to the increasing global interest in traditional, complementary, and alternative medicine, countries such as China and India have made efforts to preserve and promote their indigenous medical traditions (Chapman et al., 2005). The diverse flora and fauna of these regions have contributed to the discovery of numerous bioactive compounds with notable therapeutic applications. Additionally, Asia's cultural emphasis on herbal remedies and the preservation of traditional knowledge has firmly established it as a leader in natural product research and development (Jacobson, 2010).

Malaysia's rich biodiversity, particularly its tropical rainforests, provides an exceptional platform for the exploration and development of natural products. With approximately 15,000 species of vascular plants, many documented for their medicinal properties, the country offers immense potential for discovering bioactive compounds with applications in pharmaceuticals, cosmetics, and nutraceuticals. Indigenous communities, such as the Orang Asli, play a vital role in preserving traditional medicinal knowledge. For example, they use plants like *Streblus asper* for wound healing and *Calotropis gigantea* for pain relief, practices that have laid the groundwork for ethnopharmacological studies (Jamadon et al., 2007).

Several plants native to Malaysia have already gained recognition for their health benefits. Tongkat Ali (*Eurycoma longifolia*), traditionally used as an aphrodisiac and energy booster, has been scientifically validated for its testosterone-enhancing and anti-inflammatory properties and is now marketed in supplements worldwide. Similarly, Kacip Fatimah (*Labisia pumila*) is used by Malay women for postpartum recovery and hormonal health, with studies confirming its antioxidant and phytoestrogenic activities. Other examples include Misai Kucing (*Orthosiphon stamineus*), known for treating kidney issues and hypertension, and Temu Lawak (*Curcuma xanthorrhiza*), which has hepatoprotective and anti-inflammatory properties (Ahmed, 2021; Samy et al., 2008).

There remains an abundance of medicinal plants yet to be explored for their potential pharmaceutical applications. Currently, researchers are actively seeking to identify new plant species with therapeutic properties that could be developed into commercial medicinal products. This highlights the need for continued exploration of diverse plant species, particularly those with proven medicinal value and high consumer demand. Among these, the Piperaceae family stands out as a group with significant potential. Renowned for its high medicinal value, this plant family has been a cornerstone of traditional medicine for decades, making it an important focus for further scientific investigation.

1.2 Problem Statement

The Piperaceae family had long been regarded as a cornerstone of traditional Malaysian medicine, largely due to its rich phytochemical diversity and significant biological activities. Within this family, the genus *Piper* stood out for its wide spectrum of phytochemicals, many of which exhibited diverse pharmacological properties. Species including *P. aduncum, P. nigrum* and *P. betle* had been extensively studied and well documented in Malaysia for their medicinal applications and bioactivities. However, the therapeutic potential of many lesser known species remained underexplored. One such species, *P. rostratum*, had received minimal scientific attention, particularly concerning its essential oil composition and phytochemical profile, resulting in a critical gap in understanding its possible medicinal value.

This study was undertaken to address that gap. The investigation was set to analyze the essential oils of *P. rostratum*, isolate key phytochemicals, and evaluate their biological activities. This approach was expected to not only expand the existing knowledge of the *Piper* genus but also to uncover novel bioactive compounds with potential pharmaceutical significance. Given the increasing global interest in natural products for drug development, the study held promise for contributing to innovative pharmaceutical applications derived from *P. rostratum*. Ultimately, identifying the plant's phytochemical composition and therapeutic potential was anticipated to bridge traditional medicinal knowledge with modern scientific discovery.

1.3 Objectives of Study

The study objectives are:

1. To investigate the chemical composition of the essential oil from the leaves of *P. rostratum* using gas chromatography with flame ionisation detection (GC-FID) and gas chromatography–mass spectrometry (GC-MS).
2. To isolate phytochemicals from *P. rostratum* leaf extracts and characterize their structures using spectroscopic techniques, including nuclear magnetic resonance (NMR), infrared (IR), and mass spectrometry (MS).
3. To determine the antibacterial activity of the crude extracts and selected phytochemicals of *P. rostratum* using the microdilution method.

1.4 Scopes of Study

The study was structured into three distinct phases. In the first phase, essential oil was extracted from the leaves of *P. rostratum* by utilizing the hydrodistillation method. The chemical composition of the essential oils was analyzed using gas chromatography–flame ionization detection (GC-FID) and gas chromatography–mass spectrometry (GC-MS), and Kovats Indices. The second phase focused on isolating phytochemicals from the dried leaves of *P. rostratum* through various chromatographic techniques, including column chromatography. The phytochemical structures of the isolated compounds were elucidated using spectroscopic methods such as NMR, IR, and MS. The final phase was dedicated to evaluating the biological activities of both the phytochemicals and crude extracts, with particular attention to their antibacterial properties, assessed using minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays.