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INVESTIGATION OF ESSENTIAL OIL, PHYTOCHEMICALS AND ANTIBACTERIAL ACTIVITY OF *Piper frustratum* Boerl.

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ABSTRACT

The aim of this study was to investigate the essential oil composition, phytochemicals, and antibacterial activity of *Piper frustratum*, Piperaceae. Essential oil was obtained from the leaves of *P. frustratum via* hydrodistillation and its chemical composition was determined using gas chromatography (GC) and gas chromatography mass spectrometry (GC-MS). Cold extraction was performed to obtain the leaf extract, followed by isolation of phytochemicals using column chromatography techniques, and structural elucidation was achieved *via* various spectroscopic methods in comparison with literature. Antibacterial activity was evaluated using micro-dilution method. Results showed that nineteen chemical constituents were identified in the essential oil of *P. frustratum* leaf, accounting for 99.6% of the total composition. The major constituents include β-caryophyllene (12.6%), β-asarone (10.7%), myristicin (10.4%), α-copaene (9.6%), methyl eugenol (7.2 %), and β-cubebene (6.5%). Isolation and purification of the *P. frustratum* leaf extracts yielded eight phytochemicals including taraxerol, ursolic acid, α-asarone, γ-asarone, 3′,4′,5,5′,7-pentamethoxyflavone, asaricin, elemicin, and *N*-isobutyl-(2*E*,4*E*)-tetradecadienamide. In addition, 3′,4′,5,5′,7-pentamethoxyflavone exhibited the strongest antibacterial activity with equal minimum inhibitory concentration (MIC) and minimum bactericidal activity (MBC) values (12.5 µg/mL) against both *Escherichia coli* and *Salmonella typhi*. In conclusion, the essential oil of *P. frustratum* was dominated by sesquiterpene hydrocarbons, while phytochemical analysis of the leaf extracts revealed the presence of terpenes, phenylpropanoids, flavone, and amides. These findings indicate that *P. frustratum* is a potential natural source for antibacterial compounds for the development of new antimicrobial agents.

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PENYIASATAN MINYAK PATI, FITOKIMIA DAN AKTIVITI ANTIBAKTERIA OLEH *Piper frustratum* Boerl.

ABSTRAK

Tujuan kajian ini adalah untuk menyiasat kandungan minyak pati, fitokimia, dan aktiviti antibakteria oleh *Piper frustratum*, Piperaceae. Minyak pati diperolehi daripada daun *P. frustratum* melalui penyulingan hidro dan komposisi kimianya ditentukan menggunakan kromatografi gas (GC) dan kromatografi gas spektrometri jisim (GC-MS). Pengekstrakan sejuk dijalankan untuk memperoleh ekstrak daun, diikuti dengan pengasingan fitokimia menggunakan teknik kromatografi turus dan penentuan struktur dicapai melalui pelbagai kaedah spektroskopi serta perbandingan dengan literatur. Aktiviti antibakteria telah dinilai menggunakan kaedah pencairan mikro. Keputusan menunjukkan sembilan belas kandungan kimia telah dikenal pasti dalam minyak pati daun *P. frustratum*, merangkumi 99.6% daripada jumlah keseluruhan komposisi. Kandungan utama termasuk β-kariofilena (12.6%), β-asarona (10.7%), miristisin (10.4%), α-kopaena (9.6%), metil eugenol (7.2%), dan β-kubebena (6.5%). Pengasingan dan penulenan ekstrak daun *P. frustratum* menghasilkan lapan fitokimia termasuk taraxerol, asid ursolik, α-asarona, γ-asarona, 3′,4′,5,5′,7-pentametoksiflavon, asarisin, elemisin, dan *N*-isobutil-(2*E*,4*E*)-tetradekadienamida. Selain itu, 3′,4′,5,5′,7-pentametoksiflavon menunjukkan aktiviti antibakteria terkuat dengan nilai kepekatan perencatan minimum (MIC) dan kepekatan bakterisid minimum (MBC) yang sama (12.5 µg/mL) terhadap kedua-dua *Escherichia coli* dan *Salmonella typhi*. Kesimpulannya, minyak pati *P. frustratum* didominasi oleh hidrokarbon seskuiterpena, manakala analisis fitokimia terhadap ekstrak daun mendedahkan kehadiran terpena, fenilpropanoid, flavon, dan amida. Penemuan ini menunjukkan bahawa *P. frustratum* merupakan sumber semula jadi yang berpotensi bagi pembangunan agen antimikrob baharu.

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CONTENTS

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05-4506832 pustaka.upsi.edu.my Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah PustakaTBainun ptbupsi

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LIST OF TABLES

05-4506832 pustaka.upsi.edu.my Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah PustakaTBainun ptbupsi

05-4506832 pustaka.upsi.edu.my Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah PustakaTBainun ptbupsi

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LIST OF FIGURES

05-4506832 pustaka.upsi.edu.my Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah PustakaTBainun ptbupsi
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LIST OF SCHEME

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LIST OF ABBREVIATIONS

α	Alpha
Abs	Absorbance
ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
β	Beta
^{13}C	Carbon-13
CC	Column Chromatography
$CDCl_3$	Deuterated chloroform
$CHCl_3$	Chloroform
cm^{-1}	Reciprocal centimetre
COSY	Correlation spectroscopy
1D	1 Dimension
2D	2 Dimension
δ	Chemical shift
d	doublet
DCM	Dichloromethane
dd	doublet of doublets
DEPT	Distortionless Enhancement by Polarization Transfer
DPPH	2,2-Diphenyl-1-picrylhydrazyl
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
FRAP	Ferric Reducing Antioxidant Power

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GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
GC-FID	Gas Chromatography-Flame Ionisation Detector
^1H	Proton
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coherence
HPLC	High Performance Liquid Chromatography
Hz	Hertz
IC_{50}	Half-Maximal Inhibitory Concentration
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

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mL	milliliter
mm	millimeter
MS	Mass Spectrometer
NaCl	Sodium chloride
NMR	Nuclear Magnetic Resonance
nm	nanometer
PTLC	Preparative Thin Layer Chromatography
SiO_2	Silica gel
t	triplet
TLC	Thin Layer Chromatography

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CHAPTER 1

INTRODUCTION

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1.1 General Introduction

Natural products have played an important role in human health for thousands of years. Early civilizations depended on plants, animals, and minerals for medicine, food, and spiritual practices. Ancient societies such as the Egyptians, Greeks, and Chinese used herbs to treat illnesses, and many of these practices later influenced modern medicine. Scientific research on natural products began in the 19th century with the isolation of active compounds, leading to the discovery of important drugs such as quinine and morphine. Since then, natural products have remained a major source of inspiration for drug discovery, supported by advances in synthetic chemistry (Newman & Cragg, 2020).

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Natural products are widely used in medicine, agriculture, food, and industry. In medicine, many drugs are derived from natural sources, particularly plant compounds such as alkaloids, terpenoids, and flavonoids, which are effective against diseases including cancer, malaria, and cardiovascular disorders. Classic examples include penicillin and morphine (Nasim et al., 2022). In agriculture, essential oils and plant extracts are used as natural pesticides and fungicides, providing environmentally friendly alternatives to synthetic chemicals. Natural products are also important in the food industry for flavouring and preservation, and in cosmetics, where plant-based ingredients are used for skin care and therapeutic purposes (Shahbaz et al., 2022).

Malaysia has a long tradition of using medicinal plants in daily life and indigenous healthcare. Its tropical climate supports rich plant biodiversity, and many species have been used by the Malays, Orang Asli, and other ethnic groups for medicinal, culinary, and cultural purposes. Knowledge of these plants has been passed down through generations and remains widely practised today. Traditional Malaysian plants are commonly used to treat fever, pain, digestive problems, and infections (Basu & Mishra, 2019).

In recent years, traditional Malaysian plants have attracted increasing scientific attention. Plants once used only in folk medicine are now studied using modern pharmacological and phytochemical approaches to identify their active compounds and understand their biological effects. This research highlights the importance of preserving traditional knowledge while applying scientific methods to explore Malaysia's rich plant resources (Sulaiman & Mamat, 2016).

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Overall, combining traditional knowledge with modern scientific research is essential for discovering new and sustainable therapies. Phytochemistry plays a key role in identifying and validating bioactive compounds from medicinal plants, including those from the Piperaceae family. This integrated approach strengthens drug discovery efforts and connects traditional practices with modern medicine (Jantan et al., 2015).

1.2 Problem Statement

The Piperaceae family, known for its extensive diversity of phytochemicals, attracted considerable attention in scientific research due to the broad range of biological activities exhibited by its members. *Piper* species were rich in bioactive compounds such as alkaloids, amides, propenylphenols, lignans, neolignans, terpenes, steroids, kawapyrones, piperolides, chalcones, dihydrochalcones, flavones, and flavanones. These compounds were often responsible for the therapeutic effects observed in various *Piper* species, including antimicrobial, anti-inflammatory, antioxidant, and anticancer activities (Parmar et al., 1997). As a result, *Piper* plants were a significant focus in the search for natural products with potential pharmacological applications. Their chemical diversity and pharmacological properties made them valuable resources for drug discovery, particularly in the development of treatments for diseases where conventional drugs were less effective.

However, despite the growing body of research on *Piper* species, many species in Malaysia remained underexplored in terms of their chemical composition and

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biological properties. Malaysia's rich biodiversity offered a unique opportunity to study *Piper* species that might have possessed novel compounds with therapeutic potential. While certain species, such as *P. nigrum* (black pepper) and *P. betle* (betel leaf), had been well-documented, many others had not been fully investigated. This knowledge gap was particularly evident for *P. frustratum*, a species native to Malaysia. To date, there had been no studies focused on the isolation of phytochemicals or the extraction of essential oils from this plant, leaving its medicinal potential largely unexamined. The lack of research on *P. frustratum* presented a unique opportunity for further investigation, especially considering the plant's possible traditional uses in local medicine and its untapped potential for pharmaceutical applications.

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1.3 Objectives of Study

The objectives of the study are:

1. To investigate the chemical compositions of the essential oil from the leaves of *P. frustratum.*
2. To isolate the phytochemicals from the leaves of *P. frustratum* and characterize spectroscopically.
3. To determine the antibacterial activity of the crude extracts and selected phytochemicals of *P. frustratum.*

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1.4 Scopes of Study

In the first phase, essential oil was extracted from the leaves of *P. frustratum* using the hydrodistillation method. Once the essential oils were extracted, their chemical composition was analyzed using Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS). Additionally, Kovats indices were calculated to standardize the retention times of the compounds, further assisting in their identification. This phase provided valuable insights into the profile of bioactive compounds present in the essential oils of *P. frustratum*.

The second phase focused on isolating the phytochemicals from the leaf extracts of *P. frustratum* using various chromatographic techniques such as column chromatography and thin-layer chromatography (TLC). These techniques allowed for the separation of complex mixtures into individual components, enabling the isolation of bioactive compounds. After isolation, the structures of these phytochemicals were characterized using advanced spectroscopic methods, including Infrared (IR) spectroscopy, Nuclear Magnetic Resonance (NMR) spectroscopy, and Mass Spectrometry (MS).

In the final phase, the bioactivity of the crude extracts and selected phytochemicals, will be assessed, with a focus on antibacterial activity. By evaluating the antibacterial activity through the microdilution method in 96-well plates, the study aimed to determine the potential of substances derived from *P. frustratum* as therapeutic agents against infections caused by both Gram-positive and Gram-negative bacteria.

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