ANALYSIS OF ESSENTIAL OIL, PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITY FROM *Piper crassipes* Korth ex Miq.

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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 antioxidant activity from the leaves of *Piper crassipes*. Essential oil was obtained from the leaves by hydrodistillation, and its chemical compositions was determined using gas chromatography (GC) and gas chromatography mass spectrometry (GC-MS). Soxhlet extraction was performed for the leaves part, followed by isolation of phytochemicals using silica gel column chromatography. The chemical structures of isolated compounds were determined using spectral analysis (Infrared Spectroscopy, Nuclear Magnetic Resonance Spectroscopy, and Mass Spectrometry) and comparison with literature data. Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and total phenolic content. Twenty-two compounds (97.8%) were identified in the leaf oil with chavibetol (59.8%), chavibetol acetate (10.4%), γ-muurolene (5.4%), and germacrene D (4.6%) as major compounds. Ten compounds were isolated from the leaf extracts, including asaricin, chavibetol, dillapiole, β-sitosterol, 5,7-dimethoxyflavone, 4',5,7-trimethoxyflavone, *N*-(3-phenylpropanoyl)pyrole, chavicol, *N*-isobuty1-(2*E*,4*E*,14*Z*)-eicosatrienamide, and 4-allyl resorcinol. Both methanol extract and chavicol exhibited significant antioxidant activity in the DPPH assay with IC_{50} values of 44.0 and 25.5 µg/mL, respectively. In conclusion, the essential oil of *P. crassipes* contains phenylpropanoids as major compounds. Ten compounds were isolated from *P. crassipes* leaf extract and are reported for the first time in this species. These findings highlight the antioxidant activity of *Piper* extracts for potential medicinal applications.

ANALISIS MINYAK PATI, FITOKIMIA DAN AKTIVITI ANTIOKSIDAN DARIPADA *Piper crassipes* Korth ex Miq.

ABSTRAK

Tujuan kajian ini adalah untuk menyiasat komposisi minyak pati, fitokimia, dan aktiviti antioksidan daripada daun *Piper crassipes*. Minyak pati diperoleh daripada daun melalui penyulingan hidro dan komposisi kimianya dikenal pasti menggunakan kromatografi gas (GC) dan kromatografi gas-spektrometri jisim (GC-MS). Pengekstrakan soklet dijalankan pada bahagian daun, diikuti dengan pengasingan fitokimia menggunakan kromatografi turus silika gel. Struktur kimia yang dipencilkan ditentukan menggunakan analisis spektra (Spektroskopi Inframerah, Spektroskopi Resonans Magnet Nuklear, dan Spektrometri Jisim) dan perbandingan dengan data literatur. Aktiviti antioksidan dinilai menggunakan asai perencatan radikal bebas 2,2-difenil-1-pikrilhidrazil (DPPH) dan jumlah kandungan fenolik. Dua puluh dua sebatian (97.8%) telah dikenal pasti dalam minyak pati daun dengan kavibetol (59.8%), kavibetol asetat (10.4%), γ-murolena (5.4%), dan germacrena D (4.6%) sebagai sebatian utama. Sepuluh sebatian telah diasingkan daripada ekstrak daun, termasuk asarikin, kavibetol, dillapiol, β-sitosterol, 5,7-dimetoksiflavon, 4',5,7-trimetoksiflavon, *N*-(3-fenilpropanoil)pirol, kavikol, *N*-isobutil-(2*E*,4*E*,14*Z*)-eikosatrienamida, dan 4-alil resorsinol. Kedua-dua ekstrak metanol dan kavikol menunjukkan aktiviti antioksidan yang signifikan dalam asai DPPH dengan nilai IC_{50} masing-masing sebanyak 44.0 dan 25.5 μg/mL. Kesimpulannya, minyak pati *P. crassipes* mengandungi fenilpropanoid sebagai sebatian utama. Sepuluh sebatian telah diasingkan daripada ekstrak daun *P. crassipes* dan dilaporkan buat kali pertama dalam spesis ini. Penemuan ini menekankan aktiviti antioksidan oleh ekstrak *Piper* untuk potensi aplikasi perubatan.

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LIST OF ABBREVIATIONS

α	Alpha
Abs	Absorbance
β	Beta
br	broad
^{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
Hz	Hertz
IR	Infrared
J	Coupling constant
KBr	Potassium bromide
KI	Kovats Index
L	Liter
m	multiplet
M^{+}	Molecular ion
MeOH	Methanol
MHz	Megahertz
min	Minute(s)
m/z	Mass to charge ion
mg	milligram
$MgSO_4$	Magnesium sulphate
mL	milliliter
mm	millimeter

MS	Mass Spectrometer
NMR	Nuclear Magnetic Resonance
PTLC	Preparative Thin Layer Chromatography
s	singlet
SiO_2	Silica gel
t	triplet
TLC	Thin Layer Chromatography

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Throughout history, humankind has relied on nature to supply basic necessities. This also refers to the use of natural resources as a treatment for various medical conditions. Natural products are pharmacological or biological chemicals that produce naturally by living organisms that include plants, animals, insects, marine organisms, and microorganisms (Zhu et al., 2022). It is rich in variety and are valuable natural resources with high medicinal potential. Until this date, alongside the development of technology, molecular biology, pharmacology, and other fields, studies on natural products have produced beneficial discoveries, finding extensive use in medications, food, and health supplements (Zhang et al., 2023).

Natural products have contributed significantly to human health from time immemorial. In recognition of their high molecular diversity and biofunctionality, natural product practically is wonderful gems bestowed upon us by nature, serving as significant sources for the treatment of human medical conditions. In that regard, natural product plays a crucial role in drug research and development, as the present pharmaceuticals start to comprise natural products both directly and indirectly, giving these approaches remain dominant in modern new drug development (Zhu et al., 2022). For instance, capsaicin has been approved by the US Food and Drug Administration to treat neuropathic discomfort due to postherpetic neuralgia and neuropathic foot pain resulting from diabetic peripheral neuropathy in adults (Abrams et al., 2021).

Plants are a major source of biologically active chemicals, providing a rich array of natural products that have been utilized in traditional medicine and modern drug discovery. There are about 350,000 vascular plant species recorded worldwide, with new species being added each year and World Health Organization estimates that there are around 20,000 plant species now used for medical purposes (Cadoná et al., 2021). It remains a broad and uncharted field of study. In China, around 10,000 species of plants are used in traditional Chinese medicine, which it has to undergo a complex theoretical system with more than 2000 years of clinical use (Zhu et al., 2022). Besides, India is known for its traditional medicinal systems; Ayurveda, Siddha, and Unani. It offers treatment methods to cure many common diseases such as food allergies, which have few modern treatments.

Malaysia's rainforests are host to a wide variety of plant species, many of those have yet to be identified, positioning Malaysia being a medicinal plant species rich country, and currently, about 2,000 species have been listed as medicinal plants used in alternative medicine (Abu Bakar et al., 2018). A vast range of medicinal plants are utilized to produce effective nutritional, traditional medicine, and aesthetic preparations. In Malaysia, 10% of the 15,000 local flowering plants are said to have medicinal properties (Eswani et al., 2010).

Since the start, it has been a common practice for Malaysian to include the plant in their daily essentials, particularly dietary and medication. For instance, the leaves of *Carica papaya* (betik) and *Garcinia atroviridis* (asam gelugor) were used to treat gastric (Alsarhan et al., 2021). In addition, the root decoction of *Curcuma aeruginosa* (kunyit hitam), *Piper nigrum* (lada hitam hutan) and *Stenotaphrum helferi* (lipan bara) are used as a remedy for postpartum mothers to regain energy and health (Nordin et al., 2016). Moreover, the roots of *Cnestis ramiflora* (akar sembelit) and *Cinnamomum zeylanicum* (kayu manis) are used as a decoction to treat constipation (Nordin et al., 2016).

There are still countless of medicinal plants that have yet to be explored for the sake of potential pharmaceuticals. At present, experts are pursuing to discover new plants with therapeutic traits that might be commercialized as medicinal products. Therefore, more discoveries comprised of varying plant species ought to be done, exclusively plants with medicinal value and consumer-demand. Piperaceae is one of the plant families that is thought to enhance traditional medicine which existed for decades due to their high medicinal value.

1.2 Problem Statement

Malaysian traditional medicine has relied on medicinal plants from the Piperaceae family. They are notable due to their varied phytochemicals and earlier studies had reported that numerous fruitful biological activities had been achieved from the phytochemicals of Piperaceae. *Piper* is one of the genera in the Piperaceae family that has exhibited a wide variety of phytochemicals and biological properties, making them tend to be significant. Previous studies on the phytochemicals of *Piper* species have yielded several physiologically active compounds such as flavonoids, alkaloids, lignans, amides, and phenylpropanoids. It also showed varies of bioactivity such as antifungal, antimicrobial, antioxidant, and anti-inflammatory (Salleh et al., 2014).

Hypothetically, this is the first study reporting on the essential oil composition, phytochemistry, and biological activities of *P. crassipes*. Taking into account the significant role of this genus in the medicinal field, there is no doubt that an extensive range of studies on its biological activities should be documented for their pharmacological properties. These gaps provide a crucial opportunity for additional research into the phytochemistry and pharmacology of the genus *Piper*. As many *Piper* species have yet been adequately investigated, chemically or physiologically, to justify their usage. Therefore, more studies on the extraction and isolation of phytochemicals with biological activity should be done on this genus. Hence, the outcomes of the study may have future implications to the pharmaceutical industry.

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. crassipes.*
2. To isolate and characterize the phytochemicals from the leaves of *P. crassipes* and identified spectroscopically.
3. To determine the antioxidant activity of the crude extracts and selected phytochemicals of *P. crassipes*.

1.4 Scopes of Study

The study was separated into three parts. The first part was the extraction of the essential oil by hydrodistillation method from the leaf part of *P. crassipes.* The chemical compositions of the essential oils were analysed using GC-FID, GC-MS, and Kovats Indices. The second part was the isolation of phytochemicals from the dried leaves extracts of *P. crassipes* using various chromatographic methods. The chemical structures of the isolated phytochemicals were elucidated spectroscopically using Infrared spectroscopy (IR), Nuclear Magnetic Resonance (NMR), and Mass Spectroscopy (MS). Finally, the antioxidant activity of the crude extracts and selected phytochemicals of *P. crassipes* were carried out by DPPH free radical scavenging and phenolic content assay.