PHYTOCHEMICAL PROFILING, ESSENTIAL OIL COMPOSITION AND BIOACTIVITY EVALUATION OF *Boesenbergia albosanguinea* (Ridl.) Loes. (ZINGIBERACEAE)

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UNIVERSITI PENDIDIKAN SULTAN IDRIS

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ABSTRACT

The aim of this study was to determine essential oil composition, phytochemicals, and to evaluate bioactivities of *Boesenbergia albosanguinea* (Zingiberaceae) rhizomes. The essential oil was extracted using hydrodistillation and its chemical composition was determined *via* gas chromatography (GC) and gas chromatography mass spectrometry (GC-MS). The rhizome was extracted using Soxhlet extraction (*n*-hexane, ethyl acetate, and methanol). Phytochemicals of each extract were isolated by chromatographic techniques and their structures were confirmed using spectroscopic analysis and comparison with literature. The antioxidant activity was determined using the 2,2-diphenylpicrylhydrazil (DPPH) free radical scavenging assay, and the anti-inflammatory activity was evaluated using the lipoxygenase inhibition assay. The study indicates that the essential oil of *B. albosanguinea* comprised elemicin (44.0%), α-gurjunene (9.3%), β-caryophyllene (4.5%), and safrole (4.1%). Isolation and purification of the rhizome extracts afforded seven phytochemicals identified as panduratin A (72), isopanduratin A (76), pinocembrin (114), pinostrobin (115), 5,6-dehydrokawain (196), kaempferol (131), and luteolin (138). Elemicin (60) was also isolated from the rhizome essential oil. Elemicin showed the highest antioxidant activity potency with an IC_{50} value of 9.3 µg/mL, while isopanduratin A (76) demonstrated the strongest lipoxygenase inhibitory activity (84.9%). In conclusion, the essential oil of *B. albosanguinea* was found to be rich in phenylpropanoids, with flavonoids as the major phytochemicals. This study suggests *B. albosanguinea* is a potential source of antioxidant compounds, which may be beneficial in the prevention of free radical-related diseases such as atherosclerosis and diabetes.

PROFIL FITOKIMIA, KOMPOSISI MINYAK PATI DAN PENILAIAN BIOAKTIVITI *Boesenbergia albosanguinea* (Ridl.) Loes. (Zingiberaceae)

ABSTRAK

Tujuan kajian ini adalah untuk mengenalpasti komposisi minyak pati, fitokimia, dan menilai bioaktiviti rizom *Boesenbergia albosanguinea* (Zingiberaceae). Minyak pati diekstrak menggunakan penyulingan hidro dan komposisi kimianya ditentukan secara kromatografi gas (GC) dan kromatografi gas-spektrometri jisim (GC-MS). Rizom telah diekstrak menggunakan pengekstrakan Soxhlet (*n*-heksana, etil asetat, dan metanol). Fitokimia untuk setiap ekstrak telah dipisahkan secara teknik kromatografi dan strukturnya disahkan menggunakan analisis spektroskopi dan perbandingan dengan kajian literatur. Aktiviti antioksidan ditentukan menggunakan asai perencatan radikal bebas 2,2-difenilpikrilhidrazil (DPPH), dan aktiviti anti-radang dinilai menggunakan asai penghambatan lipoksigenes. Kajian mendapati minyak pati *B. albosanguinea* mengandungi elemicin (44.0%), α-gurjunen (9.3%), β-karyofailen (4.5%), dan safrol (4.1%). Pengasingan dan penulenan terhadap ekstrak rizom menghasilkan tujuh fitokimia dikenalpasti sebagai panduratin A (72), isopanduratin A (76), pinocembrin (114), pinostrobin (115), 5,6-dihidrokawain (196), kemferol (131), dan luteolin (138). Elemicin (60) juga dipencilkan daripada minyak pati rizom. Elemicin menunjukkan potensi aktiviti antioksidan tertinggi dengan nilai IC_{50} 9.3 µg/mL, manakala isopanduratin A (76) menunjukkan aktiviti penghambatan lipoksigenas terkuat (84.9%). Kesimpulannya, minyak pati *B. albosanguinea* didapati kaya dengan fenilpropanoid, dengan flavonoid sebagai fitokimia utama. Kajian ini mencadangkan *B. albosanguinea* berpotensi sebagai sumber sebatian antioksidan yang berkemungkinan bermanfaat dalam pencegahan penyakit berkaitan radikal bebas seperti aterosklerosis dan diabetes.

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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 General Introduction

Plants play a crucial role in sustaining life on Earth and are a key source of biologically active chemicals. Plants with practical uses, such as in medicine or cooking, are often classified as herbs. In the 17th century, plants were first categorized into groups like weeds, vegetables, and herbs. Earlier societies did not distinguish between foods and medicines as we do today. Herbs have held significant importance in medicine since ancient times. Traditionally, self-limiting illnesses were treated within families using herbal remedies passed down through generations, while more serious conditions were entrusted to specialized healers. For instance, foxglove, first recognized as a heart treatment in 1768, became the source of two important medications. Since then, many herbs have proven valuable to the pharmaceutical industry (Ody, 2000).

Herbal remedies, traditional Chinese medicine, homeopathy, naturopathy, osteopathy, acupuncture, chiropractic, Ayurveda, indigenous traditional medicine, and Unani medicine are among the numerous traditional medical systems practiced worldwide. India, in particular, is renowned for its traditional medicinal systems, which offer methods to treat common diseases like food allergies, for which modern treatments are limited. Additionally, 170 WHO member states have reported on the population's use of traditional medicines (Zaman et al., 2021). Approximately 40% of pharmaceutical drugs on the market today, including groundbreaking medications like aspirin and artemisinin, as well as pediatric cancer treatments, stem from traditional knowledge or nature. Closer examination reveals that many of these discoveries were predicated on the application of existing knowledge (Liu et al., 2016).

Traditional medicine is a scientific practice in healthcare that has been used for centuries. It is passed down orally, in writing, and through the traditions and beliefs of communities. This field encompasses two branches: empirical and spiritual. The empirical component involves treating illnesses using natural resources like plants, animals, and minerals, validated through scientific methods. The spiritual component includes practices such as reciting Quranic verses over items for treatment. For example, a *bomoh* (traditional healer) may pray over water for a distressed patient to drink or recite Quranic passages repeatedly to offer comfort. Since its inception, Malaysians have incorporated plants into their daily needs, especially in food and medicine (Zakaria & Mohd, 1994).

The Malay community continues to value traditional healing methods. Various health problems are addressed using plants in Malaysia (Darlina & Zuraidah, 2017).

Malay traditional medicine has been influenced by foreign medicinal practices due to interracial connections dating back to the 14th-century Malay Malacca Sultanate. Immigrants from China and India introduced numerous medicinal plants that thrived locally. Derived from ancient Indonesian traditional medicine, Malay traditional medicine has been adapted to meet contemporary demands. Both traditional and modern medicine utilize natural resources such as plants, minerals, and microorganisms. In modern medicine, these resources are processed to extract concentrated active components, while traditional medicine often uses them in their raw state (Zakaria & Mohd, 1994).

There are approximately 350,000 vascular plant species worldwide, with new species discovered annually. WHO estimates that around 20,000 plant species are used medicinally (Cadona et al., 2021). Plants are considered medicinal due to their biologically active compounds, which can serve as medicines or precursors for pharmaceutical development. While many of these compounds have been identified and isolated, numerous others remain undiscovered. The medicinal effects of a plant often arise from the synergistic interaction of multiple active compounds. Therefore, using the entire plant can be more effective than isolating a single active compound (Luengo & Arisó, 2015).

Medicinal plants have been used in traditional medicine since ancient times, with a significant increase in usage over the past three decades. For example, pregnant women often use herbal products like red raspberry leaf, which is rich in iron and helps tone the uterus, increase milk production, reduce nausea, and ease labor pain. Herbal remedies are also popular for treating ailments in people of all ages. Ginger and

peppermint alleviate motion sickness, while aloe vera soothes sunburns and minor cuts. Women benefit from herbs like chamomile and chasteberry to address menstrual irregularities. Children may use chamomile for colic or mallow for respiratory conditions. Men turn to herbs like saw palmetto for prostate health or rosemary for baldness treatment (Luengo & Arisó, 2015).

The WHO distinguishes between medicinal plants that have been thoroughly studied and those still unexplored (Lewis, 1981). Many medicinal plants serve as the foundation for prescription drugs widely used in modern medicine (Kurian & Sankar, 2007). However, countless plants remain unexplored, holding potential for new pharmaceuticals. Researchers continue to discover plants with therapeutic traits for commercialization. These plants are integral to traditional medicine globally and are traded to meet market demands (Cragg & Newman, 2013).

Natural products have historically provided numerous substances for biology, pharmacology, and medicine. Studies show that 61% of the 877 drugs launched globally have origins in natural products (Cseke et al., 2004). Their molecular diversity and unique biological activities make natural products vital for drug development, offering improved efficacy and safety (Nasim et al., 2022). Secondary metabolites, the active substances in medicinal plants, are found in various plant parts and may have different effects depending on the part used (Wyk & Wink, 2004).

The Malaysian rainforest, among the oldest globally, ranks 12th in biodiversity and 4th in Asia. With over 2,000 recognized medicinal plant species, Malaysia is rich in natural resources (Abu Bakar et al., 2018). The herbal industry in Malaysia is

thriving, driven by growing interest in health-oriented foods, supplements, and beauty products. This development blends traditional practices like Jamu, Kampo, Traditional Chinese Medicine, and Ayurveda (Ahmad et al., 2015). Research shows that secondary metabolites in Malaysian plants are effective for health and quality of life. For example, *Ardisia crispa* contains quinones with anti-cancer properties (Blin et al., 2021), while coumarin from *Calophyllum lanigerum* is in clinical development as an anti-HIV drug (Zailan et al., 2022). Other plants, like *Polygonum minus*, serve as sources for perfumes due to their aldehydes (Vikram et al., 2014).

The growing popularity of herbal medicine raises concerns about plant overexploitation. Conservation efforts, guided by initiatives like the WHO's World Conservation Guidelines of Medicinal Plants, aim to ensure sustainable use and preservation (Luengo & Arisó, 2015). In Malaysia, the National Pharmaceutical Regulatory Agency oversees the safety of natural products, ensuring compliance with global standards (Kandi & Vadakedath, 2023). Ethnobotanical plants hold immense potential for pharmaceuticals, warranting further research. Plants in the Zingiberaceae family, in particular, are notable for their significant medicinal value and long-standing use in traditional medicine.

1.2 Zingiberaceae Family

The ginger family, scientifically referred to as Zingiberaceae, is classified under the order Zingiberales. This family of flowering plants includes around 1,600 recognized species across approximately 50 genera, distributed across regions of Asia, the

Americas, and tropical Africa. Several notable genera include Zingiber, which comprises 100 species, and Aframomum, with 60 species. Renealmia boasts 75 species, while Curcuma also contains 100 species. Etlingera stands out with 110 species, and Hedychium and Hornstedia each have 50 species. Meisteria features 42 species, and Amomum includes 65 species. Additionally, Boesenbergia has 60 species, Riedelia contains 75 species, and Globba is part of this diverse group (Pitopang et al., 2019). Tropical Asia is recognized as the most abundant region for the Zingiberaceae family, containing a total of 1,449 recognized species spread across 56 genera. Among these, 21% are considered at risk. Malaysia hosts the highest number of vulnerable ginger taxa, with at least 150 species spanning 21 genera. Indonesia has 113 species across 24 genera, Thailand has 30 species from 11 genera, and Vietnam holds 26 species from 8 genera (Hilario & Altamirano, 2023).

Zingiberaceae plants are aromatic perennial herbs characterized by their pseudostems, single leaves, and distinctive inflorescences in terms of shape and color. They feature bulbous or horizontally creeping rhizomes. A defining characteristic of this family is the presence of essential oils in all parts of the plant, especially the rhizome. Most members of this family can be identified by their aromatic leaves and fresh rhizomes (Holtum, 1950). Zingiberaceae species thrive in tropical and subtropical regions with high humidity. They flourish in shaded environments with minimal light, although certain species can adapt to brighter conditions. Moreover, these plants are resilient to climate change and are highly adaptable to a variety of growing conditions (Larsen et al., 1999).

Zingiberaceae are widely recognized for their medicinal benefits. Many parts of these plants are aromatic and valued for their ornamental appeal and unique flavors. They are commonly used as ingredients in colorants, spices, medicines, essences, and dietary supplements (Kumar et al., 2013). Notable species in the family include turmeric (*Curcuma longa*), ginger (*Zingiber officinale*), galangal (*Alpinia galanga*), patumma or Siam tulip (*Curcuma alismatifolia*), and bitter ginger (*Zingiber zerumbet*) (Chumroenphat et al., 2020). These species have been utilized for various medicinal purposes. For instance, rhizome extracts of *Zingiber zerumbet* are used in traditional Malay medicine to treat inflammation, diarrhea, and helminthiasis (Sharkawi et al., 2019). Additionally, *Alpinia galanga* is effective against lumbago, rheumatic pain, sore throat, diabetes, tubercular glands, bronchitis, and catarrhal conditions (Strehlow & Hertzka, 1988). Furthermore, the leaves of *Curcuma aromatica* and *Curcuma longa* are used to treat bruises, sprains, snake bites, skin ailments, and a range of inflammatory conditions (Akter et al., 2010).

1.3 Genus *Boesenbergia*

Boesenbergia Kuntze is a genus within Zingiberaceae. It was initially named *Gastrochilus* Wall by Wallich in 1829. A year later, Kuntze identified that *Gastrochilus* Wall was an identical of the Orchidaceae genus *Gastrochilus* D.Don, from 1825. Hence the ginger genus was renamed *Boesenbergia* Kuntze by Kuntze. The natural habitat of this species includes Bangladesh and various other regions such, as Laos, Malaysia, Thailand, Cambodia, Myanmar, Philippines, China, Borneo, Sumatera, and Jawa (Eng-Chong et al., 2012).

The *Boesenbergia* genus currently includes about 99 species, with Thailand and Borneo being the main centres of diversity. In Malaysia, one of the most well-known species of *Boesenbergia* is *B. rotunda*, commonly known as fingerroot or Chinese ginger. Finger root utilized centuries in curing diuretics, swelling, leucorrhoea, stomach discomfort, dysentery, and stomachache (Jing et al., 2010). Another notable species in Malaysia is *B. pandurata*, commonly known as butterfly ginger, or cardamom ginger. The rhizome is utilized as an ingredient in various traditional medicines, including tonics and postnatal treatments, as well as for addressing numerous health conditions. For example, fungal infections, dry cough, rheumatism and muscular pain (Burkill, 1935). In addition, *B. pulchella*, also known as black ginger or Cekur Hitam, was traditionally used in treating the wound and healing process during confinement (Kassim et al., 2016).

The *Boesenbergia* genus serves a range of purposes, including food, medicinal applications, ornamental use, and ritualistic practices (Saensouk et al. 2016). Table 1.1 shows the medicinal uses of several *Boesenbergia* species. The medicinal properties of the genus *Boesenbergia* have been extensively documented, underscoring its significance in ethnobotanical and pharmaceutical applications. The data highlights the therapeutic potential of various species, their plant parts, and specific uses, showcasing the genus's versatility in traditional medicine systems. The rhizome is consistently identified as the primary medicinal part across most species, emphasizing its biochemical richness. For instance, *B. armenica* is recognized for its calming effects and its ability to enhance sleep (Elsharif et al., 2015), while *B. longiflora* suitable for inflammatory bowel illnesses and abscesses (Sudsai et al., 2014).

The genus demonstrates a remarkable range of medicinal uses. Species such as *B. rotunda* are used for treating dental inflammation, gastrointestinal disorders, and rheumatism (Bailly, 2022; Saenboonruang, 2018). Similarly, *B. pandurata* is utilized in addressing uterine inflammation, vaginal infections, and as an aphrodisiac (Heyne, 1987; Tuchinda et al., 2002). These examples highlight the broad pharmacological spectrum of this genus. Moreover, some species, like *B. quangngaiensis*, exhibit specific antimicrobial properties (Huong et al., 2021), while *B. parvula* and *B. xiphostachya* are particularly effective against gastrointestinal disorders, serving as remedies for stomachache, flatulence, and dyspepsia (Ragsasilp et al., 2022; Chuakul & Boonpleng, 2003).

The documented medicinal uses of *Boesenbergia* also reveal species-specific therapeutic roles, reflecting their diverse applications in both traditional and modern medicine. For example, *B. thorelii* is primarily used for allergy-related diseases (Madaka, 2013), and *B. baimaii* plays a role in treating flatulence and as a tonic in traditional medicine (Ragsasilp et al., 2022). Beyond medicinal use, certain species, such as *B. longiflora*, are valued in the cosmetic and spa industries, showcasing their potential for commercial applications (Theanphong et al., 2022). Furthermore, cultural and ethnopharmacological applications are evident in species like *B. stenophylla*, whose stems are historically used as charms to protect infants, alongside its pharmacological uses for treating food poisoning and convulsions (Jing et al., 2010). Despite the extensive knowledge documented, the table also highlights gaps in the current understanding of certain species, such as *B. pulchella* and *B. kingii*, which are primarily associated with treating gastrointestinal disorders.

Table 1.1

Medicinal uses of several Boesenbergia species

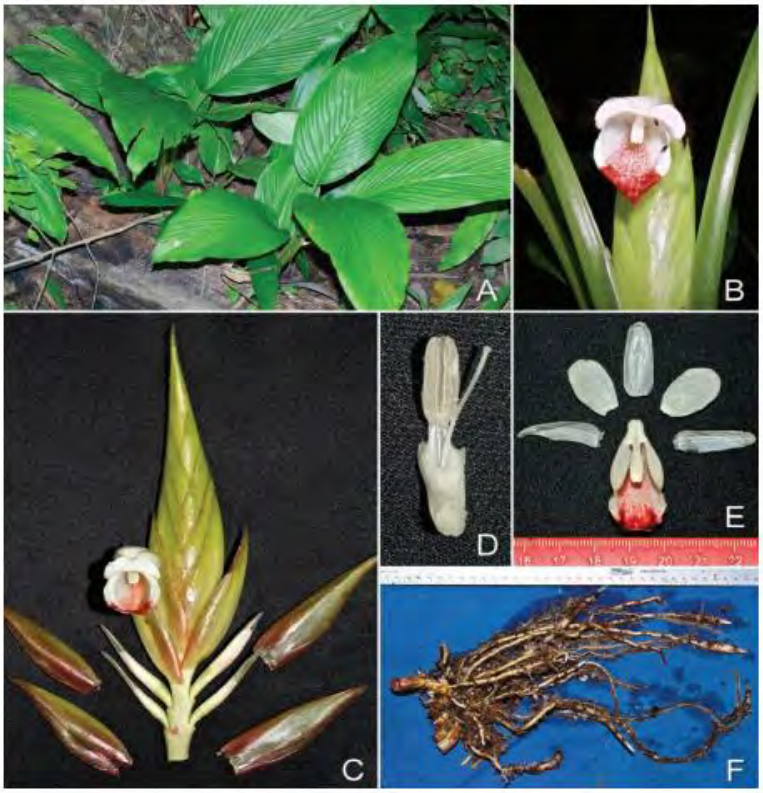
Species	Part	Medicinal Uses
B. armeniaca	Rhizome	Offers a pleasant odour and known to cause physiological effects such as inducing calmness and enhancing sleep (Elsharif et al., 2015)
B. rotunda	Rhizome	Medication of dental inflammation, treating dyspepsia, prevention and treatment of periodontitis (Bailly, 2022)
	Fingerroot	Reduce cholesterol and osteoporosis (Peltzer & Pengpid, 2019)
	Root	To cure flatulence, muscle pain, dyspepsia, carminative stomachache, gout, gastrointestinal disorders, and rheumatism (Saenboonruang, 2018)
B. longiflora	Roots	Natural components for spa, cosmetic and pharmaceutical products (Theanphong et al., 2022)
	Rhizome	Utilized in the remedy for inflammatory bowel illness and abscess (Sudsai et al., 2014)
B. quangngaiensis	Rhizome	Applied as an antimicrobial agent (Huong et al., 2021)
B. parvula	Rhizome	Cure for stomachache (Ragsasilp et al., 2022)
B. isanensis	Rhizome	Cure for stomachache and skin disease (Saensouk & Saensouk, 2020)

Species	Part	Medicinal Uses
B. pandurata	Rhizome	Medication of inflammation in women uterus and vaginal infection (Heyne, 1987)
		Utilized as an aphrodisiac, and cure for colic disorder (Tuchinda et al., 2002)
B. baimaii	Rhizome	Utilized to treat flatulence, laxatives, tonics, and stomachache (Ragsasilp et al., 2022)
B. pulchella	Leaves	Utilized for the healing process during confinement (Kassim et al., 2016)
B. kingii	Rhizome	Used to treat stomach discomfort, ulcers, inflammatory bowel disease, ulcerative colitis, and dysentery (Chuakul & Boonpleng, 2003)
B. stenophylla	Stems	Utilized by local people as a charm to protect babies from ghosts in the past (Jing et al., 2010)
	Leaves	Medication for food sickness, vomiting, and congestion (Jing et al., 2010)
	Rhizome	Defense against convulsions, prevention of intoxication and cough relief (Jing et al., 2010).
B. thorelii	Rhizome	Treatment of allergy-related diseases (Madaka, 2013)
B. xiphostachya	Rhizome	Utilized as a laxative and flatulence remedy (Chuakul & Boonpleng, 2003)

Boesenbergia albosanguinea (Figure 1.1) has been selected in this study. The main habitats of this species are Thailand (Satun Province) and Malaysia (Langkawi, Pulau Langgun). It grows best on limestone outcrops near the sea in shady spots. It thrives on limestone outcrops in shaded areas close to the sea. In the populations found in Langkawi, the plants are typically shorter and less vigorous, characterized by narrower and shorter leaves. Although the leaf sheaths display a red colour, this hue does not carry over to main axis. The inflorescences appear more tubular and are less compacted, with bracts that are marginally elongated and narrower; on some plants, these bracts may even bend away from the rachis. While the shape and colour of the flowers closely resemble those of the Thai populations, they are somewhat smaller in size (Mood et al., 2016).

Figure 1.1

Boesenbergia albosanguinea (Ridl.) Loes.



1.4 Problem Statement

The *Boesenbergia* genus, native to tropical regions like Malaysia, Indonesia, and Thailand, has captured scientific interest due to its rich repository of bioactive compounds. These compounds, including flavonoids, phenolics, terpenoids, and alkaloids, are celebrated for their diverse chemical structures and bioactivities. Numerous studies have revealed that species within this genus possess remarkable therapeutic properties, such as antioxidant, antibacterial, antifungal, and anti-inflammatory activities. These properties underscore the medicinal potential of *Boesenbergia* species, making them a valuable resource in traditional and modern medicine.

Despite extensive research on species such as *B. rotunda*, *B. pulcherrima*, *B. stenophylla*, and *B. armeniaca*, a significant gap remains in the exploration of *B. albosanguinea*. This species has yet to be reported on its essential oil composition, phytochemical profile, and biological activities. This lack of information is striking, considering the historical use of the *Boesenbergia* genus for treating various ailments and its well-documented potential as a source of bioactive compounds. Understanding the unique phytochemical and biological characteristics of *B. albosanguinea* is crucial to unlocking its potential as a medicinal plant.

The absence of research on *B. albosanguinea* not only limits our understanding of its therapeutic applications but also prevents the identification of potentially novel bioactive compounds that may be exclusive to this species. Investigating the essential oils of *B. albosanguinea* could reveal unique volatile compounds with specific

applications in pharmaceuticals, cosmetics, or agriculture. Similarly, a comprehensive study of its phytochemistry might uncover new flavonoids, terpenoids, or alkaloids with significant biological activities, thereby contributing to drug discovery and development.

Furthermore, exploring the biological activity of *B. albosanguinea* could validate its traditional medicinal uses and provide scientific evidence for its pharmacological applications. For instance, testing its extracts against microbial pathogens, oxidative stress, or inflammatory conditions could position *B. albosanguinea* as a viable candidate for developing natural therapies. Additionally, understanding its bioactivity could expand its application to industries beyond healthcare, such as food preservation or crop protection, where natural bioactive compounds are increasingly in demand.

Given the increasing global focus on natural products as safer alternatives to synthetic chemicals, the investigation of *B. albosanguinea* is both timely and essential. Comprehensive studies addressing its essential oil constituents, phytochemistry, and bioactivities are necessary to harness the full potential of this underexplored species. Such research would not only fill existing knowledge gaps but also strengthen the role of the *Boesenbergia* genus as a cornerstone in natural product research and sustainable development.

1.5 Objectives of Study

The objectives of the study are:

1. To investigate the chemical compositions of the essential oil of the rhizomes of *B. albosanguinea*.
2. To isolate and characterized the phytochemicals from the rhizome extracts of *B. albosanguinea* using IR, NMR, and MS.
3. To evaluate the antioxidant and anti-inflammatory activities of the crude extracts and isolated phytochemicals of *B. albosanguinea*.

1.6 Scopes of Study

This research begins with the isolation of the essential oil from the rhizomes of *B. albosanguinea* using hydrodistillation. Then, the chemical composition of the essential oil is analyzed using a combination of advanced techniques. gas chromatography- flame ionization detection (GC-FID) is used to separate and quantify the volatile components, while gas chromatography-mass spectrometry (GC-MS) helps identify the specific molecular structures of these components. Additionally, Kovats Indices are applied to further identify compounds based on their retention times in the chromatographic column, comparing them with known standards.

The next step of the study focuses on isolating and identifying the major constituent of the essential oil. This involves the separation of the most abundant or bioactive compound for further study. The isolation methods used are likely to include

distillation, extraction, or crystallization. Once isolated, the compound undergoes a detailed identification process, where its chemical structure is determined using several spectroscopic techniques. Infrared Spectroscopy (IR) provides insight into the functional groups present in the compound by measuring how it absorbs infrared light. Nuclear Magnetic Resonance (NMR), both 1D and 2D, is employed to study the interactions of nuclei in the molecule, offering detailed structural information. Finally, Mass Spectrometry (MS) is used to determine the molecular mass and confirm the structure of the compound.

In the following phase of the study, the phytochemicals are separated from dried rhizome extracts of *B. albosanguinea* using chromatographic methods. Preparative Thin Layer Chromatography (PTLC) is first used to separate the compounds based on their chemical properties. Then, Column Chromatography is employed to achieve further separation by passing the extract through a column packed with a stationary phase. This process allows for the isolation of distinct compounds that can then be analyzed for their chemical structure. Once isolated, the chemical structures of these compounds are confirmed by comparing the spectroscopic data (IR, NMR, MS) with existing literature on similar compounds.

The final phase of the research involves testing the biological activities of the isolated compounds. The DPPH Free Radical Scavenging Test is used to evaluate the antioxidant potential of the compounds by measuring their ability to neutralize free radicals. This is important because antioxidants play a key role in protecting cells from oxidative stress. The Lipoxygenase (LOX) Inhibitory Assay assesses the anti-inflammatory potential of the compounds by measuring their ability to inhibit

lipoxygenase, an enzyme involved in the production of inflammatory mediators. These biological activity tests help determine the potential therapeutic applications of the compounds in conditions related to oxidative stress and inflammation.

Overall, this study combines chemical analysis, structural identification, and biological testing to explore the potential of *B. albosanguinea*'s essential oil and phytochemicals for medicinal applications, with a particular focus on antioxidant and anti-inflammatory effects.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to Essential Oils

Nature offers humanity a wealth of medicinal resources that benefit all aspects of life. These active ingredients are derived from nature itself, particularly from aromatic plants and herbs, and are commonly referred to as "essential oils," "essences," or "volatile oils." Plants rely on essential oils for reproduction, growth, and protection against insects or predators. For centuries, humans have recognized their therapeutic properties, making essential oils some of the oldest and most potent healing agents. Generations have utilized these oils to improve, soothe, and energize both body and mind. The Egyptians were among the earliest civilizations to use aromatic herbs and aromatherapy, which have a long history in healing; they burned aromatic oils as offerings to the gods and for embalming. In ancient Greece and Rome, perfumed oils were used as sacraments during worship rituals (Wells & Billot, 1988).

Essential oils play a significant role in traditional and folk medicine worldwide. Ancient civilizations such as Egypt, Greece, and Rome are renowned for their use of essential oils. Traditional Egyptian medicine relied on aromatic plant extracts for both physical and mental health. In the 1930s, innovative clinics in Europe began incorporating essential oils into treatments for trauma and severe illnesses such as tuberculosis and diabetes (Gnatta et al., 2016). Hippocrates, the renowned Greek physician known as the "Father of Medicine," meticulously recorded the therapeutic properties of around 300 herbs, including thyme, saffron, marjoram, cumin, and peppermint (Smith, 2024; Bhavaniramya et al., 2019).

Today, essential oils are widely used in complementary medicine to promote physical, emotional, and psychological well-being, reflecting their broad appeal and versatile applications. Numerous essential oils exhibit antibacterial, fungicidal, relaxing, stimulant, and antidepressant properties, making them highly effective medicinal agents. Their medicinal characteristics enable their use in treating various infections caused by both pathogenic and non-pathogenic agents (Hamid et al., 2011). Inhalation of essential oils or their individual volatile terpenes plays a crucial role in regulating the central nervous system. For example, in Chinese folk medicine, epilepsy treatment involves using aroma inhibitors derived from storax pill essential oil and pre-inhalation of *Acorus gramineus* rhizome essential oils (Koo et al., 2004).

Essential oils reflect the unique characteristics of growing plants that support every aspect of their existence and are widely used in aromatherapy. Synthetic materials that mimic the appearance and scent of essential oils lack the therapeutic qualities of the natural product. For medicinal purposes, only purified essential oils

derived from natural plant essences extracted via steam distillation, expression, or maceration should be used. Chemical reproductions of natural oils are ineffective for medicinal applications, regardless of their pleasant scent. The chemical composition of natural plants is the source of the healing properties found in essential oils (Sibley, 2003).

Essential oils are secondary metabolic products derived from plants and are stored in specific cells such as oil cells, glands, and trichomes (Pengelly, 2004). They are extracted from various parts of aromatic plants, including the rhizome, leaf, root, flower, stem, fruit, bark, and seeds. These oils are complex, hydrophobic mixtures containing volatile compounds. Typically, they are oily liquids at room temperature with distinct aroma and flavor. While insoluble in water, they dissolve readily in organic solvents (Maurya et al., 2021).

Extraction methods for essential oils vary depending on the plant material and desired outcome. Steam distillation involves passing steam over plant material, causing the release of oil, which is then condensed and separated from water due to density differences (Guenther, 1948). Water distillation, often used for delicate materials such as flower petals, immerses the plant material in water to prevent clumping and reduce heat damage, though it requires careful monitoring to avoid scorching (McMahon, 2004). Cold expression is specific to citrus fruits, where volatile oils are extracted by piercing or pressing the rind, separating the oil from the juice without using heat or chemicals, thus preserving the oil's natural quality. However, sourcing citrus oils from organically grown fruits is essential to avoid contamination from pesticides or herbicides (Medeiros, 2018). Carbon dioxide

extraction is an eco-friendly technique that uses pressurized CO_2 as a solvent, producing extracts with complete aromatic profiles and minimal chemical changes (Ehlers et al., 2000). Traditional methods such as enfleurage and maceration, though less commonly used today, are still applied to premium flowers like jasmine, yielding highly fragrant extracts (Manniche, 1999). Modern techniques like supercritical fluid extraction are gaining popularity for their efficiency and reduced environmental impact. Analytical methods such as gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS) are widely used to identify the chemical composition of essential oils (Baharum et al., 2010). Most essential oils are composed of terpenoid and phenylpropanoid derivatives, with bioactive compounds like ketones, aldehydes, esters, and alcohols contributing to their distinctive aroma, flavor, and therapeutic properties (Baldim et al., 2019).

Essential oils are used as raw materials in various industries, including fragrances, cosmetics, aromatherapy, phytotherapy, spices, nutrition, and pesticides (Jirovetz, 2000). The market for essential oils is growing rapidly, with increasing demand anticipated in the coming years. Applications in plant and animal industries, perfumery, cosmetics, food, and pharmaceuticals continue to expand. The rising popularity of essential oils reflects their versatile uses and increasing consumer interest. Ongoing research explores their potential applications across various bodily processes. Table 2.1 shows the medicinal uses of several common essential oils.

Table 2.1

Medicinal uses of several common essential oils

Essential oils	Species	Uses
Ajwain	*Trachys-permum ammi*	Remedial agent for flatulence, atonic dyspepsia, and diarrhea (Ranjan et al., 2012)
Anise	*Pimpinella anisum*	Utilized in the industry as a flavouring and perfuming agent (Koriem, 2015)
Basil	*Ocimum basilicum*	Relieve migraines, coughing, vomiting, and renal problem (Simon et al., 1999)
Bay laurel	*Laurus nobilis*	Utilized orally to treat the symptoms of gastrointestinal problems, such as epigastric bloating and flatulence (Qnais et al., 2012)
Bergamot	*Citrus bergamia*	Beneficial as an antimicrobial agent to facilitate wound healing (Navarra et al., 2015)
Birch	*Betula pendula*	Remedy for bone-related problems including arthritis and rheumatism (Rastogi et al., 2015)
Cedarwood	*Juniperus virginiana*	Treatment of wounds, rheumatism, bronchitis, cough, fungal infections, and hemorrhoids (Tümen et al., 2013)
Chamomile	*Matricaria chamomilla*	Medication for gastrointestinal disorders including diarrhea (Alanís et al., 2005)
Cinnamon	*Cinnamomum verum*	Enhanced flavour to the foodstuffs and quality of the food (Aparna et al., 2014)

Essential oils	Species	Uses
Clary sage	*Salvia sclarea*	Medication for sore throat, ulcers, intestinal spasms, diarrhea, and gynecological diseases (Ozdemir & Alpinar, 2015)
Clove	*Syzygium aromaticum*	Medication to heal injuries and burns, as well as to ease pain in oral health and treat discomfort in the teeth (Batiha et al., 2020)
Cumin	*Cuminum cyminum*	To cure ulcers, as well as to reduce coughing and irritation (Shivakumar et al., 2010)
Eucalyptus	*Eucalyptus radiata*	Utilized to reduce colds, fever, and bronchial infections (Jeane et al., 2003)
Frankincense	*Boswellia carterii*	Treatment for rheumatic and ulcerative colitis (Banno et al., 2006)
Ginger	*Zingiber officinale*	Used in the treatment of joint stiffness and pain as well as a tonic for the uterus, brain, and stomach (Sritoomma et al., 2014)
Jasmine	*Jasminum sambac*	Used as a fragrance in skin care products (Abdoul-Latif et al., 2010)
Lavender	*Lavandula angustifolia*	Used in aromatherapy and massage (Buchbauer et al., 1991)
Lemongrass	*Cymbopogon citratus*	Used to improve digestion, nausea, and menstruation problems such as headaches, muscle cramps, and rheumatism (Shah et al., 2011)

Essential oils	Species	Uses
Moringa	*Moringa oleifera*	Useful for treating malaria, parasite infections, arthritis, inflammation, wounds, skin conditions, genito-urinary problems, high blood pressure, and diabetic (Leone et al., 2015)
Mugwort	*Artemisia vulgaris*	Treatment of diabetes and epilepsy, and in combination with psychoneurosis, irritability, insomnia, and anxiety (Walter & Memory, 2003)
Neroli	*Citrus aurantium*	Treating gastrointestinal disorders, tachycardia, and rheumatism, to minimize central nervous system disorders (Moraes et al., 2009)
Patchouli	*Pogostemon cablin*	Antifungal agents in the food industry to preserve root vegetables during storage and to extend the shelf life of baked foods (Galovičová et al., 2022)
Peppermint	*Mentha piperita*	Used for treating certain stomach disorders such as indigestion and cough (Alankar, 2009)
Roman chamomile	*Anthemis nobilis*	Treatment of fever, inflammation, menstrual disorders, insomnia, ulcers, wounds, gastrointestinal disorders, and hemorrhoids (Ali et al., 2015)
Rose geranium	*Pelargonium graveolens*	Relieving discomfort caused by post-herpetic neuralgia, treating diarrhea, hemorrhoids, and inflammatory (Peterson et al., 2006)

Essential oils	Species	Uses
Rosemary	*Rosmarinus officinalis*	Treatment of diabetes, asthma, gastrointestinal issues, and inflammation (Bakirel et al., 2008)
Sandalwood	*Santalum album*	Treatment of bleeding piles, diarrhea, eye infections, hemorrhage, hiccoughs, poisoning, urticaria, and vomiting (Desai & Hirenath, 1991)
Spearmint	*Mentha spicata*	Treatment of colds, gastralgia, hemorrhoids, and stomachache (Tetika et al., 2013)
Tarragon	*Artemisia dracunculus*	Treatment of fever, diabetes, and bacterial or parasitic infections (Raeisi et al., 2012)
Tea tree	*Melaleuca alternifolia*	Used for coughs and colds through inhalation and skin diseases (Çalişkan & Özfenerci, 2018)
Vetiver	*Vetiveria zizanioides*	Treatment of ulcer, fever, epilepsy, rheumatism, headache, and malaria (Luqman et al., 2005)
Wintergreen	*Gaultheria procumbens*	Relieve back pain, cold symptoms, colitis, migraines, fever, skin conditions, throat discomfort and decaying teeth (Chevallier, 1996)
Ylang-ylang	*Cananga odorata*	To treat depression and nervousness (Saedi & Crawford, 2006)
Cajuput	*Melaleuca cajuputi*	It is an excellent antiseptic for respiratory tract by minimising feverish temperature and release flu toxin from the body.

Essential oils	Species	Uses
Juniper	*Juniperus communis*	It is well known for its detoxifying properties as it clears the body toxin, particularly when too much alcohol been consumed.
Sweet marjoram	*Origanum majorana*	It is known for its soothing effect on the digestive system, relieving cramps, indigestion, constipation and flatulence, and aiding the clearing of toxins.
Fennel	*Foeniculum vulgare*	It is an excellent tonic for the digestive system, working on ailments such as indigestion caused by stress, nausea, vomiting and colic.
Coriander	*Coriandrum sativum*	It has a soothing, warming effect on stomach, relieving wind and stomach cramps.
Ravensara	*Ravensara aromatica*	It is a sedative oil that helps build up the immune system, figh infection and ease coughs.
Niaouli	*Melaleuca quinquenervia*	It heals skin eruptions, ulcers and is useful for washing infected wounds
Angelica	*Angelica archangelica*	Good for fatigue, migraine, stress-related disorders, muscular aches and pains, accumulation of toxins and dull, congested skin.
Benzoin	*Styrax benzoin*	Tonic for the lungs, and beneficial for respiratory disorders, bronchitis, asthma, coughs, colds and laryngitis.

Essential oils	Species	Uses
Citronella	*Cymbopogon nardus*	Its deodorizing and stimulating properties refresh sweaty, tired feet.
Elemi	*Canarium kuzonicum*	Its antiseptic properties good for infected cuts and wounds.
Galbanum	*Ferula galbaniflua*	Good for aches and pains, and for poor circulation.
Immortelle	*Helichrysum angustifolia*	Its cell-regenerating properties make it useful for scars, acne, dermatitis and abscesses.
Manuka	*Leptospermum scoparium*	Beneficial for rheumatoid arthritis, acne, dermatitis and allergic rashes.
Palmarosa	*Cymbopogon martinii*	Vitalizes and regenerates skin cells. Antiseptic, hydrating and soothing for the skin.
Yarrow	*Achillea millefolium*	Helps with injuries, skin conditions, hypertension, sleep disorders, fevers, colds, and coughs.

Despite these concerns, further toxicological research is necessary to fully understand the interactions between essential oils and medications, as well as their effects on various organ systems. With proper safety measures and dosage adjustments, essential oils can provide a valuable alternative to synthetic chemical preparations.

2.2 Chemical Composition of *Boesenbergia* Essential Oils

Numerous studies have documented the chemical constituents of *Boesenbergia* essential oils from 2001 to 2024. Table 2.2 provides details of nine different *Boesenbergia* species: *B. pandurata*, *B. rotunda*, *B. curtisii*, *B. armeniaca*, *B. pulcherrima*, *B. quangngaiensis*, *B. plicata*, *B. longiflora*, and *B. stenophylla*, along with their identified major components from various localities around the world.

Reports on *Boesenbergia* essential oils have primarily originated from Malaysia (8 studies) and Thailand (6 studies), with additional studies from Indonesia and India (2 each), as well as Cambodia and Vietnam (1 each). The oils were mainly extracted from rhizomes, roots, and leaves, with *B. pandurata* and *B. rotunda* being the most extensively studied species. The diverse major components of *Boesenbergia* oils contribute to their unique properties. For instance, geraniol **(16)** and camphor **(42)** were the most frequently reported components. Geraniol **(16)** accounted for 26.0% of the oil from Indonesian *B. pandurata* (Jantan et al., 2001), while camphor **(42)** was found in significant amounts (57.9%) in Malaysian *B. pandurata* (Sukari et al., 2008).

Monoterpenes are prevalent in most *Boesenbergia* essential oils, with 1,8-cineole (45) being a hallmark compound known for its fresh, camphor-like scent. This compound, reported in eight studies, reached its highest concentration (67.8%) in *B. rotunda* from Thailand. It is associated with anti-inflammatory and respiratory benefits (Theanphong et al., 2021). Other oxygenated monoterpenes, such as geraniol **(16),** camphor **(42)**, and methyl (*E*)-cinnamate **(33**), were also present in various species (Ahmad & Jantan, 2003).

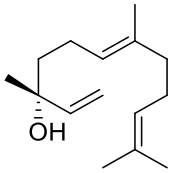
Sesquiterpenes are another significant group of compounds found in *Boesenbergia* essential oils. Analysis of *B. armeniaca* revealed that its leaf oil contained a high proportion of oxygenated sesquiterpenes (47.95%), followed by sesquiterpene hydrocarbons (20.69%), including (E)-nerolidol **(1)**, β-caryophyllene **(3)**, and β-bisabolene **(5)**. In contrast, *B. stenophylla* oils contained sesquiterpene hydrocarbons (6.44%) and oxygenated sesquiterpenes (0.51%) (Nor & Ibrahim, 2018). The rhizome oil of *B. stenophylla* was particularly rich in sesquiterpenes such as δ-elemene **(38)**, β-elemene **(11)**, α-santalene **(40)**, spathulenol **(7)**, α-humulene **(36)**, and kaur-16-ene **(37)** (Ahmad & Jantan, 2003). Other notable compounds include α-selinene **(15)** (Omar et al., 2015) and germacrene D **(14)** (Huong et al., 2021).

Each species displays a distinct chemical profile. For example, *B. armeniaca* is rich in (E)-nerolidol **(1)** (42.5%) and linalool **(2)** (11.6%) in Malaysian leaves, while *B. pulcherrima* from Indian rhizomes contains predominately palmitic acid **(6)** (75.5%). Variations in chemical profiles also occur within the same species depending on plant part and location. For instance, in *B. longiflora*, β-phellandrene **(22)** (15.2%) dominates in the root oil, while γ-terpinene **(26)** (26.7%) is prominent in the rhizome oil from Thailand. Rhizomes generally yield higher essential oil percentages compared to leaves. For example, the rhizomes of *B. stenophylla* yield 3.39%, whereas its leaves yield only 0.08%. Additionally, the dominant compounds vary; *B. pandurata* shows high camphor levels (57.9%) in Malaysian rhizomes but geraniol (24.6%) predominates in other regions. These variations emphasize the need for optimized extraction methods and careful selection of plant parts based on the intended application.

Recurring compounds across species and regions highlight the prevalence of certain bioactive components within the *Boesenbergia* genus. Key compounds such as camphor **(42)**, 1,8-cineole **(45)**, and geraniol **(16)**, found in *B. pandurata*, *B. rotunda*, and *B. stenophylla*, are notable for their antimicrobial, anti-inflammatory, and antioxidant properties. Additionally, fatty acids like palmitic acid and linolenic acid, found in *B. pulcherrima* and *B. plicata*, add nutritional and therapeutic value. Terpenoids such as (E)-nerolidol **(1)** and β-caryophyllene **(3)**, present in *B. armeniaca* and *B. quangngaiensis*, further demonstrate the pharmaceutical potential of the genus.

The table also underscores how regional and environmental factors influence the chemical composition of essential oils. For example, *B. rotunda* from Thailand is rich in (Z)-β-ocimene **(52)** (36.7%), while Malaysian samples contain higher nerol concentrations (39.5%). Similarly, *B. pandurata* shows significant variations in camphor content, ranging from 57.9% in Malaysian rhizomes to 16.9% in Thailand. These differences highlight the influence of climate, soil, and ecological factors on the phytochemical profiles of *Boesenbergia* species.

The identified constituents in *Boesenbergia* essential oils hold significant potential for diverse applications. Compounds like camphor **(42)**, 1,8-cineole **(45)**, and geraniol **(16)** are particularly valuable in pharmaceuticals due to their anti-inflammatory and antimicrobial properties. Fatty acids like palmitic acid **(6)** offer nutritional benefits, while volatile terpenoids such as γ-terpinene **(26)** and β-caryophyllene **(3)** show promise in agriculture as natural pest repellents. Additionally, aromatic compounds like linalool and citronellyl formate **(10)** make these oils suitable for cosmetics and aromatherapy.



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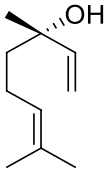

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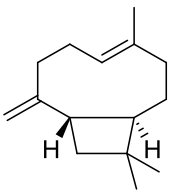

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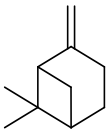

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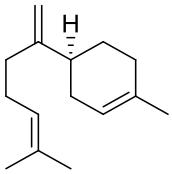

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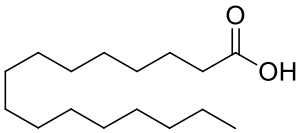

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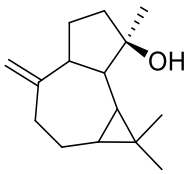

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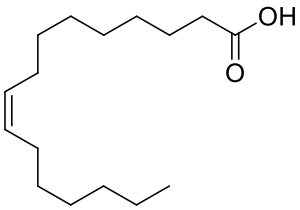

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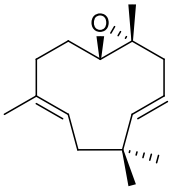

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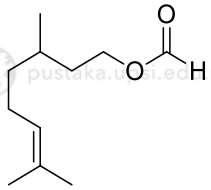

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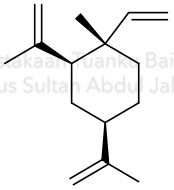

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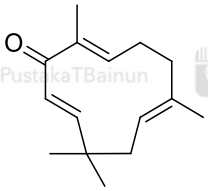

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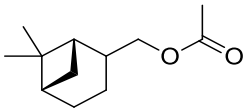

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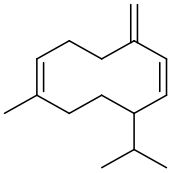

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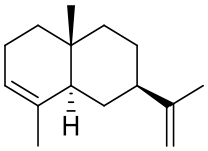

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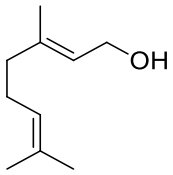

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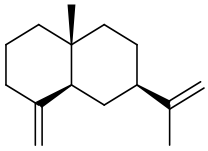

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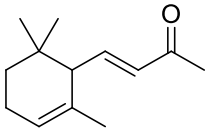

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Table 2.2

Major components identified from several Boesenbergia essential oils

Species	Locality (Parts)	Total, %	Yield %	Major components
B. armeniaca	Malaysia (Leaves)	38, 99.2%	0.01	(*E*)-Nerolidol (1) (42.5%), linalool (2) (11.6%), β-caryophyllene (3) (6.2%), β-pinene (4) (4.8%), β-bisabolene (5) (3.9%) (Nor & Ibrahim, 2018)
B. pulcherrima	India (Rhizome)	21, 100%	NM	Palmitic acid (6) (75.5%), spathulenol (7) (7.5%), palmitoleic acid (8) (5.5%), humulene epoxide II (9) (3.0%), citronellyl formate (10) (3.0%) (Byahatti & Thangadurai, 2019)
B. quang-ngaiensis	Vietnam (Rhizome)	47, 86.2%	0.16	β-Elemene (11) (18.4%), zerumbone (12) (11.4%), myrtenyl acetate (13) (10.6%), germacrene D (14) (3.9%), β-caryophyllene (3) (3.7%) (Huong et al., 2021)
B. plicata	Malaysia (Leaves)	18, 73.4%	NM	(*E*)-Nerolidol (1) (15.7%), α-selinene (15) (9.2%), geraniol (16) (6.6%), β-selinene (17) (5.2%), α-ionone (18) (5.0%) (Omar et al., 2015)

Species	Locality (Parts)	Total, %	Yield %	Major components
B. plicata	Malaysia (Leaves)	18, 93.6%	NM	Linolenic acid (19) (35.2%), palmitic acid (6) (32.0%), β-pinene (4) (11.4%), phytol (20) (5.9%), α-pinene (21) (2.9%) (Omar et al., 2015)
B. longiflora	Thailand (Root)	53, 97.7%	0.32	β-Phellandrene (22) (15.2%), α-phellandrene (23) (14.7%), δ-3-carene (24) (8.6%), β-terpinene (25) (8.5%), α-pinene (21) (7.7%) (Theanphong et al., 2022)
	Thailand (Rhizome)	52, 97.3%	0.36	γ-Terpinene (26) (26.7%), γ-elemene (27) (20.8%), α-terpinene (28) (9.5%), β-phellandrene (22) (7.5%), α-pinene (21) (7.3%) (Theanphong et al., 2022)
	India (Rhizome)	13, 99.5%	2.3	Longipinocarvone (29) (81.6%), β-caryophyllene (3) (3.4%), 9-epi-β-caryophyllene (30) (1.5%), β-patchoulene (31) (2.9%), borneol (32) (2.3%) (Kar et al., 2015)
B. stenophylla	Malaysia (Leaves)	23, 100%	0.08	Methyl (*E*)-cinnamate (33) (83.1%), β-pinene (4) (4.8%), α-caryophyllene (34) (3.5%) (Nor & Ibrahim, 2018)

Species	Locality (Parts)	Total, %	Yield %	Major components
B. stenophylla	Malaysia (Leaves)	28, 92.5%	1.63	Methyl (*E*)-cinnamate (33) (49.9%), β-calacorene (35) (7.7%), spathulenol (7) (5.6%), α-humulene (36) (5.3%), kaur-16-ene (37) (3.9%) (Ahmad & Jantan, 2003)
	Malaysia (Rhizome)	28, 94.3%	3.39	Methyl (*E*)-cinnamate (33) (53.4%), δ-elemene (38) (7.4%), γ-muurolene (39) (5.1%), α-santalene (40) (3.1%), germacrene B (41) (2.1%) (Ahmad & Jantan, 2003)
B. pandurata	Malaysia (Rhizome)	32, 68.1%	NM	Camphor (42) (57.9%), geraniol (16) (6.2%), *trans*-2-hexanyl-n-propionate (43) (5.5%), 9-epi-β-caryophylene (30) (3.4%), cyclohexyl-n-propionate (44) (2.7%) (Sukari et al., 2008)
	Malaysia (Rhizome)	54, 92.3%	3.30	Camphor (42) (32.1%), geraniol (16) (16.2%), 1,8-cineole (45) (13.9%), camphene (46) (5.8%), methyl (*E*)-cinnamate (33) (5.8%) (Jantan et al., 2001)

Species	Locality (Parts)	Total, %	Yield %	Major components
B. pandurata	Malaysia (Rhizome)	54, 71.7%	2.42	Geraniol (16) (24.6%), camphor (42) (24.1%), (*E*)-β-ocimene (47) (19.0%), 1,8-cineole (45) (8.0%), camphene (46) (6.0%) (Jantan et al., 2001)
	Thailand (Rhizome)	54, 92.7%	2.62	(*E*)-β-ocimene (47) (22.8%), geraniol (16) (20.8%), camphor (42) (16.9%), 1,8-cineole (45) (7.8%), camphene (46) (5.4%) (Jantan et al., 2001)
	Thailand (Rhizome)	7, 93%	NM	(*E*)-β-Ocimene (47) (27.0%), camphor (42) (24.0%), 1,8-cineole (45) (17.0%), geraniol (16) (11.0%), camphene (46) (8.0%) (Phanthong et al., 2013)
	Thailand (Rhizome)	8, 100%	0.26	γ-Terpinene (26) (44.0%), geraniol (16) (20.6%), 6-camphenone (48) (18.7%), 1,8-cineole (45) (12.8%), methyl (*E*)-cinnamate (33) (2.1%) (Natta et al., 2008)
	Indonesia (Rhizome)	54, 96.6%	2.94	Geraniol (16) (26.0%), (*E*)-β-ocimene (47) (23.7%), camphor (42) (16.1%), 1,8-cineole (45) (7.5%), camphene (46) (5.5%) (Jantan et al., 2001)

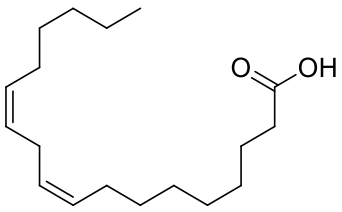
Species	Locality (Parts)	Total, %	Yield %	Major components
B. pandurata	Indonesia (Rhizome)	21, 98.7%	0.96	(*Z*)-β-Ocimene (52) (19.3%), camphor (42) (18.8%), geraniol (16) (15.5%), 1,8-cineole (45) (12.0%), (*E*)-β-ocimene (47) (9.1%) (Oktavianawati et al., 2018)
	Indonesia (Rhizome)	21, 79.6%	0.70	(*E*)-β-ocimene (47) (21.5%), camphor (42) (20.3%), 1,8-cineole (45) (10.1%), Z-citral (49) (9.0%), methyl (*E*)-cinnamate (33) (7.6%) (Oktavianawati et al., 2018)
B. rotunda	Malaysia (Rhizome)	19, 100%	NM	Nerol (50) (39.5%), camphor (42) (36.0%), 1,8-cineole (45) (9.4%), methyl (*E*)-cinnamate (33) (6.8%), α-fenchene (51) (2.0%) (Baharudin et al., 2015)
	Malaysia (Rhizome)	33, 89.4%	3.30	Camphor (42) (32.1%), geraniol (16) (16.2%), 1,8-cineole (45) (13.9%), methyl (*E*)-cinnamate (33) (5.8%), camphene (46) (5.8%) (Jantan et al., 2008)

Species	Locality (Parts)	Total, %	Yield %	Major components
B. rotunda	Thailand (Rhizome)	24, 100%	NM	(*Z*)-β-Ocimene (52) (36.7%), geraniol (16) (25.2%), camphor (42) (14.9%), 1,8-cineole (45) (8.9%), camphene (46) (4.4%) (Apinundecha et al., 2023)
	Thailand (Root)	20, 96.7%	5.98	δ-3-Carene (24) (35.2%), camphor (42) (28.0%), methyl (*E*)-cinnamate (33) (15.1%), sabinene (53) (7.1%), citral (54) (3.0%) (Suwannayod et al., 2019)
	Thailand (Root)	54, 99.1%	0.18	Camphor (42) (22.3%), geraniol (16) (18.4%), camphene (46) (16.0%), 1,8-cineole (45) (9.4%), (*E*)-β-ocimene (47) (7.8%) (Theanphong et al., 2021)
	Thailand (Rhizome)	54, 97.9%	0.20	Camphor (42) (41.1%), (*Z*)-β-ocimene (52) (16.2%), 1,8-cineole (45) (13.1%), geraniol (16) (9.3%), camphene (46) (6.8%) (Theanphong et al., 2021)
	Thailand (Root)	54, 98.7%	0.22	(*Z*)-β-Ocimene (52) (27.7%), camphor (42) (20.0%), geraniol (16) (16.2%), 1,8-cineole (45) (13.8%), camphene (46) (6.3%) (Theanphong et al., 2021)

Species	Locality (Parts)	Total, %	Yield %	Major components
B. rotunda	Thailand (Rhizome)	54, 99.2%	0.26	Camphor (42) (25.7%), 1,8-cineole (45) (20.1%), (*Z*)-β-ocimene (52) (17.2%), geraniol (16) (11.8%), camphene (46) (6.8%) (Theanphong et al., 2021)
	Thailand (Root)	54, 98.2%	0.19	1,8-Cineole (45) (67.8%), β-caryophyllene (3) (7.5%), p-cymene (55) (4.8%), limonene (56) (3.6%), camphor (42) (2.5%) (Theanphong et al., 2021)
	Thailand (Rhizome)	54, 97.8%	0.21	1,8-Cineole (45) (65.8%), camphor (42) (7.9%), *p*-menth-8-en-1,2-diol (57) (7.6%), α-terpineol (58) (6.5%), geraniol (16) (4.4%) (Theanphong et al., 2021)
	Thailand (Rhizome)	22, 100%	0.86	(*E*)-β-Ocimene (47) (40.8%), geraniol (16) (16.8%), camphor (42) (16.0%), 1,8-cineole (45) (11.9%), camphene (46) (4.1%) (Sanguansermsri et al., 2024)
	Cambodia (Rhizome)	30 98.2%	0.34	(*Z*)-β-Ocimene (52) (27.6%), geraniol (16) (24.0%), camphor (42) (19.1%), 1,8-cineole (45) (5.5%), camphene (46) (5.4%) (Houdkova et al., 2018)

Species	Locality (Parts)	Total, %	Yield %	Major components
B. curtisii	Malaysia (Leaves)	20, 95.7%	0.14	(*E*)-Nerolidol (1) (16.4%), α-selinene (15) (7.9%), linalool (2) (7.5%), β-selinene (17) (7.0%), borneol (32) (6.2%) (Salleh et al., 2023)
B. xiphostachya	Thailand (Rhizome)	15, 78.7%	1.98	3,6-dimethoxy-2-ethylbenzaldehyde (59) (38.4%), elemicin (60) (15.4%), α-asarone (61) (12.9%), cis-isoelemicin (62) (4.3%), methyleugenol (63) (4.2%) (Suphrom et al., 2019)

*NM-not mentioned



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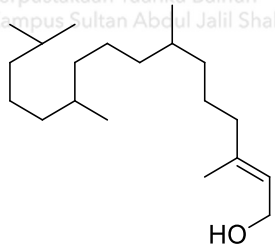

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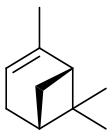

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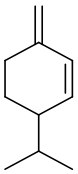

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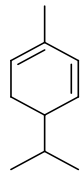

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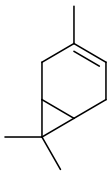

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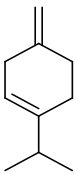

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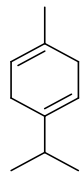

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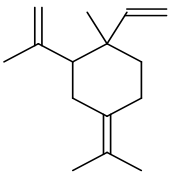

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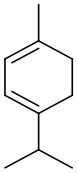

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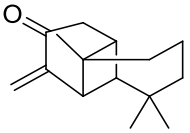

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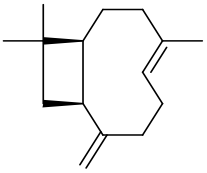

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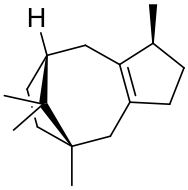

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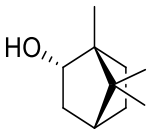

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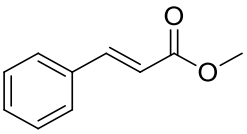

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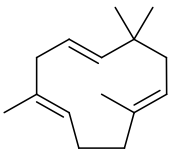

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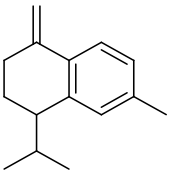

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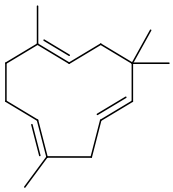

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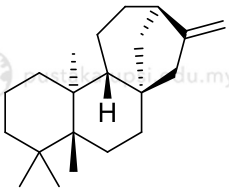

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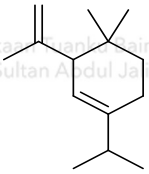

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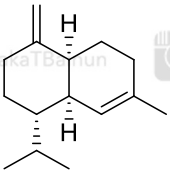

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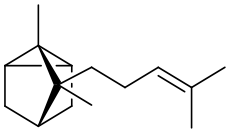

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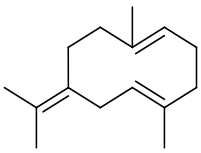

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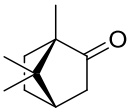

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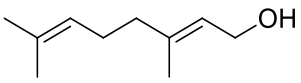

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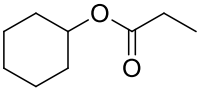

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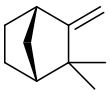

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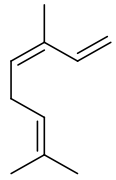

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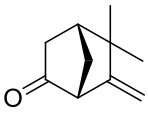

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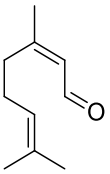

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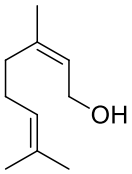

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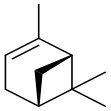

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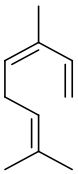

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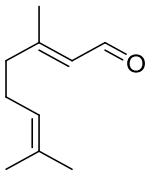

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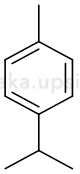

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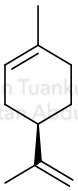

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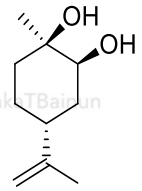

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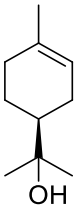

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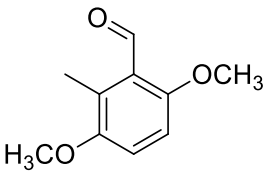

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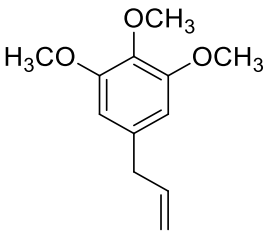

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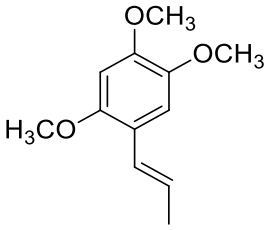

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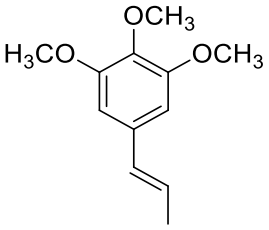

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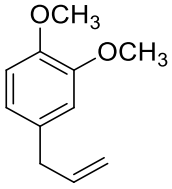

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2.3 Phytochemical Studies of *Boesenbergia* Species

2.3.1 Flavonoids

Flavonoids can be divided into 12 subgroups, including flavonols, aurones, proanthocyanidins, isoflavones, chalcones, flavanones, anthocyanins, phlobaphenes, dihydroflavonols, stilbenes, leucoanthocyanidins, and flavones. The formation of numerous flavonoid compounds is driven by acylation and other molecular polymerization processes (Liu et al., 2021). In *Boesenbergia* extracts, flavonoids represent the major group of phytochemicals isolated from various species, particularly *B. rotunda* and *B. pandurata*. The identified flavonoids are summarized in Table 2.3. Studies on flavonoids from *Boesenbergia* species have been documented from 2007 to 2023.

Six Boesenbergia species; *B. pandurata*, *B. rotunda*, *B. kingii*, *B. longiflora*, *B. pulchella*, and *B. armeniaca* have been reported to contain approximately 77 compounds (**64-140**). These include chalcones (44 compounds, **64-107**), flavanones (22 compounds, **108-129**), flavonols (6 compounds, **130-135**), flavones (4 compounds, **136-139**), and one flavonolignan **(140)**. The rhizome of *Boesenbergia* species is primarily utilized for flavonoid extraction, yielding compounds such as panduratin A **(72)**, cardamonin **(102)**, pinocembrin **(114)**, pinostrobin **(115)**, and alpinetin **(116)** (Widyananda et al., 2023).

The chalcones dominate the list, primarily isolated from the rhizomes of *B. pandurata* and *B. rotunda*. Notable representatives include (±)-krachaizin A **(64)**, (±)-

krachaizin B **(65)**, and (±)-panduratin A **(72)**, highlighting the prominence of *B. pandurata* in producing unique chalcones. The identification of structurally related compounds such as panduratin B1 and B2 **(79)**, and panduratin M **(87)** indicates a rich biosynthetic pathway for chalcones in this species. Flavanones also feature prominently, with compounds like (±)-pinocembrin **(114)** and (2R)-8-geranylpinostrobin **(108)** identified across multiple species, including *B. pandurata*, *B. rotunda*, and *B. kingii*. The geranyl-substituted flavanones demonstrate structural diversity within this group and underscore the biosynthetic versatility of *Boesenbergia*.

Flavonols, including rutin **(130)**, kaempferol **(131)**, and quercetin **(134)**, have been identified in *B. rotunda*, *B. pulchella*, and *B. armeniaca*. These compounds are renowned for their potent antioxidant activities, contributing significantly to the therapeutic potential of *Boesenbergia* extracts. Flavonols such as kaempferol **(131)** derivatives such as kaempferol-3,7,4'-trimethyl ether **(132)** and quercetin **(134)** are consistently found in rhizomes, further emphasizing the importance of this plant part for isolating bioactive flavonoids. Flavones, such as luteolin **(138)** and diosmin **(139)**, were predominantly isolated from *B. armeniaca* and *B. pulchella*, reflecting relatively less explored flavonoid profiles. Additionally, the identification of silybin **(140)**, a flavonolignan, in *B. rotunda* rhizomes highlights a unique phytochemical characteristic of this species, further diversifying its functional applications. Geographically, most flavonoids were isolated from rhizomes of species native to Southeast Asia, such as *B. pandurata* and *B. rotunda*.

Table 2.3

Flavonoids isolated from several Boesenbergia species

Compound	Species	Part	Reference
CHALCONES			
(±)-Krachaizin A (64)	*B. pandurata*	Rhizome	Morikawa et al., 2008
(±)-Krachaizin B (65)	*B. pandurata*	Rhizome	Morikawa et al., 2008
4-Hydroxynicolaioidesin A (66)	*B. pandurata*	Rhizome	Nguyen et al., 2017
Nicolaioidesin A (67)	*B. pandurata*	Rhizome	Nguyen et al., 2017
Nicolaioidesin B (68)	*B. pandurata*	Rhizome	Nguyen et al., 2017
	B. pandurata	Rhizome	Win et al., 2007
Nicolaioidesin C (69)	*B. pandurata*	Rhizome	Nguyen et al., 2017
Isopanduratin A1 (70)	*B. pandurata*	Rhizome	Nguyen et al., 2017
(1'*R*,2'*S*,6'*R*)-2-Hydroxyisopanduratin A (71)	*B. pandurata*	Rhizome	Win et al., 2007
(±)-Panduratin A (72)	*B. pandurata*	Rhizome	Tewtrakul et al., 2009
	B. rotunda	Rhizome	Widyananda et al., 2023
(±)-Hydroxypanduratin A (73)	*B. pandurata*	Rhizome	Nguyen et al., 2017
	B. pandurata	Rhizome	Morikawa et al., 2008
(±)-6-Methoxypanduratin A (74)	*B. pandurata*	Rhizome	Win et al., 2007
(±)-3''-Hydroxymethyl panduratin A (75)	*B. pandurata*	Rhizome	Nguyen et al., 2017
(±)-Isopanduratin A (76)	*B. rotunda*	Rhizome	Widyananda et al., 2023

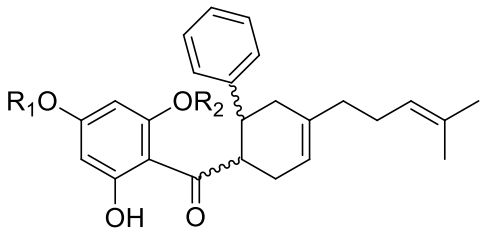
Compound	Species	Part	Reference
(±)-Isopanduratin A2 (77)	*B. pandurata*	Rhizome	Win et al., 2007
Panduratin C (78)	*B. pandurata*	Rhizome	Tewtrakul et al., 2009
Panduratin B1 & B2 (79)	*B. pandurata*	Rhizome	Win et al., 2008
Panduratin D (80)	*B. pandurata*	Rhizome	Win et al., 2008
Panduratin E (81)	*B. pandurata*	Rhizome	Win et al., 2008
Panduratin F (82)	*B. pandurata*	Rhizome	Win et al., 2008
Panduratin G (83)	*B. pandurata*	Rhizome	Win et al., 2008
Panduratin J (84)	*B. pandurata*	Rhizome	Nguyen et al., 2017
Panduratin K (85)	*B. pandurata*	Rhizome	Nguyen et al., 2017
Panduratin L (86)	*B. pandurata*	Rhizome	Nguyen et al., 2017
Panduratin M (87)	*B. pandurata*	Rhizome	Nguyen et al., 2017
Panduratin N (88)	*B. pandurata*	Rhizome	Nguyen et al., 2017
Panduratin O (89)	*B. pandurata*	Rhizome	Nguyen et al., 2017
Panduratin T (90)	*B. rotunda*	Rhizome	Nguyen et al., 2021
Panduratin U (91)	*B. rotunda*	Rhizome	Nguyen et al., 2021
Panduratin V (92)	*B. rotunda*	Rhizome	Nguyen et al., 2021
Panduratin W (93)	*B. rotunda*	Rhizome	Nguyen et al., 2021
Panduratin X (94)	*B. rotunda*	Rhizome	Nguyen et al., 2021
Panduratin Y (95)	*B. rotunda*	Rhizome	Nguyen et al., 2021
Boesenbergin A (96)	*B. rotunda*	Rhizome	Chahyadi et al., 2014
Boesenbergin B (97)	*B. pandurata*	Rhizome	Chahyadi et al., 2014
Rubranine (98)	*B. rotunda*	Rhizome	Widyananda et al., 2023

Compound	Species	Part	Reference
2',4'-Dihydroxy-3'-(1"-geranyl)-6'-methoxychalcone (99)	*B. pandurata*	Rhizome	Win et al., 2007
Flavokawain A (100)	*B. rotunda*	Rhizome	Widyananda et al., 2023
Flavokawain C (101)	*B. pandurata*	Rhizome	Win et al., 2007
Cardamonin (102)	*B. rotunda*	Rhizome	Widyananda et al., 2023
	B. pandurata	Rhizome	Tewtrakul et al., 2009
Pinostrobin chalcone (103)	*B. rotunda*	Rhizome	Han et al., 2023
Pinocembrin chalcone (104)	*B. pandurata*	Rhizome	Tewtrakul et al., 2009
Helichrysetin (105)	*B. pandurata*	Rhizome	Tewtrakul et al., 2009
Uvangoletin (106)	*B. pandurata*	Rhizome	Tewtrakul et al., 2009
2,6-Dihydroxy-4-methoxy-dihydrochalcone (107)	*B. rotunda*	Rhizome	Morikawa et al., 2008
FLAVANONES			
(2*R*)-8-Geranylpinostrobin (108)	*B. pandurata*	Rhizome	Win et al., 2007
(2*S*)-6-Geranylpinostrobin (109)	*B. pandurata*	Rhizome	Win et al., 2007
5,7-Dihydroxy-8-geranylflavanone (110)	*B. pandurata*	Rhizome	Morikawa et al., 2008
	B. rotunda	Rhizome	Widyananda et al., 2023
7-Methoxy-5-hydroxy-8-geranylflavanone (111)	*B. pandurata*	Rhizome	Morikawa et al., 2008

Compound	Species	Part	Reference
(-)-6-Geranylpinocembrin (112)	*B. pandurata*	Rhizome	Win et al., 2007
7,4'-Dihydroxy-5-methoxyflavanone (113)	*B. pandurata*	Rhizome	Morikawa et al., 2008
	B. rotunda	Rhizome	Widyananda et al., 2023
(±)-Pinocembrin (114)	*B. rotunda*	Rhizome	Widyananda et al., 2023
	B. pandurata	Rhizome	Win et al., 2007
(±)-Pinostrobin (115)	*B. kingii*	Rhizome	Sudsai et al., 2016
	B. pandurata	Rhizome	Win et al., 2007
	B. rotunda	Rhizome	Atun et al., 2017
	B. longiflora	Rhizome	Sudsai et al., 2014
Alpinetin (116)	*B. rotunda*	Rhizome	Widyananda et al., 2023
Sakuranetin (117)	*B. pandurata*	Rhizome	Tuchinda et al., 2002
	B. rotunda	Rhizome	Widyananda et al., 2023
5,7,2'-trihydroxy-8-methoxyflavanone (118)	*B. rotunda*	Rhizome	Taechowisan et al., 2024
5,2',5'-trihydroxy-7,8-dimethoxyflavanone (119)	*B. rotunda*	Rhizome	Taechowisan et al., 2024
Panduratin Q (120)	*B. rotunda*	Rhizome	Nguyen et al., 2021
Panduratin R (121)	*B. rotunda*	Rhizome	Nguyen et al., 2021
Panduratin S (122)	*B. rotunda*	Rhizome	Nguyen et al., 2021
Rotundaflavone Ia (123)	*B. rotunda*	Rhizome	Morikawa et al., 2008
Rotundaflavone Ib (124)	*B. rotunda*	Rhizome	Morikawa et al., 2008

Compound	Species	Part	Reference
Rotundaflavone IIa (125)	*B. rotunda*	Rhizome	Morikawa et al., 2008
Rotundaflavone IIb (126)	*B. rotunda*	Rhizome	Morikawa et al., 2008
(2*R*,7"*S*)-8-(1-phenyl-2-car-boxyethyl)pinocembrin (127)	*B. pandurata*	Rhizome	Nguyen et al., 2020
Hesperidin (128)	*B. pulchella*	Rhizome	Jing et al., 2010
Naringin (129)	*B. rotunda*	Rhizome	Jing et al., 2010
	B. pulchella	Rhizome	Jing et al., 2010
	B. armeniaca	Rhizome	Jing et al., 2010
FLAVONOLS			
Rutin (130)	*B. rotunda*	Rhizome	Jing et al., 2010
	B. pulchella	Rhizome	Jing et al., 2010
	B. armeniaca	Rhizome	Jing et al., 2010
Kaempferol (131)	*B. rotunda*	Rhizome	Jing et al., 2010
	B. pulchella	Rhizome	Jing et al., 2010
	B. armeniaca	Rhizome	Jing et al., 2010
Kaempferol-3,7,4'-trimethyl ether (132)	*B. kingii*	Rhizome	Sudsai et al., 2016
	B. longiflora	Rhizome	Sudsai et al., 2013
Kaempferol-7,4'-dimethyl ether (133)	*B. longiflora*	Rhizome	Sudsai et al., 2014
	B. kingii	Rhizome	Sudsai et al., 2016
Quercetin (134)	*B. rotunda*	Rhizome	Jing et al., 2010
	B. pulchella	Rhizome	Jing et al., 2010
	B. armeniaca	Rhizome	Jing et al., 2010

Compound	Species	Part	Reference
Rhamnazin (135)	*B. longiflora*	Rhizome	Sudsai et al., 2014
	B. kingii	Rhizome	Sudsai et al., 2016
FLAVONES			
Tectochrysin (136)	*B. pandurata*	Rhizome	Win et al., 2007
3,5,7-Trihydroxyflavone (137)	*B. stenophylla*	Rhizome	Primus et al., 2022
Luteolin (138)	*B. armeniaca*	Rhizome	Jing et al., 2010
Diosmin (139)	*B. pulchella*	Rhizome	Jing et al., 2010
	B. armeniaca	Rhizome	Jing et al., 2010
FLAVONOLIGNANS			
Silybin (140)	*B. rotunda*	Rhizome	Rosdianto et al., 2020



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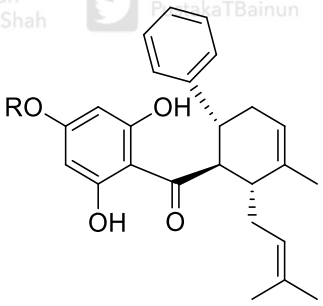

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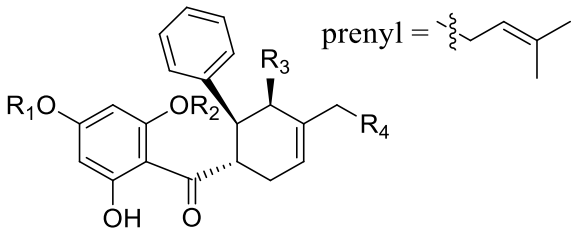

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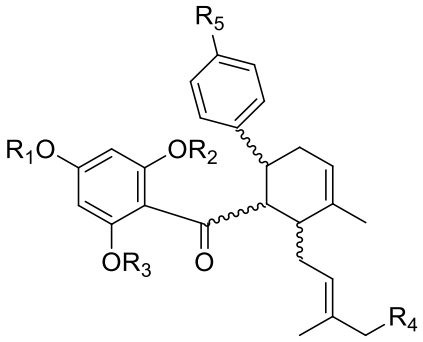

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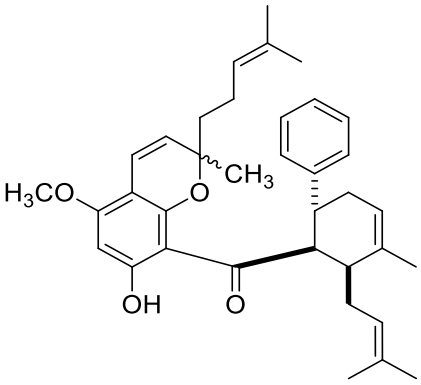

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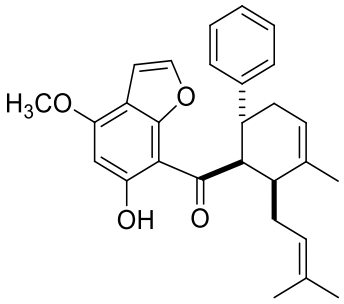

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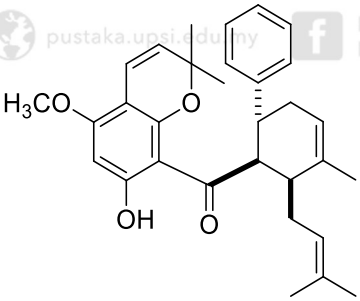

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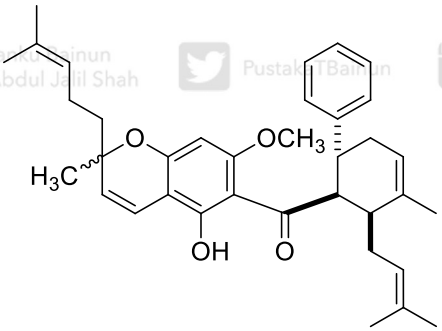

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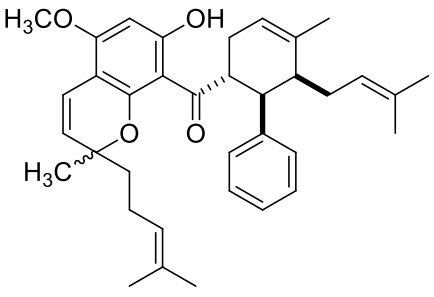

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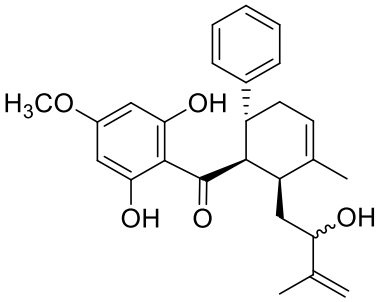

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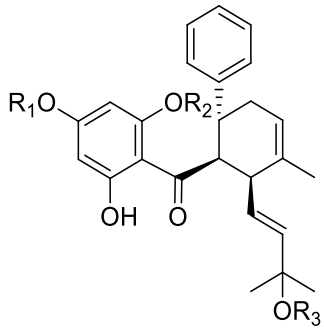

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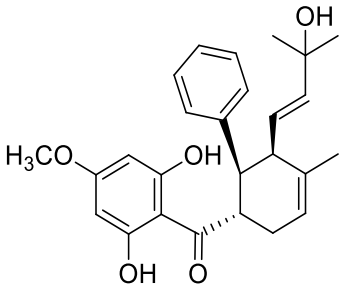

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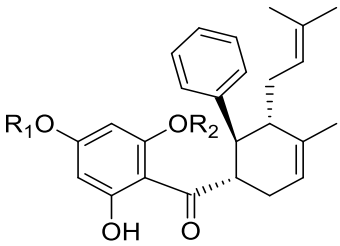
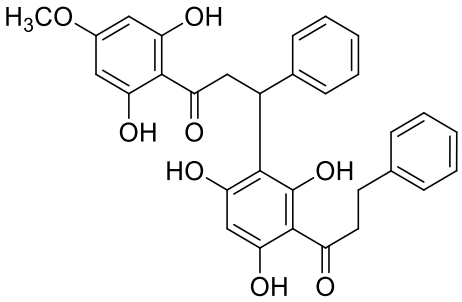


(88) $R_1 = CH_3$; $R_2 = H$
(89) $R_1 = H$; $R_2 = CH_3$



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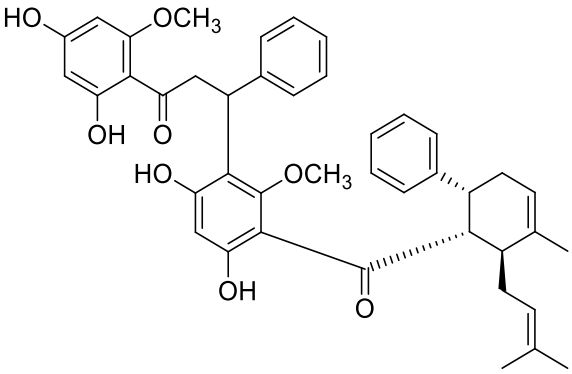
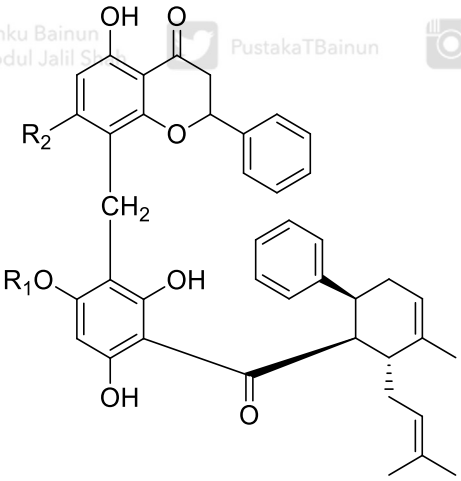
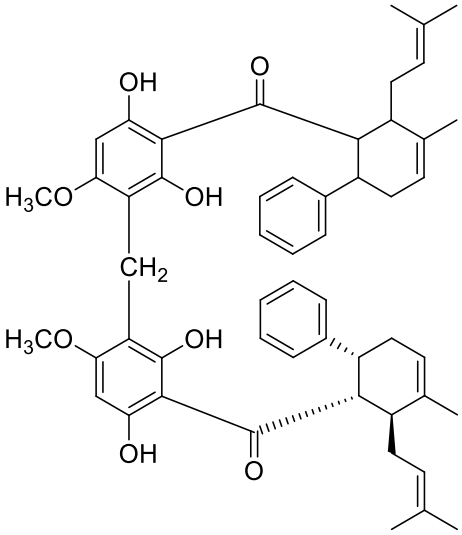


(91)



(92) $R_1 = CH_3$; $R_2 = H$
(93) $R_1 = H$; $R_2 = CH_3$
(94) $R_1 = R_2 = CH_3$



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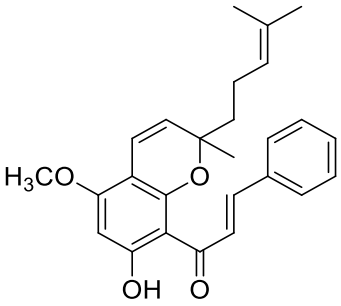

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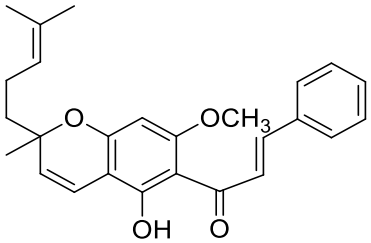

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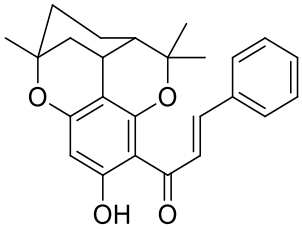

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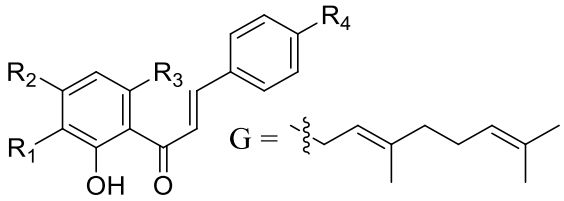
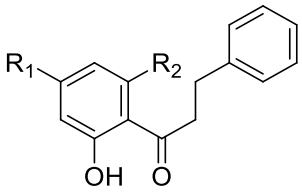
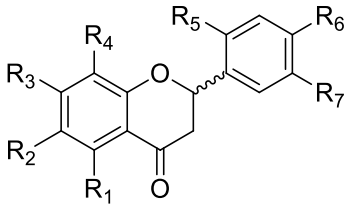
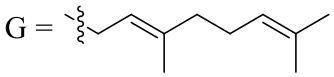


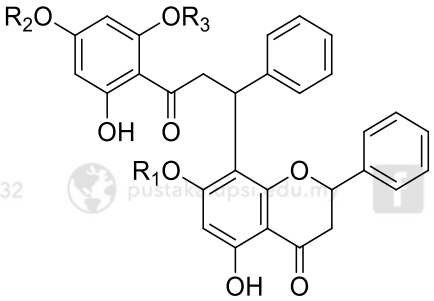
(99) R_1 = G; R_2 = OH; R_3 = OCH_3; R_4 = H
(100) R_1 = H; R_2 = R_3 = R_4 = OCH_3
(101) R_1 = H; R_2 = R_3 = OCH_3; R_4 = OH
(102) R_1 = R_4 = H; R_2 = OH; R_3 = OCH_3
(103) R_1 = R_4 = H; R_2 = OCH_3; R_3 = OH
(104) R_1 = R_4 = H; R_2 = R_3 = OH
(105) R_1 = H; R_2 = R_4 = OH; R_3 = OCH_3



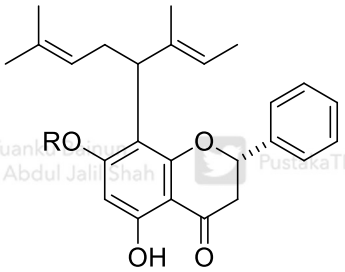
(106) R_1 = OH; R_2 = OCH_3
(107) R_1 = OCH_3; R_2 = OH

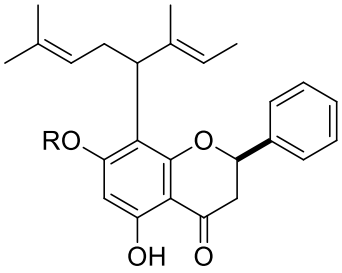
(108) R_1 = OH; R_2 = R_5 = R_6 = R_7 = H; R_3 = OCH_3; R_4 = G
(109) R_1 = OH; R_2 = G; R_3 = OCH_3; R_4 = R_5 = R_6 = R_7 = H
(110) R_1 = R_3 = OH; R_2 = R_5 = R_6 = R_7 = H; R_4 = G
(111) R_1 = OH; R_2 = R_5 = R_6 = R_7 = H; R_3 = OCH_3; R_4 = G
(112) R_1 = R_3 = OH; R_2 = G; R_4 = R_5 = R_6 = R_7 = H
(113) R_1 = OCH_3; R_2 = R_4 = R_5 = R_7 = H; R_3 = R_6 = OH
(114) R_1 = R_3 = OH; R_2 = R_4 = R_5 = R_6 = R_7 = H
(115) R_1 = OH; R_2 = R_4 = R_5 = R_6 = R_7 = H; R_3 = OCH_3
(116) R_1 = OCH_3; R_2 = R_4 = R_5 = R_6 = R_7 = H; R_3 = OH
(117) R_1 = R_6 = OH; R_3 = OCH_3; R_2 = R_4 = R_5 = R_7 = H
(118) R_1 = R_3 = R_5 = OH; R_2 = R_6 = R_7 = H; R_4 = OCH_3
(119) R_1 = R_5 = R_7 = OH; R_2 = R_6 = H; R_3 = R_4 = OCH_3



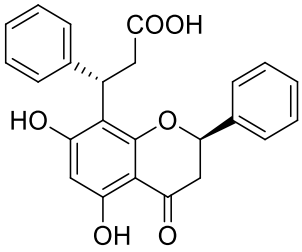
(120) R_1 = CH_3; R_2 = R_3 = H
(121) R_1 = R_3 = H; R_2 = CH_3
(122) R_1 = R_2 = H; R_3 = CH_3



(123) R = CH_3
(124) R = H



(125) R = CH_3
(126) R = H



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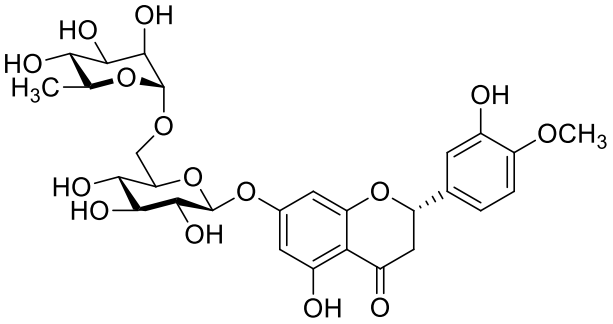
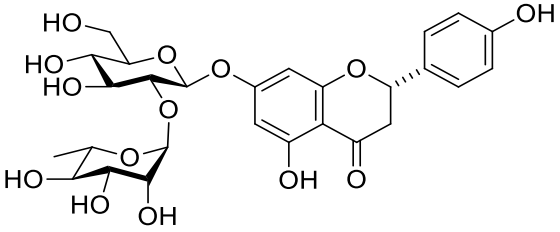


(128)



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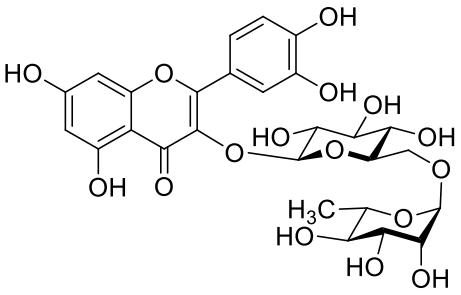
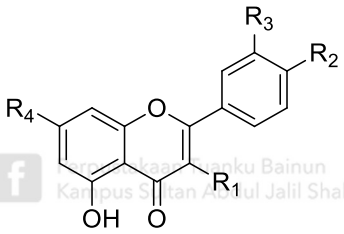
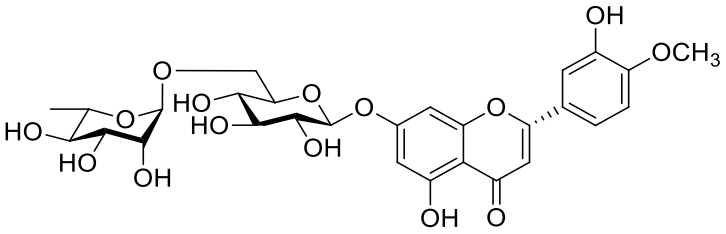


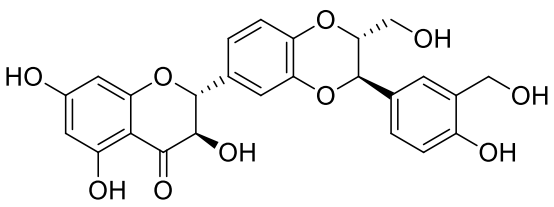
(130)



(131) $R_1 = R_2 = R_4 = OH$; $R_3 = H$
(132) $R_1 = R_2 = OCH_3$; $R_3 = R_4 = H$
(133) $R_1 = OH$; $R_2 = R_4 = OCH_3$; $R_3 = H$
(134) $R_1 = R_2 = R_3 = R_4 = OH$
(135) $R_1 = R_2 = OH$; $R_3 = R_4 = OCH_3$
(136) $R_1 = R_2 = R_3 = H$; $R_4 = OCH_3$
(137) $R_1 = R_4 = OH$; $R_2 = R_3 = H$
(138) $R_1 = H$; $R_2 = R_3 = R_4 = OH$



(139)



(140)

2.3.2 Diarylheptanoids

Two aromatic rings bonded to seven carbon chains make up the skeleton of diarylheptanoids, which are complex phenolic chemicals (Amalraj et al., 2017). A wide range of structural diversity is exhibited by diarylheptanoids, which have been discovered in various plant parts, such as seeds, fruits, leaves, roots, rhizomes, and barks (Ibrahim et al., 2017). Multiple research projects regarding diarylheptanoids within *Boesenbergia* plants have been reported between 2014 and 2021. Three *Boesenbergia* species have been reported on diarylheptanoids which were from *B. kingii*, *B. longiflora*, and *B. stenophylla*.

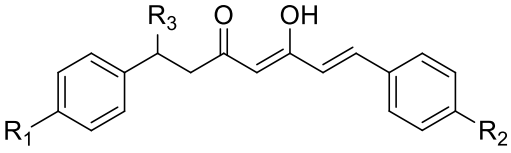
One prominent diarylheptanoid found in *Boesenbergia* species is curcumin **(144)**, that gives turmeric its characteristic yellow colour. Extracts from *B. kingii* and *B. longiflora* are being widely studied because many medicinal properties. In addition, demethoxycurcumin **(145)** was also reported from the same species. It has shown promising anti-cancer effects (Sudsai et al., 2016). Table 2.4 contains a list of additional isolated diarylheptanoid compounds.

Table 2.4 details the diarylheptanoids isolated from *Boesenbergia* species. These include compounds like dihydrobisdemethoxycurcumin, curcumin, and bisdemethoxycurcumin, commonly found in *B. longiflora* and *B. kingii*. Stenophyllols A, B, and C **(147-149)**, identified in *B. stenophylla*, are notable for their structural uniqueness. Such compounds have demonstrated many bioactivities, including anti-inflammatory and antioxidant properties, aligning with their structural similarity to curcumin, a well-known bioactive compound.

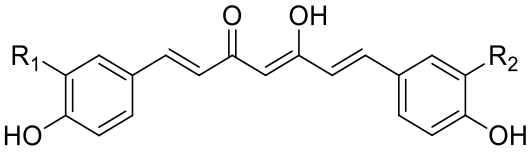
Table 2.4

Diarylheptanoids isolated from several Boesenbergia species

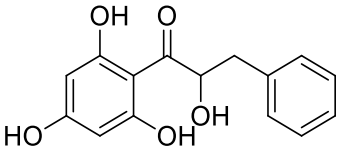
Compound	Species	Part	Reference
Dihydrobisdemethoxycurcumin (141)	*B. longiflora*	Rhizome	Sudsai et al., 2014
	B. kingii	Rhizome	Sudsai et al., 2016
1-Hydroxy-dihydrobis-demethoxycurcumin (142)	*B. kingii*	Rhizome	Sudsai et al., 2016
Dihydrobisdemethoxycurcumin-4',4''-diacetate (143)	*B. longiflora*	Rhizome	Sudsai et al., 2014
	B. kingii	Rhizome	Sudsai et al., 2016
Curcumin (144)	*B. longiflora*	Rhizome	Sudsai et al., 2014
	B. kingii	Rhizome	Sudsai et al., 2016
Demethoxycurcumin (145)	*B. longiflora*	Rhizome	Sudsai et al., 2014
	B. kingii	Rhizome	Sudsai et al., 2016
Bisdemethoxycurcumin (146)	*B. longiflora*	Rhizome	Sudsai et al., 2014
	B. kingii	Rhizome	Sudsai et al., 2016
Stenophyllol A (147)	*B. stenophylla*	Rhizome	Primus et al., 2022
Stenophyllol B (148)	*B. stenophylla*	Rhizome	Primus et al., 2022
Stenophyllol C (149)	*B. stenophylla*	Rhizome	Primus et al., 2022
7-(4-hydroxy-3-methoxyphenyl)-1-phenylhept-4-en-3-one (150)	*B. stenophylla*	Rhizome	Primus et al., 2022
5*R*-hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-phenylheptan-3-one (151)	*B. stenophylla*	Rhizome	Primus et al., 2022
1,7-diphenylhept-4-en-3-one (152)	*B. stenophylla*	Rhizome	Primus et al., 2022



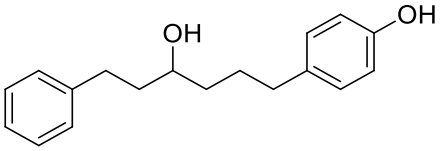
(141) $R_1 = R_2 = OH$; $R_3 = H$
(142) $R_1 = R_2 = R_3 = OH$
(143) $R_1 = R_2 = OAc$; $R_3 = H$



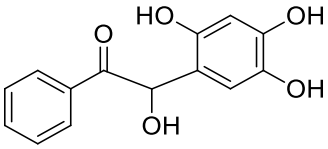
(144) $R_1 = R_2 = OCH_3$
(145) $R_1 = H$; $R_2 = OCH_3$
(146) $R_1 = R_2 = H$



(147)



(148)



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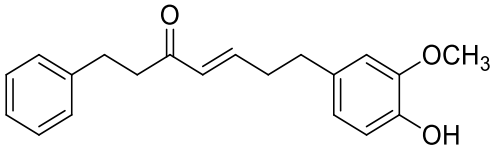

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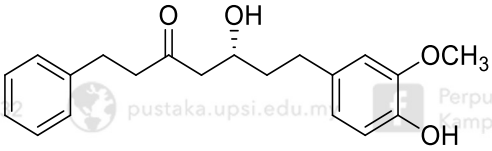
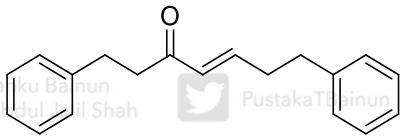


(151)



(152)

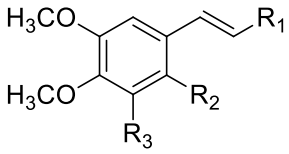
2.3.3 Phenylpropanoids

Study on the phenylpropanoids of *Boesenbergia* species were successfully isolated ten compounds **(153-162)** from rhizome of *B. thorelii*. Table 2.5 provides an overview of phenylpropanoids isolated from *B. thorelii*, specifically from the rhizomes. These compounds are notable for their diverse structural characteristics and potential bioactive properties, highlighting the phytochemical richness of this species. The identified phenylpropanoids include 3,4-dimethoxycinnamyl alcohol **(153)**, 3,4-

dimethoxycinnamaldehyde **(154)**, and 3,4-dimethoxycinnamic acid **(155)**, among others.

The identification of multiple methoxy derivatives, such as 3,4,5-trimethoxycinnamyl alcohol **(156)**, 3,4,5-trimethoxycinnamaldehyde **(157)**, and 2,4,5-trimethoxystyrene **(161)**, suggests that *B. thorelii* may possess significant antioxidant and anti-inflammatory properties. Additionally, compounds such as (E)-asarone **(158)** and isoasarone **(159)**, which are also present in the rhizomes, are recognized for their neuroprotective and antimicrobial properties. The presence of acoraminol A **(160)** further expands the functional diversity of *B. thorelii*, highlighting its potential as a source of bioactive metabolites with broad pharmacological applications.

The consistent isolation of these phenylpropanoids from the rhizome underscores the importance of this plant part in storing and synthesizing secondary metabolites. Rhizomes act as a reservoir for defense compounds, protecting the plant against pathogens, herbivores, and environmental stressors. The ecological role of phenylpropanoids in rhizomes, combined with their bioactive properties, suggests that *B. thorelii* has adapted to produce a chemically rich profile to thrive in its natural habitat.

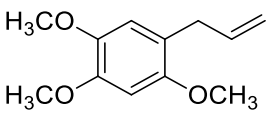


(153) $R_1 = CH_2OH$; $R_2 = R_3 = H$
(154) $R_1 = CHO$; $R_2 = R_3 = H$
(155) $R_1 = COOH$; $R_2 = R_3 = H$
(156) $R_1 = CH_2OH$; $R_2 = H$; $R_3 = OCH_3$
(157) $R_1 = CHO$; $R_2 = H$; $R_3 = OCH_3$
(158) $R_1 = CH_3$; $R_2 = OCH_3$; $R_3 = H$

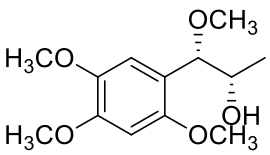
Table 2.5

Phenylpropanoids isolated from several Boesenbergia species

Compound	Species	Part	Reference
3,4-Dimethoxycinnamyl alcohol (153)	*B. thorelii*	Rhizome	Thongphichai et al., 2019
3,4-Dimethoxycinnamaldehyde (154)	*B. thorelii*	Rhizome	Thongphichai et al., 2019
3,4-Dimethoxycinnamic acid (155)	*B. thorelii*	Rhizome	Thongphichai et al., 2019
3,4,5-Trimethoxycinnamyl alcohol (156)	*B. thorelii*	Rhizome	Thongphichai et al., 2019
3,4,5-Trimethoxy-cinnamaldehyde (157)	*B. thorelii*	Rhizome	Thongphichai et al., 2019
(*E*)-Asarone (158)	*B. thorelii*	Rhizome	Thongphichai et al., 2019
Isoasarone (159)	*B. thorelii*	Rhizome	Thongphichai et al., 2019
Acoraminol A (160)	*B. thorelii*	Rhizome	Thongphichai et al., 2019
2,4,5-Trimethoxystyrene (161)	*B. thorelii*	Rhizome	Thongphichai et al., 2019
1-methoxyethyl-2,4,5-trimethoxybenzene (162)	*B. thorelii*	Rhizome	Thongphichai et al., 2019



(159)



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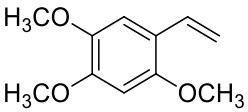
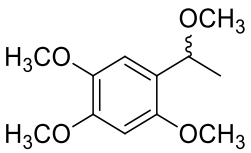


(161)



(162)

2.3.4 Miscellaneous Phytochemicals

Other secondary metabolites have been identified from *Boesenbergia* species such as benzenoids **(163-167)**, lignans **(168-172)**, cyclohexane **(173-175)**, diterpene acid **(176)**, phenolic acids **(177-181)**, diterpenoids **(182-187)**, esters **(188-192)**, sesquiterpenes **(193-195)**, kavalactones **(196-197)** and, sterol **(198)**. The various phytochemicals studies reported in the literatures are discussed below. Table 2.6 showcases a diverse group of compounds, including benzonoids, lignans, diterpenoids, sesquiterpenes, and kavalactones.

Lignans such as thoreliins A **(168)** and B **(169)**, along with benzonoids like 3,4-dimethoxybenzaldehyde **(164)**, are prominent in *B. thorelii*. These molecules are often associated with anticancer and antimicrobial activities. *B. rotunda* appears particularly versatile, yielding diterpenoids (seikphoochinal A) and phenolic acids (caffeic acid), which are linked to antioxidative properties. Additionally, kavalactones such as 5,6-dehydrokawain from *B. rotunda* are notable for their sedative and anxiolytic effects, showcasing the species' ethnopharmacological significance. The phytochemical diversity highlighted in these tables illustrates the immense therapeutic potential of *Boesenbergia* species. The dominance of rhizome-derived compounds suggests targeted harvesting strategies to optimize phytochemical yield. Furthermore, the repeated presence of bioactive classes like diarylheptanoids and phenylpropanoids across species underscores their evolutionary and functional significance.

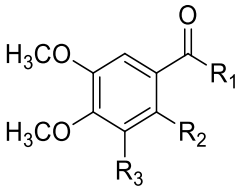
Table 2.6

Miscellaneous phytochemicals isolated from several Boesenbergia species

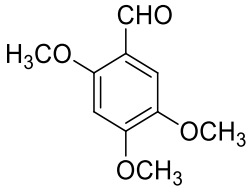
Compound	Species	Part	Reference
BENZENOIDS			
3,4-Dimethoxy-benzaldehyde (163)	*B. thorelii*	Rhizome	Thongphichai et al., 2019
3,4-Dimethoxybenzoic acid (164)	*B. thorelii*	Rhizome	Thongphichai et al., 2019
Asaraldehyde (165)	*B. thorelii*	Rhizome	Thongphichai et al., 2019
3,4,5-Trimethoxy-benzaldehyde (166)	*B. thorelii*	Rhizome	Thongphichai et al., 2019
Asaronaldehyde (167)	*B. thorelii*	Rhizome	Madaka et al., 2013
LIGNANS			
Thoreliin A (168)	*B. thorelii*	Rhizome	Thongphichai et al., 2019
Thoreliin B (169)	*B. thorelii*	Rhizome	Thongphichai et al., 2019
(-)-Pachypostaudin B (170)	*B. thorelii*	Rhizome	Thongphichai et al., 2019
Pellucidin A (171)	*B. thorelii*	Rhizome	Thongphichai et al., 2019
Acortatarinowin E (172)	*B. thorelii*	Rhizome	Thongphichai et al., 2019
CYCLOHEXANE			
(+)-Zeylenol (173)	*B. rotunda*	Rhizome	Widyananda et al., 2023
Crotepoxide (174)	*B. rotunda*	Rhizome	Widyananda et al., 2023
Boesenboxide (175)	*B. pandurata*	Rhizome	Pancharoen *et al*., 1984
DITERPENE ACID			
Isopimaric acid (176)	*B. rotunda*	Rhizome	Widyananda et al., 2023

Compound	Species	Part	Reference
PHENOLIC ACIDS			
Caffeic acid (177)	*B. rotunda*	Rhizome	Jing et al., 2010
	B. pulchella	Rhizome	Jing et al., 2010
	B. armeniaca	Rhizome	Jing et al., 2010
p-Coumaric acid (178)	*B. rotunda*	Rhizome	Jing et al., 2010
	B. pulchella	Rhizome	Jing et al., 2010
	B. armeniaca	Rhizome	Jing et al., 2010
Ferulic acid (179)	*B. pulchella*	Rhizome	Jing et al., 2010
	B. armeniaca	Rhizome	Jing et al., 2010
Sinapic acid (180)	*B. pulchella*	Leaves/stem	Jing et al., 2010
Chlorogenic acid (181)	*B. rotunda*	Rhizome	Jing et al., 2010
	B. pulchella	Rhizome	Jing et al., 2010
	B. armeniaca	Rhizome	Jing et al., 2010
DITERPENOIDS			
Seikphoochinal A (182)	*B. rotunda*	Rhizome	Win et al., 2019
Galanal A (183)	*B. rotunda*	Rhizome	Win et al., 2019
Galanal B (184)	*B. rotunda*	Rhizome	Win et al., 2019
Boesenmaxanes A (185)	*B. maxwellii*	Rhizome	Moe et al., 2020
Boesenmaxanes B (186)	*B. maxwellii*	Rhizome	Moe et al., 2020
Boesenmaxanes C (187)	*B. maxwellii*	Rhizome	Moe et al., 2020
ESTERS			
Panduratin H (188)	*B. pandurata*	Rhizome	Win et al., 2008

Compound	Species	Part	Reference
Panduratin I (189)	*B. pandurata*	Rhizome	Win et al., 2008
2,4-Dihydroxy-6-phenethyl-benzoic acid methyl ester (190)	*B. rotunda*	Rhizome	Widyananda et al., 2023
	B. rotunda	Rhizome	Morikawa et al., 2008
Geranyl-2,4-dihydroxy-6-phenethylbenzoate (191)	*B. rotunda*	Rhizome	Morikawa et al., 2008
	B. pandurata	Rhizome	Win et al., 2007
Protocatechuic acid methyl ester (192)	*B. thorelii*	Rhizome	Madaka et al., 2013
SESQUITERPENES			
Longiferone A (193)	*B. longiflora*	Rhizome	Sudsai et al., 2014
	B. kingii	Rhizome	Sudsai et al., 2016
Longiferone B (194)	*B. longiflora*	Rhizome	Sudsai et al., 2014
	B. kingii	Rhizome	Sudsai et al., 2016
Longiferone C (195)	*B. longiflora*	Rhizome	Sudsai et al., 2014
	B. kingii	Rhizome	Sudsai et al., 2016
KAVALACTONES			
5,6-Dehydrokawain (196)	*B. rotunda*	Rhizome	Yoshikawa et al., 2008
Dihydro-5,6-dehydrokawain (197)	*B. pandurata*	Rhizome	Tuchinda et al., 2002
STEROL			
β-Sitosterol-D-glucoside (198)	*B. longiflora*	Rhizome	Sudsai et al., 2014
	B. kingii	Rhizome	Sudsai et al., 2016



(163) $R_1 = R_2 = R_3 = H$
(164) $R_1 = OH$; $R_2 = R_3 = H$
(165) $R_1 = R_3 = H$; $R_2 = OCH_3$
(166) $R_1 = R_2 = H$; $R_3 = OCH_3$



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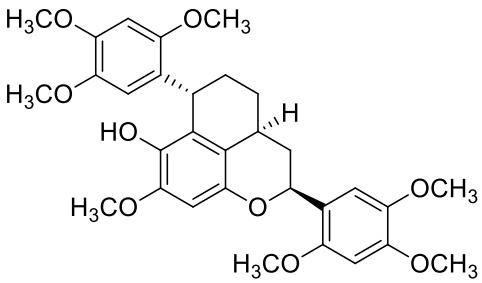

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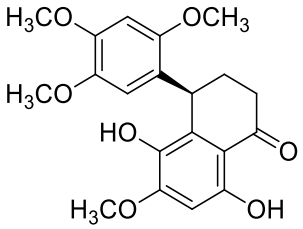

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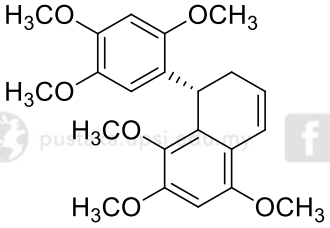

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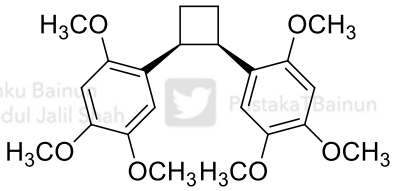

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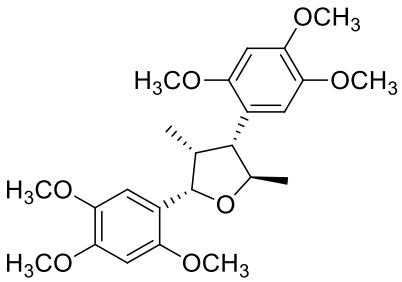

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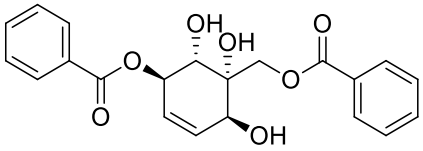

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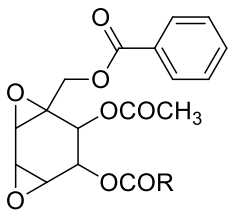
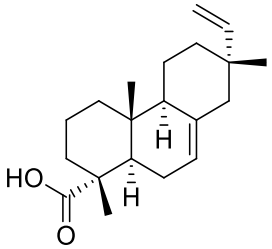


(174) $R = CH_3$
(175) $R = Ph$



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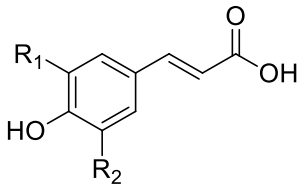
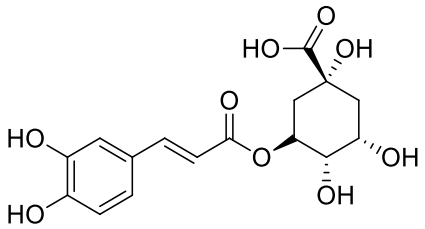


(177) R_1 = OH; R_2 = H
(178) R_1 = R_2 = H
(179) R_1 = OCH_3; R_2 = H
(180) R_1 = R_2 = OCH_3



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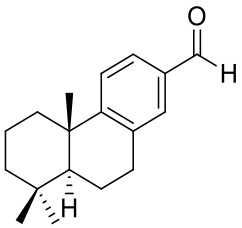

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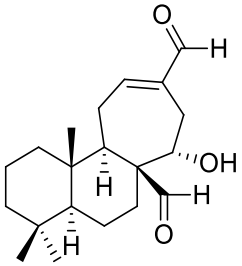

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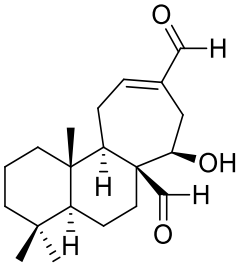

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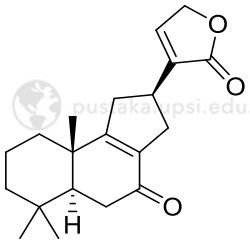

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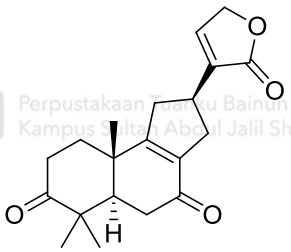

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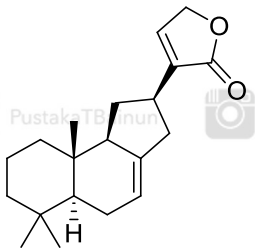

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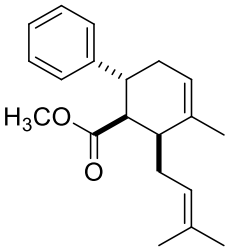

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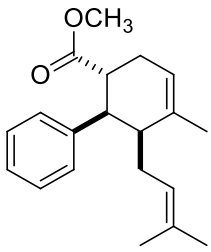

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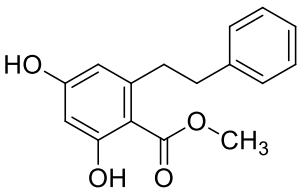

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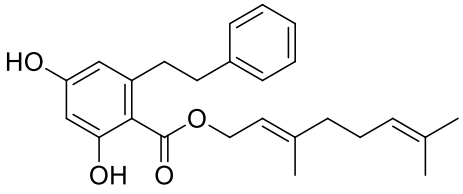

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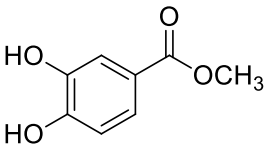

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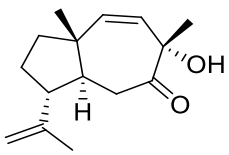

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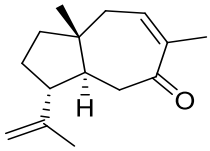

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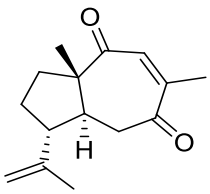

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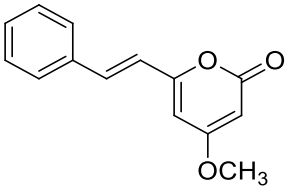

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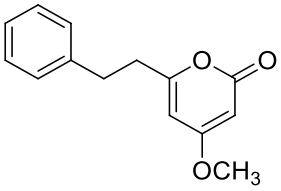

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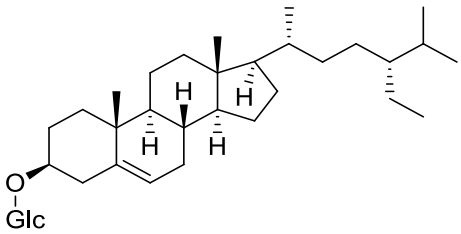

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2.4 Biological Activities of *Boesenbergia* Species

Numerous researchers are drawn to the exploration of biological activities due to the immense range of natural products that hold promise for use in medicine, agriculture, and industry. Plant compounds, in particular, have been found to possess properties that make them suitable for use as antioxidants, anti-inflammatory, and antibacterial agent. In the course of literature review, it has been learnt that *Boesenbergia* essential oils, phytochemicals, as well as their crude extracts demonstrated an array of bioactivities. *Boesenbergia pandurata*, in particular, has received huge attention and has been widely studied.

2.4.1 Biological Activities of *Boesenbergia* Essential Oils

The provided document summarizes the bioactivities of essential oil extracted from various *Boesenbergia* species, highlighting their therapeutic and practical applications. These essential oils exhibit a diverse range of bioactivities, such as antimicrobial and antioxidant properties to antifungal and repelling agent properties, highlighting the genus' pharmaceutical and ecological significance.

Several studies have highlighted the potential therapeutic benefits of *Boesenbergia* essential oils, including antimicrobial, antifungal, antibiofilm, antioxidant, anti-inflammatory, antibacterial, repellent, and larvicidal activities. For instance, (*E*)-β-ocimene **(47)**, one of the primary compounds found in *B. pandurata*, has been shown to inhibit COX-2 and reduce PGE2 levels, thereby reducing inflammation in the body (Homnan et al., 2020). Furthermore, camphor **(42)**, geraniol **(16)**, and 1,8-cineole **(45)** were identified as the main contributors to the antifungal activity of *B. pandurata* oils (Jantan et al., 2003).

Moreover, *B. pulcherrima* root extract shows promise as an environmentally friendly agricultural product with antifungal activity against *Fusarium oxysporum*, with α-asarone **(61)** exhibiting the highest activity (Park et al., 2020). Notably, methyl (*E*)-cinnamate **(33)**, extracted from *B. pandurata* essential oil, has demonstrated strong coccidiocidal efficacy, making it a promising candidate for anti-coccidial activity (Jitviriyanon et al., 2016). Table 2.7 provides a detailed overview of the bioactivities of several *Boesenbergia* essential oils.

Table 2.7

Biological activities of several Boesenbergia essential oils

Bioactivity	Species	Description
Antimicrobial	*B. quangngaiensis*	The rhizome oil has excellent activity towards Pseudomonas aeruginosa, having MIC of 7.68 µg/mL (Huong et al., 2021)
	B. pulcherrima	The essential oil was efficient against *Shigella flexneri*, *Escherichia coli*, along with *Staphylococcus epidermidis*, showing zones of inhibition of 17.1, 16.5, and 14.5 mm, respectively (Byahatti & Thangadurai, 2019)
	B. pandurata	The rhizome oil displayed great activity against *Listeria monocytogenes* and *Salmonella enterica* by inhibiting all strains at ≤0.4% (Thongson et al., 2005)
	B. rotunda	The essential oil showed a significant effect against *B. cereus*, with MIC as well as MBC levels of 62.5 and 125 µg/mL, correspondingly (Tasfiyati et al., 2023)
Antibiofilm	*B. rotunda*	The essential oil was significantly reduced *Enterococcus faecalis*, *Fusobacterium nucleatum*, and *Treponema denticola* in the biofilms for all incubation periods (Widyarman et al., 2019)

Bioactivity	Species	Description
Anticoccidial	*B. pandurata*	The essential oil showed strong sporulation inhibition against *Eimeria tenella* with IC_{50} value 0.13 mg/mL (Jitviriyanon et al., 2016)
Antioxidant	*B. rotunda*	The rhizome oil had a strong inhibition action against *Staphylococcus aureus* with MIC value of 4 mg/mL (Apinundecha et al., 2023)
	B. longiflora	The rhizome oil exhibited high activity with EC_{50} value 18.8 μg/mL (DPPH), 29.3 μg/mL (superoxide anion radicals) and 27.0 μg/mL (hydroxyl radicals) (Theanphong et al., 2022)
Anti-inflammatory	*B. pandurata*	The rhizome oil possessed high inhibitory effect on COX-2 (IC_{50} value 51.2 μg/mL) and PGE2 levels (IC_{50} value 41.0 μg/mL) (Homnan et al., 2020)
Anti-inflammatory	*B. pandurata*	The essential oil displayed efficient repellent against *Leptotrombidium imphalum* with ED_{50} value 30.4 mg and EC_{50} value 152.4% (Rodkvamtook et al., 2012)
Antifungal	*B. pulcherrima*	The root oil showed high activity on *F. oxysporum* (EC_{50} value 43.1 μg/mL) (Park et al., 2020)
Larvicidal	*B. rotunda*	The roots and rhizome oil exhibited against *Culex quinquefasciatus* larvae (LT_{50} of 1.7 min) (Phukerd & Soonwera, 2013)

Bioactivity	Species	Description
Antibacterial	*B. pandurata*	The rhizome oil exhibits high efficacy against *Bacillus cereus*, *Salmonella typhi*, *Shigella flexneri*, and *Escherichia coli*, with MIC of 100 μg/mL (Phanthong et al., 2013)
Antifungal	*B. pandurata*	The rhizome oil exhibited the lowest MIC values of 0.63 μg/μL (*Mucor sp.*), 1.25 μg/μL (*Aspergillus niger*), and 2.5 μg/μL (*Trichophyton rubrum* and *Epidermophyton floccosum*) (Jantan et al., 2003)
	B. rotunda	The essential oil showed great inhibition of growth and AFB_1 production of *Aspergillus parasiticus* with IC_{50} value 0.52 mg/mL (Jantapan et al., 2017)
Repellent	*B. rotunda*	The rhizome oil displayed high repellencies against *Periplaneta americana* (90%), and *Blattella germanica* (95%) (Thavara et al., 2007)
Repellent	*B. rotunda*	The root and rhizome oils were the most effective repellent against *Culex quinquefasciatus*, with a protection time of 4.3 hours, comparable to the chemical repellent 20% N,N-diethyl-meta-toluamide (Phukerd & Soonwera, 2014)

The essential oils of *Boesenbergia* species exhibit strong antimicrobial properties, targeting diverse pathogens. For example, *B. quangngaiensis* oil exhibits strong anti-*Pseudomonas aeruginosa* action, having low MIC that is 7.68 μg/mL. Similarly, *B. pulcherrima* demonstrates efficacy against *Shigella flexneri*, *Escherichia coli*, as well as Staphylococcus epidermidis, having inhibitory zones that up to 17.1 mm. *B. pandurata* and *B. rotunda* also reveal significant antimicrobial effects, particularly against foodborne pathogens such as *Listeria monocytogenes* and Salmonella enterica, indicating potential use in food safety applications.

B. rotunda essential oil significantly disrupts biofilm formation by pathogens like Enterococcus faecalis and Treponema denticola, crucial for combating persistent infections. Additionally, the anticoccidial activity of *B. pandurata*, with strong sporulation inhibition of *Eimeria tenella* (IC_{50} value 0.13 mg/mL), highlights its relevance in veterinary medicine and poultry health management. The oils demonstrate notable antioxidant capabilities, as seen in *B. longiflora* with EC50 values against DPPH and superoxide anion radicals. These properties suggest their role in combating oxidative stress-related disorders. Furthermore, the anti-inflammatory properties of *B. pandurata*, showing its inhibitory to COX-2 and PGE2 levels, underline its therapeutic potential in managing inflammatory conditions.

B. pulcherrima and *B. rotunda* exhibit robust antifungal activities against species like Fusarium oxysporum and Aspergillus parasiticus, with low IC_{50} and MIC values. These properties are vital for agricultural and clinical applications. The value 1.7 minutes, positions it as an effective natural alternative to chemical insecticides. Essential oils from *B. rotunda* display excellent repellent activities against pests such

as Periplaneta americana and Blattella germanica, with efficacy comparable to commercial chemical repellents. This suggests potential applications in pest management and public health. Additionally, antibacterial and antifungal activities of *B. pandurata* and *B. rotunda* further emphasize their broad-spectrum bioactivities, with potential for pharmaceutical and agricultural use.

The comprehensive bioactivity profile of *Boesenbergia* essential oils showcases their immense potential in various domains, from medicine to agriculture and pest control. Their antimicrobial, antioxidant, anti-inflammatory, and repellent advantages make this genus an excellent source of natural products having practical uses. Further study may concentrate in isolating and characterizing active constituents to further explore their mechanisms of action and optimize their use in targeted applications.

2.4.2 Biological Activities of *Boesenbergia* Phytochemicals

Several researchers have previously isolated various biologically active compounds from *Boesenbergia* species, including flavonoids, diarylheptanoids, phenylpropanoids, and benzenoids. These chemicals are well recognized because of their anti-inflammatory, anti-tumor, anti-proliferative, antimutagenic, anticancer, antimicrobial, antioxidant, and anti-allergic properties. The most bioactive flavonoids have been found to be panduratin A **(72)** and cardamonin **(102)** from chalcones, as well as pinocembrin **(114)** and pinostrobin **(115)**. Table 2.8 provides bioactivities exhibited by several phytochemicals from *Boesenbergia* species.

Table 2.8

Biological activities of several phytochemicals from Boesenbergia species

Compound	Species	Biological activities
CHALCONE		
(±)-Krachaizin B (65)	*B. rotunda*	Anti-inflammatory: Inhibited cytotoxicity caused by TNF-α L929 cells (IC_{50} value 6.1 µg/mL) with 54.0% and 65.0% at 30 µM (Morikawa et al., 2008)
Nicolaioidesin B (68)	*B. pandurata*	Cytotoxicity: Showed preferential activity against human pancreatic PANC-1 tumour with PC_{100} value 2.5 µM (Win et al., 2007)
Nicolaioidesin C (69)	*B. pandurata*	Antiausterity: Exhibited action against PANC-1 human pancreatic cancer cells with PC_{50} value 0.84 µM (Nguyen et al., 2017)
Isopanduratin A1 (70)	*B. pandurata*	Antiausterity: Exhibited potent activity against PANC-1 human pancreatic cancer cells with PC_{50} value 1.0 µg/mL(Nguyen et al., 2017)
(±)-Panduratin A (72)	*B. rotunda*	Anti-SARS-CoV-2: IC_{50} value 5.30 µM against SARSCoV-2 infection (Kanjanasirirat et al., 2020)
		DEN-2 virus NS3 protease: Good inhibition towards dengue 2 virus NS3 protease with the Ki value 25 µM (Kiat et al., 2006)

Compound	Species	Biological activities
(±)-Panduratin A (72)	*B. rotunda*	Anti-inflammatory: Inhibit trypsin and N-aminopeptidase (APN) activity with 61.4% and 95.2% at 30 µM (Morikawa et al., 2008)
	B. pandurata	Anti-inflammatory: Inhibited ear edema brought on by TPA in a dose-dependent way having ID_{50} value 12 µg/ear (Tuchinda et al., 2002)
		Anti-inflammatory: IC_{50} values 10.5 and 60.3 µM on prostaglandin E2 and tumour necrosis factor-alpha (TNF-α) production, respectively (Tewtrakul et al., 2009)
		Anti-inflammatory: IC_{50} value 5.3 µM on LPS-induced NO release by RAW264.7 cells (Tewtrakul et al., 2009)
		Antibacterial: Showed <25% inhibitory in biofilm formation and prevented biofilm growth by >50% at MIC (8 µg/mL) (Yanti et al., 2009)
		Neuroprotective: Exerted effects against L-glutamate toxicity in N18-RE-105 cells, having EC_{50} value 13 µM (Shindo et al., 2006)
		Antioxidant: Decreased cell development inhibition with t-BHP and pretreatment by 10-15 µM (Sohn et al., 2005)

Compound	Species	Biological activities
(±)-Panduratin A (72)	*B. pandurata*	Antimutagen: Inhibited mutation with existence of 50 ng of Trp-P-1 in *Salmonella typhimurium* TA98, having IC_{50} value 12.1 µM (Trakoontivakorn et al., 2001)
		Antimicrobial: Inhibited MBC value 4 µg/mL against *Prevotella intermedia* and *Propionibacterium acnes* (Rukayadi et al., 2009)
		Cytotoxicity: IC_{50} values 9.6 and 8.2 µM for melanogenesis and tyrosinase (Lee et al., 2010)
(±)-Hydroxy panduratin A (73)	*B. rotunda*	Cytotoxicity: IC_{50} value 10.6 µM on melanin synthesis and 10.5 µM on tyrosinase melan-a cells (Yoon et al., 2007)
		Cytotoxicity: IC_{50} value 23.2 µM on melanin synthesis and >30 µM on tyrosinase activity of melan-a cells (Yoon et al., 2007)
(±)-Hydroxy panduratin A (73)	*B. rotunda*	Antimutagen: Inhibited mutation with existence of 50 ng of Trp-P-1 in *Salmonella typhimurium* TA98, having IC_{50} value 12.7 µM (Trakoontivakorn et al., 2001)
		Vasorelaxant: Showed the effect on porcine coronary artery with EC_{50} value 17.8 µM (Adhikari et al., 2020)

Compound	Species	Biological activities
(±)-Hydroxy panduratin A (73)	*B. pandurata*	Anti-inflammatory: Inhibited ear edema brought on by TPA in a dose-dependent way having ID_{50} value 84 µg/ear (Tuchinda et al., 2002)
(±)-Isopanduratin A (76)	*B. rotunda*	Anti-inflammatory: Inhibited tumor necrosis factor-alpha (TNF-α) in L929 cells, having IC_{50} value 6.1 µg/mL (Morikawa et al., 2008)
Boesenbergin A (96)	*B. rotunda*	Anticancer: Inhibited development in MCF-7, HT-29 cancer cells with IC_{50} value 3.75 and 6.56 µg/mL (Kirana et al., 2007)
Cardamonin (102)	*B. rotunda*	Anti-BACE1: Showed strong beta-site amyloid precursor protein cleaving enzyme-1 (BACE1) with IC_{50} value 4.35 µM (Youn & Jun, 2019)
		Cytotoxicity: Exhibited activity against HK1 cells with IC_{50} value 27.0 µg/mL (Break et al., 2020)
		Anti-tumor: Inhibition of Epstein Barr virus (EBV) activation, having IC_{50} 3.1 µM (Murakami et al., 1993)
		Anti-HIV-1 Protease: Exhibited mild inhibitory towards HIV-1 protease with IC_{50} value 75.1 µM (Tewtrakul et al., 2003)

Compound	Species	Biological activities
Pinocembrin chalcone (104)	*B. pandurata*	Antimutagen: Inhibited mutation with existence of 50 ng of Trp-P-1 in *Salmonella typhimurium* TA98, having IC_{50} 5.2 µM (Trakoontivakorn et al., 2001)
Helichrysetin (105)	*B. pandurata*	Anti-HIV-1 protease: Possessed potent anti-HIV-1 PR action, having IC_{50} value 18.7 µM (Cheenpracha et al., 2006)
FLAVANONES		
5,7-Dihydroxy-8-geranylflavanone (110)	*B. rotunda*	Anti-atherosclerosis: Bind to the atherosclerosis-related proteins (CETP, ACAT1, OSC, and sPLA2) active sites with Pa>0.7 value (Widyananda et al., 2023)
7,4'-Dihydroxy-5-methoxy-flavanone (113)	*B. rotunda*	Anti-atherosclerosis: Bind to the atherosclerosis-related proteins (CETP, ACAT1, OSC, and sPLA2) active sites with Pa>0.7 value (Widyananda et al., 2023)
(±)-Pinocembrin (114)	*B. rotunda*	Anti-atherosclerosis: Bind to the atherosclerosis-related proteins (CETP, ACAT1, OSC, and sPLA2) active sites with Pa>0.7 value (Widyananda et al., 2023)

Compound	Species	Biological activities
(±)-Pinostrobin (115)	*B. rotunda*	Antioxidant: IC value of 92.6 µg/mL using DPPH method (Atun et al., 2017)
	B. kingii	Wound healing: Significantly accelerated the formation of collagen around 75.0 µg/mL (Sudsai et al., 2016)
	B. pandurata	Anti-ulcerogenic: Reduced the incidence of ulcers by inhibition of 92.03% (Abdelwahab et al., 2011)
Alpinetin (116)	*B. rotunda*	Anti-inflammatory: Inhibited TNF-α in L929 cells, having IC_{50} value 6.1 µg/mL (Morikawa et al., 2008)
Sakuranetin (117)	*B. rotunda*	Anti-atherosclerosis: Bind to the active sites of atherosclerosis-related proteins with Pa>0.7 value (Widyananda et al., 2023)
FLAVONOLS		
Kaempferol-3,7,4'-trimethyl ether (132)	*B. longiflora*	Anti-inflammatory: Exhibited against NO release with IC_{50} value 23.5 µM (Sudsai et al., 2013)
FLAVONES		
3,5,7-Trihydroxy-flavone (137)	*B. stenophylla*	Anti-neuroblastoma: Excellent activity against PI3K and/or AKT1 which reduces the cell viability to 20% (Primus et al., 2022)

Compound	Species	Biological activities
DIARYLHEPTANOIDS		
Dihydrobisdemethoxycurcumin (141)	*B. longiflora*	Anti-inflammatory: Exhibited action towards NO release, having IC_{50} value 23.0 μM (Sudsai et al., 2013)
Curcumin (144)	*B. kingii*	Antioxidant: IC_{50} value 21.0 μM in DPPH (Sudsai et al., 2016)
	B. longiflora	Anti-inflammatory: Exhibited action towards NO release, having IC_{50} value 4.5 μM (Sudsai et al., 2013)
Demethoxy-curcumin (145)	*B. longiflora*	Anti-inflammatory: Exhibited action towards NO release, having IC_{50} value 11.7 μM (Sudsai et al., 2013)
Bisdemethoxy-curcumin (146)	*B. longiflora*	Anti-inflammatory: Exhibited action towards NO release, having IC_{50} value 15.7 μM (Sudsai et al., 2013)
Stenophyllol B (148)	*B. stenophylla*	Anti-neuroblastoma: Excellent activity against PI3K and/or AKT1 which reduces the cell viability to 30% (Primus et al., 2022)
		Antiproliferative: Significant effects in TNBC cells (HCC1937 and Hs578T) with IC_{50} values 17.8 and 27.4 μM, respectively (Lee et al., 2023)

Compound	Species	Biological activities
BENZENOIDS		
Asaronaldehyde (167)	*B. thorelii*	Anti-allergic: Showed appreciable effect with IC_{50} value 24.3 µM (Madaka et al., 2013)
LIGNAN		
Thoreliin A (168)	*B. thorelii*	Anti-HIV-1: Exhibited syncytium reduction with EC_{50} value 20.6 µM (Thongphichai et al., 2019)
DITERPENE ACID		
Isopimaric acid (176)	*B. rotunda*	Anti-atherosclerosis: Bind to the atherosclerosis-related proteins (CETP, ACAT1, OSC, and sPLA2) active sites with Pa>0.7 value (Widyananda et al., 2023)
STEROL		
β-Sitosterol-D-glucoside (198)	*B. kingii*	Wound healing: Strongly stimulated collagen production at 96.7 µg/mL (Sudsai et al., 2016)
DITERPENOID		
Galanal A (183)	*B. rotunda*	Antiproliferative: Exhibited significant activity against lung LK-2, A549 with IC_{50} 4.38 and 28.2 µM (Win et al., 2019)

2.4.3 Biological Activities of *Boesenbergia* Extracts

The document provides an extensive overview regarding diverse bioactivities of extracts taken from various *Boesenbergia* species. These activities span an entire range of therapeutic as well as biomedical applications, demonstrating pharmacological value of this plant genus. Several extracts of *Boesenbergia* species, especially the rhizome extract of *B. rotunda* and *B. pandurata* exhibited many different kinds of biological properties, notably anticancer, anti-inflammatory, antimicrobial, antioxidant, antidiabetic, andaphrodisiac and more bioactivities. Table 2.9 listed pharmacological studies of several extracts of *Boesenbergia* species.

Boesenbergia extracts exhibit notable antimicrobial effects against bacteria and fungi. For instance, extracts from *B. rotunda* inhibit *Staphylococcus aureus*, *S. epidermidis*, as well as *Bacillus subtilis* with varying MIC values, showcasing broad-spectrum activity. Additionally, antifungal effects against *Candida albicans* and *Saccharomyces cerevisiae* highlight the potential of *Boesenbergia* in addressing fungal infections. These activities are attributed to the rich phytochemical composition of the extracts, which can disrupt microbial growth and survival. The antibiofilm properties of *B. rotunda* and *B. pandurata* are particularly significant, as biofilms are a major challenge in treating chronic infections. These extracts effectively inhibit biofilm formation with exhibitin IC_{50} and MIC values, indicating their utility in clinical applications.

Table 2.9

Biological activities of several Boesenbergia extracts

Bioactivity	Species	Description
Antibiofilm	*B. rotunda*	The extract significantly reduced growth of biofilm with IC_{50} 17.7 µg/mL (Kanchanapiboon et al., 2020)
	B. pandurata	The extract significantly prevented biofilm formation with MIC 7.81 µg/mL (Limsuwan et al., 2008)
Anti-ulcerogenic	*B. rotunda*	The intestinal mucosa was protected by pretreatment with the extract, shown from decreased area of ulcers along with mucosa content, as well as decreased or absent submucosal edema and leucocyte infiltration (Abdelwahab et al., 2011)
Wound healing	*B. rotunda*	The extract significantly improved wound healing in the HaCaT cell monolayer, as well as cell growth and scar healing, by promoting the MAPK and PI3K/Akt signaling mechanisms (Ruttanapattanakul et al., 2021)
	B. longiflora	The extract exhibited activity through fibroblasts migratory throughout the first day (77.3%) and the second day (100%), which also increased collagen stimulation (187.5 µg/mL) (Sudsai et al., 2013)
Antibacterial	*B. rotunda*	The extract neutralized the cultured *Staphylococci* with a MIC 16 µg/mL (Teethaisong et al., 2018)

Bioactivity	Species	Description
Anti-inflammatory	*B. longiflora*	The extract showed potent effect through NO inhibitory activity (IC_{50} value of 5.5 μg/mL), as well as reduced carrageenan-induced rodent paw inflammation (ED_{50} value of 222.7 mg/kg) (Sudsai et al., 2013)
	B. pandurata	Greatly lowered in aged rats when extract (200 mg/kg day-1) was given oral (Kim et al., 2018)
Anti-apoptotic	*B. rotunda*	All markedly decreased within the blood samples of DOX-treated mice by the extract (Zhang et al., 2023)
Anti-SARS-CoV-2	*B. rotunda*	Treatment using the extract post infection with viruses significantly inhibited. SARS-CoV-2 infected Vero E6 cells, having IC_{50} value of 3.62 μg/mL (Kanjanasirirat et al., 2020)
α-Glucosidase	*B. rotunda*	The extract significantly inhibited the activity of enzyme α-glucosidase as well as pancreatic lipase (100% inhibition) (Chatsumpun et al., 2017)
Anti-leptospiral	*B. rotunda*	The extract's MICs towards 24 pathogenic leptospiral bacteria were 125 μg/mL (Chander et al., 2016)
Cytotoxicity	*B. rotunda*	The methanol as well as hexane extracts reduced HK1 proliferation with IC_{50} values of 136 and 66 μg/mL, respectively (Break et al., 2021)

Bioactivity	Species	Description
Antimicrobial	*B. rotunda*	The displayed potential activity by inhibiting *S. aureus, S. epidermidis* and *B. subtilis* with MIC between 0.04 to 25 mg/mL (Jitvaropas et al., 2012)
Antifungal	*B. rotunda*	The displayed potential activity by inhibiting *Candida albicans* and *Saccharomyces cerevisiae* with MIC ranging between 0.16 to 25 mg/mL (Jitvaropas et al., 2012)
Antioxidant	*B. rotunda*	The FRAP and DPPH concentrations measured 22.2 µM/µg and 76.3 mg/mL, correspondingly (Jitvaropas et al., 2012)
Sexual behavior in rats	*B. rotunda*	Every dosage of extract greatly boosted relative testicular weight and seminiferous tubule diameter. A single dosage of 60 mg/kg considerably raised the relative weight of the seminal vesicle (Sudwan et al., 2007)
Immuno-stimulant	*B. pandurata*	The extract improves effectiveness delivered by the Pseudomonas sp. vaccination mainly strengthening immunity as well as resistance to illness in tilapia (Hardi et al., 2019)
Antioxidant	*B. pandurata*	The extract inhibited lipid peroxidation within brain of the mice homogenate (IC_{50} value of 26 µg/mL) (Shindo et al., 2006)

The anti-inflammatory properties of *B. longiflora* and *B. pandurata*, demonstrated through reduced nitric oxide production and downregulation of pro-inflammatory markers, suggest potential in managing inflammation-related disorders. The antioxidant capabilities of *Boesenbergia* extracts are noteworthy. For example, *B. rotunda* exhibits strong DPPH and FRAP assays. These properties could help combat inflammation along with its related problems, such as neurodegenerative illness.

The extracts of *Boesenbergia* species demonstrate promising effects on metabolic health. For instance, *B. pandurata* demonstrates anti-obesity activity by reducing body mass growth and levels of cholesterol. Additionally, the α-glucosidase inhibitory activity of *B. rotunda* points to its potential in managing diabetes by controlling postprandial glucose levels. Wound healing activities, particularly from *B. rotunda* and *B. longiflora*, underscore their regenerative properties. These extracts enhance fibroblast migration, collagen production, and key signaling pathways, making them suitable for skin repair applications.

The immune-boosting properties of *B. pandurata*, demonstrated by enhanced disease resistance in aquatic animals and increased mitochondrial biogenesis, highlight its role in immunomodulation. Moreover, *B. rotunda* shows significant antiviral action towards SARS-CoV-2, causing it a possible alternative in further exploration in antiviral therapies. Extracts from *Boesenbergia* species also display anti-ulcerogenic properties, sexual health benefits, and antiproliferative activity against cancer cells. For example, *B. rotunda* is proven to protect the intestinal membrane and improve testicular weight and seminal vesicle size in animal studies, indicating its potential for gastrointestinal and reproductive health.

CHAPTER 3

EXPERIMENTAL

3.1 Plant Materials

The rhizomes of *B. albosanguinea* (voucher number PL-13/23) were collected from Langgun Island, Langkawi (6.43449° or 6° 26' 4" N, 99.89486° or 99° 53' 42" E) in August 2023. The sample was identified by Dr. Shamsul Khamis (Universiti Kebangsaan Malaysia) and deposited at UKM Herbarium.

3.2 General Experimental Procedures

Hydrodistillation is a common method for extracting essential oils from plants, especially those with volatile compounds like leaves, flowers, seeds, and rhizomes. The process begins by preparing the plant material, usually by cleaning, chopping, and

sometimes drying it. Steam is generated and passed through the plant, causing the cells to break and release the essential oils, which evaporate with the steam. The steam and oil vapors travel through a tube to a condenser, where they cool and condense into liquid. The essential oil separates from the water-based component, called hydrosol, and floats on top due to its lower density. The oil is then separated, usually by decanting or using a separating funnel, and filtered to remove impurities. Hydrodistillation is a gentle, solvent-free method that preserves the essential oil's integrity, though it can be time-consuming and may cause loss or degradation of heat-sensitive compounds.

GC-FID and GC-MS are techniques used to analyze essential oils. In GC-FID, the sample is injected into a column where compounds are separated. After separation, they pass through a flame ionization detector (FID) that burns them, generating a current proportional to their concentration. This makes GC-FID sensitive for detecting hydrocarbons but provides limited structural information. In GC-MS, compounds are separated and then ionized in a mass spectrometer, where they are broken into fragments. These fragments create a mass spectrum, which helps identify the compounds. GC-MS gives detailed information on both the quantity and structure of compounds, making it more informative than GC-FID, though it's more complex to interpret.

Soxhlet extraction is a method used to extract lipophilic (fat-soluble) compounds from plant materials. This method is efficient and works well for extracting essential oils, fats, and waxes, but it can be time-consuming and requires careful monitoring to avoid overheating or excessive evaporation. Gravity column chromatography (CC), thin layer chromatography (TLC), and preparative thin layer chromatography (PTLC)

are techniques used to separate and purify compounds based on their chemical properties, such as polarity and solubility. Thin layer chromatography (TLC) is a fast and simple method to check the progress of a reaction or the purity of a compound. Preparative thin layer chromatography (PTLC) is a larger-scale version of TLC used to purify bigger amounts of compounds. Both methods are important for separating and purifying compounds, with the choice depending on the sample size and purity needed.

Structural elucidation is the process of determining the chemical structure of a compound, including the arrangement of atoms and functional groups. This is done using spectroscopic techniques like Infrared Spectroscopy (IR), Nuclear Magnetic Resonance (NMR), and Mass Spectrometry (MS), which provide complementary information. IR measures how a compound absorbs infrared light, revealing information about its functional groups based on bond vibrations. NMR analyzes how atomic nuclei interact with an external magnetic field, providing details on the types of atoms, their connectivity, and arrangement within the molecule. MS measures the molecular weight and fragmentation pattern of a compound. Together, these techniques allow for complete structural elucidation: IR identifies functional groups, NMR reveals atomic details and connectivity, and MS confirms molecular weight and fragmentation.

3.3. Extraction and Analysis of Essential Oil

The fresh rhizomes were cut into small pieces before being placed in a round bottom flask 5 L. After that, distilled water was added up to the point when it completely covers all samples. The mixture was put through a Clevenger apparatus in the flask and

the hydrodistillation process lasted for 4 h. Diethyl ether was used to extract the essential oil and water mixture, followed by drying the mixture over anhydrous $MgSO_4$ and filtering it. The essential oil was obtained after the filtrate was evaporated at room temperature.

The GC-FID analysis was carried out on a Shimadzu GC-2010 Plus (Shimadzu) gas chromatograph that fitted with an HP-5 column (25 m in length, 0.33 µm thick, and 0.20 mm in diameter). At a flow rate of 1.0 mL/min, helium was used as a carrier gas. The temperature of the flame ionisation detector (FID) injector was set to 250°C, while the temperature of the detector was set to 280°C. The oven's temperature was maintained at 50°C for 15 min before being gradually raised to 280°C at 5°C/min. The diluted samples (1/100 in diethyl ether, v/v) of 1.0 µL were injected manually (split ratio 10:1) (Salleh et al., 2016).

The Agilent GC-MS 7890A/5975C Series MSD (70 eV direct inlet) was used to record the GC-MS chromatograms together with the HP-5MS capillary column (30 m 0.25 mm internal diameter 0.25 µm film thickness). At a flow rate of 1 mL/min, helium was used as the carrier gas. The temperature of the injector was set at 250°C. The oven temperature was designed to rise from 50°C (5 min hold) to 250°C at 10 C/min, then to 117 for 15 min. For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. A scan rate of 0.5 s (cycle time: 0.2 s) was applied covering a mass range from 50 to 400 amu (Salleh et al., 2016).

The chemical components of the essential oil were identified by comparing their mass spectra to reference spectra from a library, such as the Wiley Library, which

contains a vast database of mass spectra for known compounds. Kovats Indices are numerical values based on the retention times of compounds in a chromatographic column, helping to confirm the identity of the components. These values are compared to literature data (Adams, 2001), which lists retention times for various volatile compounds. By comparing the retention times of compounds in the essential oil with those in the literature, their identity can be verified. For quantitative analysis, Flame Ionization Detection (FID) data is used.

3.4 Extraction and Analysis of Phytochemicals

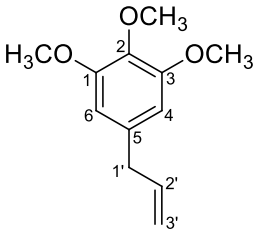
The soxhlet extraction method was implemented to obtain the crude extract from the dried rhizomes of *B. albosanguinea* using *n*-hexane, DCM, and MeOH as solvents. Gravity column chromatography (CC) was carried out using Merck SiO_2 (70-230 Mesh). Thin layer chromatography (TLC) analysis was performed on 0.20 mm precoated silica gel aluminium sheets (Merck Kieselgel $60F_{254}$). Preparative thin layer chromatography (PTLC) was conducted using a 1 mm thin glass plate of Merck SiO_2 $60F_{254}$. *n*-Hexane, EtOAc, DCM, $CHCl_3$, Et_2O, and MeOH were used as solvent systems in the chromatographic method. The spots on the TLC plate were detected by ultraviolet (UV) illumination at 254 nm and 365 nm, before being sprayed with vanillin-sulphuric acid reagent.

1D and 2D NMR spectra were recorded on a JEOL Spectrometer (JNM-ECX-500). Chemical shifts were reported in ppm. Residual solvents ($CDCl_3$ and DMSO) were used as an internal standard. The IR spectra were recorded on Perkin Elmer series

1600 spectrophotometer (KBr pellet for solid and NaCl discs for liquid sample). The UV spectra were recorded on the Shimadzu UV 1601PC spectrophotometer. Mass spectral data were acquired from Thermo Fisher Scientific's Liquid Chromatography Mass Spectrometry.

3.5 Isolation and Characterization of Major Component from Essential Oil

Thin-layer chromatography (TLC) analysis of the rhizome oil of *B. albosanguinea* revealed a major spot when developed with a solvent system of *n*-hexane and $CHCl_3$ (0.5:4.5). Preparative thin-layer chromatography (PTLC) purification of this spot yielded compound **(60)** as colourless oil (8.0 mg).



(60)

Elemicin **(60)**. Colourless oil (8.0 mg); R_f value 0.65 (Hex:EtOAc); IR (NaCl) υ_{max} (cm^{-1}): 3014 (sp^2 C-H), 2938 (sp^3 C-H), 1625 and 1500 (C=C), 1081 cm^{-1} (C-O); ^{1}H NMR (ppm): δ 3.36 (2H, d, *J* = 6.8 Hz, H-1'), 3.85 (3H, s, 2-OCH_3), 3.87 (6H, s, 1/3-OCH_3), 5.11 (1H, dd, *J* = 10.1 and 1.8 Hz, H-3'a), 5.13 (1H, dd, *J* = 17.0 and 1.8 Hz, H-3'b), 5.98 (1H, m, H-2'), 6.43 (2H, s, H-4/H-6); ^{13}C NMR (ppm): δ 40.5 (C-1'), 56.0 (1-OCH_3), 56.0 (3-OCH_3), 60.8 (2-OCH_3), 105.5 (C-4/C-6), 115.9 (C-3'a/C-3'b), 136.4 (C-2), 137.2 (C-2'), 153.2 (C-1/C-3), 135.7 (C-5); MS: *m/z* 208.1 [M^+] $C_{12}H_{16}O_3$.

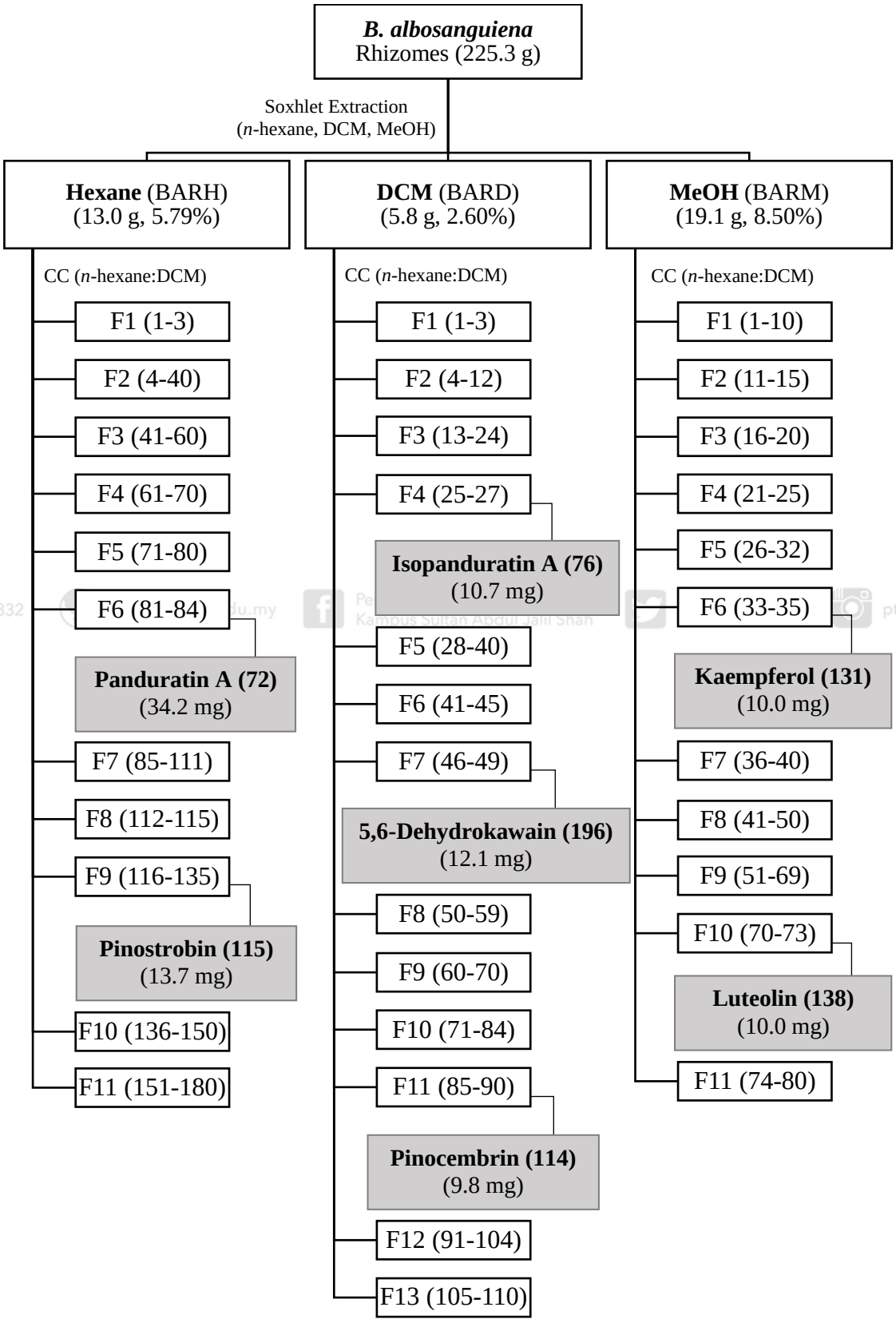
3.6 Isolation and Characterization of Phytochemicals

The dried rhizomes of *B. albosanguinea* were processed using soxhlet extraction using a polarity gradient of *n*-hexane, DCM, and MeOH as solvents. The sample was placed inside a thimble, which was then loaded into a soxhlet extractor. The soxhlet extractor was positioned on a flask containing the respective extraction solvent and equipped with a condenser. The solvent was heated to its reflux temperature, ensuring that any solvent vapor cooled and dripped back into the chamber housing the solid material. As the chamber filled with the warm solvent, some of the desired compounds dissolved. Once the soxhlet chamber was nearly full, the contents were emptied *via* the siphon and the solvent was returned to the distillation flask. This cycle was repeated for each solvent over the course of a day.

The *n*-hexane extract (13.0 g) was subjected to CC and eluted with *n*-hexane:DCM as solvent system to afford 11 major fractions (BARH F1-F11). Fraction F6 (50:50) had yielded compound **(72)** as white crystalline needle, whereas F9 (40:60) had yielded compound **(115)** as white solid. The DCM extract (5.8 g) was subjected to CC using *n*-hexane:DCM as solvent and managed to get 13 major fractions (BARD F1-F13). Fraction F4 (60:40) was successfully isolated compound **(76)** as yellow oil, while fraction F7 (40:60) had yielded compound **(196)** as white solid. In addition, fraction F11 (20:80) was successfully accomplished compound **(114)** as yellow solid. The MeOH extract (19.1 g) was subjected to CC using *n*-hexane:DCM to afford 11 major fractions (BARM F1-F11). Fraction F6 and F10 were further purified by washing with cold hexane to yield compound **(131)** and compound **(138)**, both as white solids. The purification process are summarized in Figure 3.1.

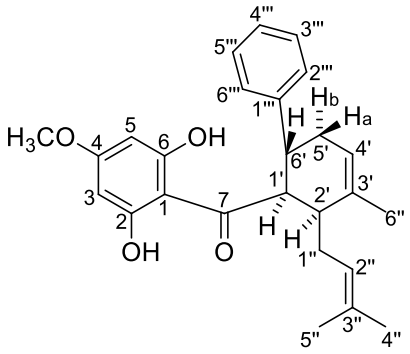
Figure 3.1

Flow chart for the purification process of B. albosanguinea rhizome extracts



3.7 Spectral Data of Isolated Phytochemicals

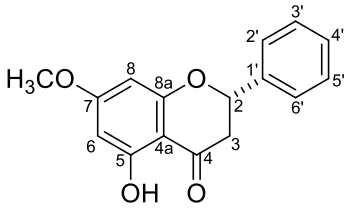
Modern spectroscopic techniques, including IR, NMR, and MS were utilized to successfully isolate seven compounds from the rhizome of *B. albosanguinea*. The characterization of these compounds was further confirmed through comparison with literature reports.



(72)

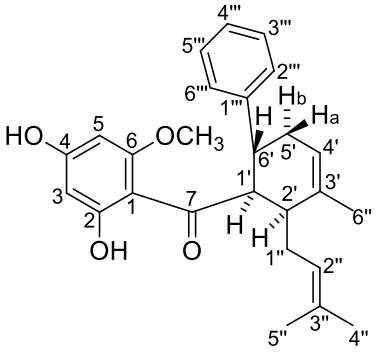
Panduratin A **(72)**. White crystalline needle (34.2 mg); R_f value 0.65 ($CHCl_3$:MeOH); IR (KBr) υ_{max} cm^{-1}: 3252 (OH), 2963 (*sp*^{2} C-H), 2906 (*sp3* C-H), 1630 (C=O), 1574 (C=C), 1163 (C-O stretching); ^{1}H NMR (ppm): δ 1.51 (6H, s, H-4"/H-5"), 1.77 (3H, s, H-6"), 2.05 (1H, ddd, *J* = 3.0, 11.8, 18.0 Hz, H-5'b), 2.16 (1H, ddd, *J* = 7.0, 7.5, 15.0 Hz, H-1″a), 2.27 (1H, ddd, *J* = 7.3, 7.3, 14.9 Hz, H-1″b), 2.38 (1H, ddd, *J* = 4.6, 7.0, 17.7 Hz, H-5'a), 2.62 (1H, ddd, *J* = 4.7, 7.4, 7.8 Hz, H-2'), 3.42 (1H, ddd, *J* = 6.2, 10.8, 10.9 Hz, H-6'), 3.74 (3H, s, 4-OCH_3), 4.67 (1H, dd, *J* = 4.7 Hz, H-1'), 4.85 (1H, t, *J* = 6.9 Hz, H-2"), 5.42 (1H, dd, *J* = 3.0, 5.0 Hz, H-4'), 5.87 (2H, s, H-3/H-5), 7.10 (1H, m, H-4'''), 7.21 (4H, m, H-2'''/H-3'''/H-5'''/H-6'''); ^{13}C NMR (ppm): δ 17.3 (C-5"), 22.9 (C-6"), 25.5 (C-4"), 29.6 (C-1"a/C-1"b), 35.8 (C-5'a/C-5'b), 37.1 (C-6'), 41.8 (C-2'), 54.0 (C-1'), 55.0 (4-OCH_3), 96.6 (C-3/C-5), 105.6 (C-1), 122.6 (C-4'), 124.1 (C-

2"), 125.6 (C-4'"), 127.2 (C-2'"/C-6'"), 127.9 (C-3'"/C-5'"), 132.4 (C-3"), 137.3 (C-3'), 145.7 (C-1'"), 167.6 (C-4), 167.6 (C-2), 207.0 (C-7); MS: *m/z* 407.2 $[M+H]^+$ $C_{26}H_{31}O_4$.



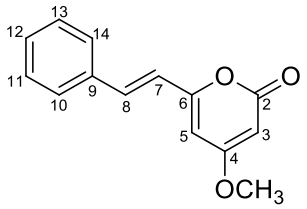
(115)

Pinostrobin **(115)**. White solid (13.7 mg); R_f value 0.42 (Hex:EtOAc); IR (KBr) υ_{max} (cm^{-1}): 3227 (OH), 2975 (sp^3 C-H), 1618 (C=O), 1576 (C=C), 1284 (C-O); ^{1}H NMR (ppm): δ 2.85 (1H, dd, *J* = 3.0, 17.1 Hz, H-3b), 3.12 (1H, dd, *J* = 13.0, 17.1 Hz, H-3a), 3.84 (3H, s, 7-OCH_3), 5.45 (1H, dd, *J* = 3.0, 13.1 Hz, H-2), 6.09 (1H, d, *J* = 2.3 Hz, H-8), 6.11 (1H, d, *J* = 2.2 Hz, H-6), 7.42 (1H, m, H-3'/H-4'/H-5'), 7.47 (1H, m, H-2'/H-6'), 12.05 (1H, s, 7-OH); ^{13}C NMR (ppm): δ 43.4 (C-3a/b), 55.7 (7-OCH_3), 79.3 (C-2), 94.3 (C-8), 95.2 (C-6), 103.2 (C-4a), 126.2 (C-2'/C-6'), 128.9 (C-3'/C-4'/C-5'), 138.4 (C-1'), 162.8 (C-8a), 164.2 (C-5), 168.0 (C-7), 195.8 (C-4); MS: *m/z* 271.0 $[M+H]^+$ $C_{16}H_{15}O_4$.



(76)

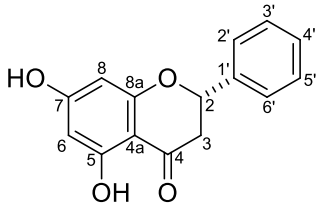
Isopanduratin A **(76)**. Yellow oil (10.7 mg); R_f value 0.60 ($CHCl_3$:MeOH); IR (NaCl) υ_{max} (cm^{-1}): 3288 (OH), 3021 (sp^2 C-H), 2923 (sp^3 C-H), 1622 (C=O), 1593 (C=C), 1164 (C-O); ^{1}H NMR (ppm): δ 1.52 (6H, s, H-4''/H-5''), 1.81 (3H, s, H-6''), 2.05 (1H, m, H-5'b), 2.10 (1H, m, H-1'a), 2.26 (1H, m, H-1'b), 2.43 (1H, m, H-5'a), 2.52 (1H, m, H-2'), 3.44 (1H, m, H-6'), 3.92 (3H, s, 6-OCH_3), 4.52 (1H, dd, *J* = 4.7 and 11.3 Hz, H-1'), 4.88 (1H, t, *J* = 7.0 Hz, H-2''), 5.45 (1H, br.s, H-4'), 5.94 (1H, s, H-3), 5.96 (1H, s, H-5), 7.11 (1H, m, H-4'''), 7.21 (2H, m, H-2'''/H-6'''), 7.23 (2H, m, H-3'''/H-5'''); ^{13}C NMR (ppm): δ 17.8 (C-5''), 22.9 (C-6''), 25.6 (C-4''), 28.9 (C-1''a/b), 35.8 (C-5'a/b), 37.1 (C-6'), 42.6 (C-2'), 54.1 (C-1'), 55.7 (6-OCH_3), 90.9 (C-5), 96.8 (C-3), 106.6 (C-1), 120.9 (C-4'), 124.1 (C-2''), 125.5 (C-4'''), 127.0 (C-2'''/6'''), 128.3 (C-3'''/5'''), 131.8 (C-3''), 137.2 (C-3'), 147.1 (C-1'''), 162.7 (C-4/6), 167.5 (C-2), 206.3 (C-7); MS: *m/z* 405.2 $[M-H]^+$ $C_{26}H_{29}O_4$.



(196)

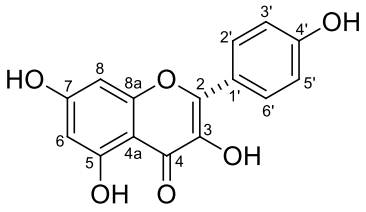
5,6-Dehydrokawain **(196)**. White solid (12.1 mg); R_f value 0.50 ($CHCl_3$:MeOH); IR (KBr) υ_{max} (cm^{-1}): 3076 (sp^2 C-H), 2951 (sp^3 C-H), 1720 (C=O), 1554 and 1447 (C=C), 1256 (C-O); ^{1}H NMR (ppm): δ 3.85 (3H, s, 4-OCH_3), 5.52 (1H, d, *J* = 2.2 Hz, H-3), 5.97 (1H, d, *J* = 2.1 Hz, H-5), 6.61 (1H, d, *J* = 16.0 Hz, H-7), 7.36-7.42 (1H, m, H-10/H-11/H-12/H-13/H-14), 7.54 (1H, m, H-8); ^{13}C NMR (ppm): δ 55.9 (4-OCH_3), 88.8 (C-3), 101.3 (C-5), 118.6 (C-7), 127.4 (C-10/C-14), 128.9 (C-11/C-13),

129.4 (C-12), 135.2 (C-8), 135.8 (C-9), 158.6 (C-4), 163.9 (C-6), 171.0 (C-2); MS: *m/z* 229.0 $[M+H]^+$ $C_{14}H_{13}O_3$.



(114)

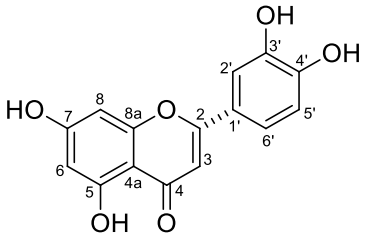
Pinocembrin **(114)**. Yellow solid (9.8 mg); R_f value 0.55 ($CHCl_3$:MeOH); IR (KBr) υ_{max} (cm^{-1}): 3157 (OH), 2891 (sp^3 C-H), 1630 (C=O), 1582 (C=C), 1166 (C-O); ^{1}H NMR (ppm): δ 2.86 (1H, dd, *J* = 3.0, 17.1 Hz, H-3b), 3.12 (1H, dd, *J* = 3.0, 17.1 Hz, H-3a), 5.45 (1H, dd, *J* = 3.0, 13.0 Hz, H-2), 6.00 (2H, s, H-8), 6.03 (2H, s, H-6), 7.41-7.49 (1H, m, H-2'/H-3'/H-4'/H-5'/H-6'), 12.07 (1H, s, 5-OH); ^{13}C NMR (ppm): δ 43.4 (C-3a/b), 79.3 (C-2), 95.5 (C-8), δ 96.8 (C-6), 103.3 (C-4a), 126.2 (C-2'/C-6'), 128.9 (C-3'/C-4'/C-5'), 138.3 (C-1'), 163.1 (C-8a), 164.4 (C-5), 164.5 (C-7), 195.8 (C-4); MS: *m/z* 257.0 $[M+H]^+$ $C_{15}H_{13}O_4$.



(131)

Kaempferol **(131)**. White solid (10.0 mg); R_f value 0.55 ($CHCl_3$:MeOH); IR (KBr) υ_{max} (cm^{-1}): 3421 (OH), 1661 (C=O), 1604 and 1447 (C=C), 1256 (C-O); ^{1}H NMR (ppm): δ 6.15 (1H, d, *J* = 2.0 Hz, H-6), 6.40 (1H, d, *J* = 2.0 Hz, H-8), 6.89 (1H, d, *J* = 8.8 Hz, H-3'/H-5'), 8.00 (1H, d, *J* = 8.8 Hz, H-2'/H-6'), 12.44 (1H, s, 5-OH); ^{13}C

NMR (ppm): δ 94.0 (C-8), 98.7 (C-6), 103.5 (C-4a), 115.9 (C-3'/C-5'), 122.2 (C-1'), 130.0 (C-2'/C-6'), 136.2 (C-3), 147.3 (C-2), 156.7 (C-8a), 159.7 (C-4'), 161.2 (C-5), 164.4 (C-7), 176.4 (C-4); MS: *m/z* 287.1 $[M+H]^+$ $C_{15}H_{11}O_6$.



(138)

Luteolin **(138)**. White solid (10.0 mg): R_f value 0.62 ($CHCl_3$:MeOH); IR (KBr) υ_{max} (cm^{-1}): 3222 (OH), 1661 (C=O), 1603 and 1447 (C=C), 1256 (C-O); ^{1}H NMR (ppm): δ 6.00 (1H, d, *J* = 2.0 Hz, H-6), 6.25 (1H, d, *J* = 2.0 Hz, H-8), 6.47 (1H, s, H-3), 6.74 (1H, d, *J* = 8.1 Hz, H-5'), 7.28 (1H, d, *J* = 1.7 Hz, H-6'), 7.31 (1H, d, *J* = 2.3 Hz, H-2'), 12.89 (1H, s, 5-OH); ^{13}C NMR (ppm): δ 94.8 (C-8), 100.1 (C-6), 102.3 (C-3), 102.8 (C-4a), 112.8 (C-2'), 116.3 (C-5'), 119.4 (C-6'), 120.4 (C-1'), 146.9 (C-3'), 152.4 (C-4'), 158 (C-8a), 161.8 (C-5/C-7), 164.1 (C-2); MS: *m/z* 285.0 $[M-H]^+$ $C_{15}H_9O_6$.

3.8 Antioxidant Activity

The DPPH free radical scavenging assay of the crude extracts and phytochemicals were investigated as designated by Salleh et al. (2016) with slight modifications. The DPPH solution was freshly prepared in MeOH. The samples in MeOH (200 µL) with a series of concentrations (200, 150, 100, 50, and 25 µg/mL) were mixed with the DPPH solution (3.8 mL). The mixture was kept aside for 30 min at room temperature in the

dark, and the absorbance was measured at 517 nm. The percentage inhibition of DPPH (%) was calculated using the following formula:

$$I\% = \left[A_{blank} - A_{sample} / A_{blank}\right] \times 100$$

The IC_{50} value, which is the amount of antioxidant required to reduce the initial DPPH concentration by 50%, will be determined from the obtained result. As a standard, ascorbic acid was used and diluted to the same concentration as the samples. The tests were conducted in triplicate.

3.9 Anti-inflammatory Activity

The anti-inflammatory of the crude extracts and isolated phytochemicals was assessed using the lipoxygenase assay, with slight adjustments to the method described by Salleh et al. (2016).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Analysis of Essential Oil Composition

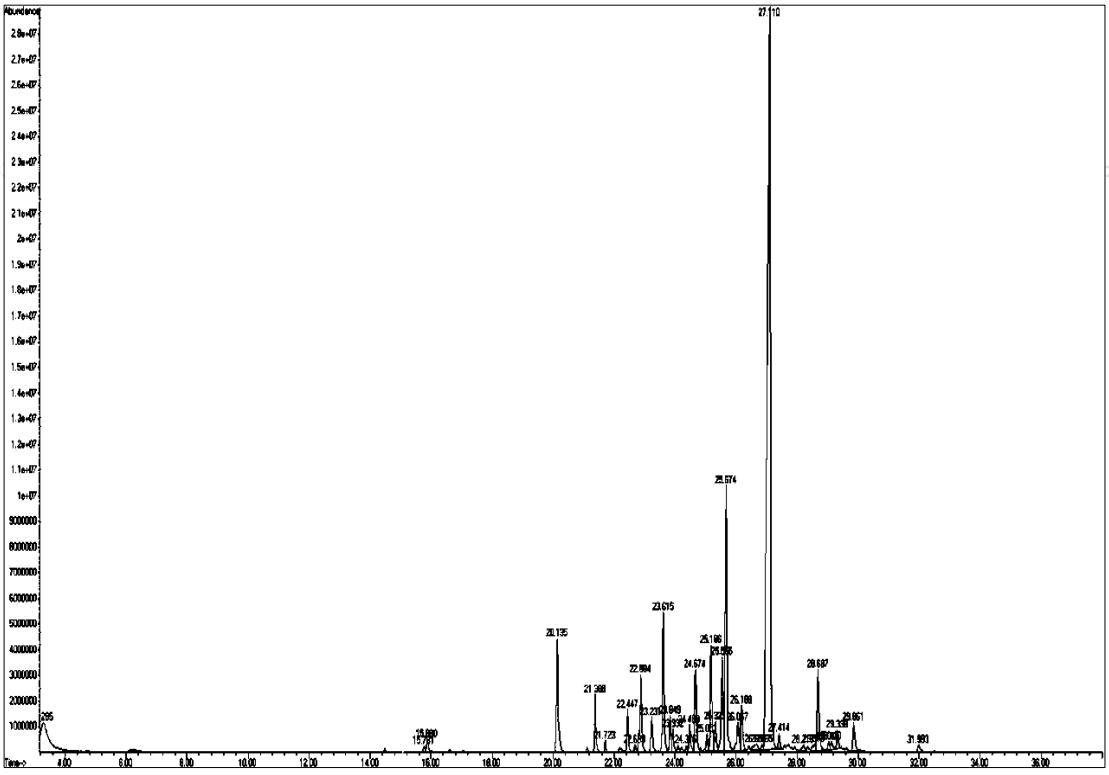
This study investigated the essential oil composition of *B. albosanguinea*. The essential oil was extracted from the fresh rhizome (400 g) by using the hydrodistillation method for 4 h in a Clevenger-type apparatus. The essential oil had a cloudy, opaque, and white appearance, with the percentage yield of 0.5% based on the fresh weight basis.

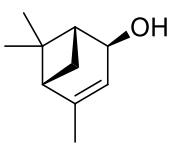
The GC-FID and GC-MS analysis (Figure 4.1) of the rhizome oil of the *B. albosanguinea* led to the identification of 34 components, representing 96.7% of the oil. The chemical components identified are listed in Table 4.1. Analysis of the chemical components successfully identified the rhizome oil consist of several groups of components with phenylpropanoid (54.3%) as the major group components, followed by sesquiterpene hydrocarbons (39.0%), oxygenated sesquiterpenes (2.6%), and

oxygenated monoterpenes (0.8%). The most abundant components were elemicin **(60)** (44.0%), α-gurjunene **(206)** (9.3%), β-caryophyllene **(3)** (4.5%), and safrole **(200)** (4.1%). The rhizome oil also contained substantial amounts of sesquiterpene hydrocarbons, comprising twenty components that accounted for 39.0% of the total composition. This fraction was predominantly constituted by α-gurjunene **(206)** (9.3%), β-caryophyllene **(3)** (4.5%), germacrene D **(14)** (3.6%), bicyclogermacrene **(213)** (3.4%), and α-elemene **(212)** (3.3%).

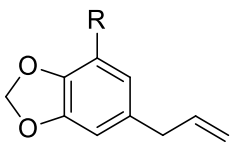
Figure 4.1

Chromatogram of B. albosanguinea essential oil

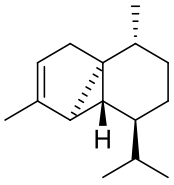




(199)



(200) R = H
(215) R = OCH_3



(201)

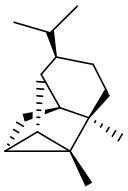
Table 4.1

Chemical components identified from B. albosanguinea essential oil

No	Components	KI[a]	KI[b]	Percentage (%)
1	Linalool (2)	1095	1095	0.2
2	*trans*-Verbenol (199)	1140	1140	0.4
3	Camphor (42)	1142	1141	0.2
4	Safrole (200)	1285	1285	4.1
5	α-Cubebene (201)	1342	1345	0.3
6	Cyclosativene (202)	1370	1369	0.2
7	α-Copaene (203)	1375	1374	1.8
8	Isoledene (204)	1376	1376	0.3
9	β-Cubebene (205)	1385	1387	0.2
10	β-Elemene (11)	1390	1389	2.4
11	Methyl eugenol (63)	1402	1403	1.2
12	α-Gurjunene (206)	1410	1409	9.3
13	Aristolene (207)	1415	1416	0.4
14	β-Caryophyllene (3)	1418	1417	4.5
15	β-Copaene (208)	1430	1430	1.5
16	γ-Elemene (27)	1436	1434	2.6
17	Aromandendrene (209)	1440	1439	1.2
18	α-Humulene (36)	1450	1452	1.0
19	*epi*-Cubebol (210)	1452	1453	1.6
20	Alloaromadendrene (211)	1460	1458	0.4
21	α-Elemene (212)	1476	1477	3.3

No	Components	KI[a]	KI[b]	Percentage (%)
22	γ-Muurolene (39)	1478	1478	0.7
23	Germacrene D (14)	1484	1484	3.6
24	Bicyclogermacrane (213)	1502	1500	3.4
25	α-Bourbonene (214)	1505	1509	0.2
26	Myristicin (215)	1515	1517	2.9
27	δ-Cadinene (216)	1520	1522	1.7
28	Elemicin (60)	1552	1555	44.0
29	γ-Asarone (217)	1570	1572	0.7
30	Spathulenol (7)	1580	1578	0.3
31	Guaiol (218)	1600	1602	0.3
32	(*Z*)-Asarone (219)	1615	1616	1.2
33	τ-Muurolol (220)	1645	1644	0.4
34	(*E*)-Asarone (158)	1675	1675	0.2
Group components				
	Phenylpropanoids			54.3
	Sesquiterpene hydrocarbons			39.0
	Oxygenated sesquiterpenes			2.6
	Oxygenated monoterpenes			0.8
Total identified				96.7

KI[a] – Kovats index experimental
KI[b] – Kovats index from literature (Adams, 2001)



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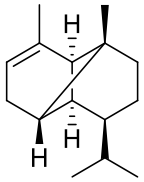

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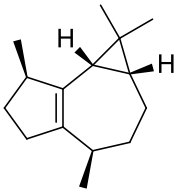

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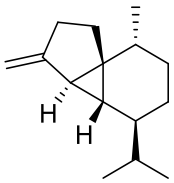

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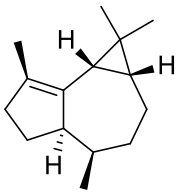

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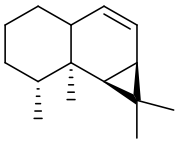

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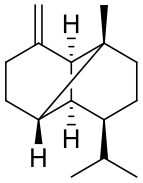

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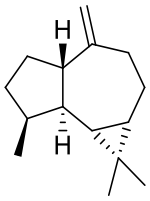

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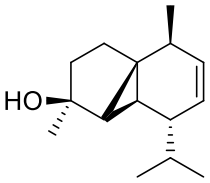

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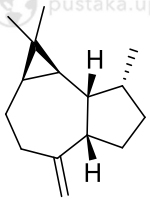

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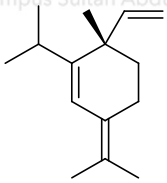

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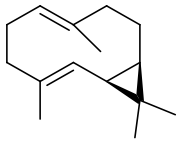

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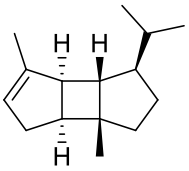
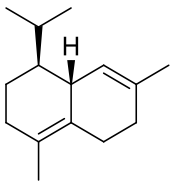
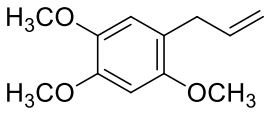


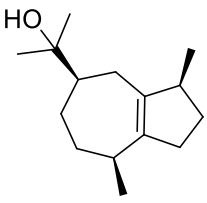
(214)



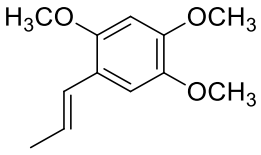
(216)



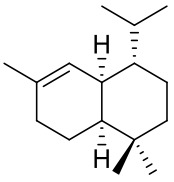
(217)



(218)



(219)



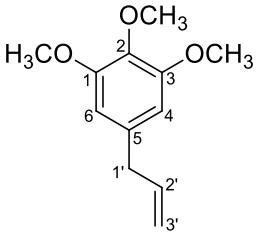
(220)

The composition of the rhizome oil exhibited both similarities and differences compared to previous reports in the literature. Consistent with prior studies, α-pinene **(21)**, β-pinene **(4)**, and limonene **(56)** emerge as major components, reflecting the typical monoterpene hydrocarbons found in *Boesenbergia* species (Hieu et al., 2023). However, notable differences may exist in the relative abundance or presence of minor components. A review of the existing literature on essential oils of the genus *Boesenbergia* revealed that elemicin **(60)** was found predominantly in *B. xiphostachya* (Suphrom et al., 2019) and from the hexane fraction of *B. pulcherrima* roots (Park et al., 2020). These subtle differences in the chemical components may be attributed to the differences in environmental and genetic factors, chemotype, and nutritional status of the plants, which may influence their oil composition (Salleh et al., 2016).

Elemicin **(60)**, an alkenylbenzene constituent of natural oils of several plant species, is widely distributed in food, dietary supplements, and medicinal plants. It shows extensive pharmacological effects, including antimicrobial, antioxidant, anti-acetylcholinesterase, and antiviral activity. Recently, it has attracted attention due to its potential for eliciting toxicity and hallucinatory side-effects (Zhang et al., 2022; Andrea et al., 2008).

4.2 Isolation of Major Component from *B. albosanguinea* Essential Oil

4.2.1 Elemicin (60)



(60)

Compound A **(60)** was isolated as a colourless oil and gave a pungent smell. The IR spectrum (Figure 4.2A) revealed absorption bands corresponding to stretching vibrations at 3014 cm^{-1} (sp^2 C-H), 2938 cm^{-1} (sp^3 C-H), 1625 and 1500 cm^{-1} (C=C), and 1081 cm^{-1} (C-O).

The ^{1}H NMR spectrum (Figure 4.2B) reveals a three singlet signals δ 3.87 and 3.85 corresponded to methoxyl group at C-1, C-2 and C-3. Another singlet signal resonance at δ 6.43 indicating the presence of aromatic protons of H-4 and H-6. A doublet signal appeared at δ 3.36 with a coupling constant of 6.8 Hz, characteristic of methylene proton of H-1'. Besides, a doublet of doublets signal was displayed at δ 5.11 which attributed to proton H-3'a and H-3'b. A multiplet signal at δ 5.98 integrated for was assigned to methine proton, H-2'. Assignment of the above protons were further established by the COSY spectrum (Figure 4.2C) by the correlation of prenyl groups of H-1' (δ 3.36), H-2' (δ 5.98) and H-3' (δ 5.11-5.13) were found correlated to each other.

The ^{13}C NMR spectrum (Figure 4.2D) showed the presence of twelve carbons which attributed to four quaternary, three methine, two methylene, and three methoxyl carbons. The DEPT spectra (Figure 4.2E) revealed the existence of quaternary carbons resonate at δ 153.2 (C-1/C-3) and 136.4 (C-2). The methine carbons were observed at δ 105.5 (C-4/C-6) and 137.2 (C-2'). The methylene carbons resonate at δ 40.5 (C-1') and 115.9 (C-3'). The methoxyl carbons were presented at δ 56.0 (1/3-OCH_3) and 60.8 (2-OCH_3). The ^{13}C NMR data were supported by the MS spectrum (Figure 4.2F) which gave the molecular ion peak at *m/z* 208.1, in accordance with a molecular formula $C_{12}H_6O_3$. The complete ^{1}H and ^{13}C NMR data are tabulated in Table 4.2.

The HMQC spectrum (Figure 4.2G) revealed the signal at δ 6.43 (H-4/ H-6) correlated with the carbon signal at δ 105.5 confirming these protons are attached to C-4 and C-6. The doublet at δ 3.36 (H-1') were correlated with the carbon signal at δ 40.5 (C-1'). The multiplet at δ 5.98 (H-2'), correlating with the carbon signal at δ 137.2 (C-2′). The double doublets at δ 5.11-5.13 (H-3') correlating with the carbon signal at δ 115.9 (C-3'). In addition, the HMBC spectrum (Figure 4.2H), the methoxyl protons at δ 3.87 (1/3-OCH_3) and 3.85 (2-OCH_3) show correlations with the aromatic carbons, specifically at δ 153.2 (C-1/C-3) and δ 136.4 (C-2), further confirming the positions of the methoxy groups in the aromatic ring.

Based on the comparison of spectral data, compound **(60)** was identified as 5-allyl-1,2,3-trimethoxybenzene or commonly known as elemicin. This compound was reported previously from the hexane fraction of *B. pulcherrima* roots extract (Park et al., 2020).

Table 4.2

*NMR data of elemicin **(60)** and literature*

C	δ_H (ppm) (Int. mult. *J* in Hz) ($CDCl_3$, 500 MHz)	δ_C (ppm)	δ_H (ppm) (Int. mult. *J* in Hz) ($CDCl_3$, 400 MHz)[a]	δ_C (ppm)[a]
1	-	153.2	-	153.0
2	-	136.4	-	136.1
3	-	153.2	-	153.0
4	6.43 (2H, s)	105.5	6.43 (2H, s)	104.5
5	-	135.7	-	135.7
6	6.43 (2H, s)	105.5	6.43 (2H, s)	104.5
1'	3.36 (2H, d, 6.8)	40.5	3.30 (2H, d, 6.6)	40.4
2'	5.98 (1H, m)	137.2	5.91 (1H, m)	137.1
3'a	5.11 (1H, dd, 10.1, 1.8)	115.9	5.03 (1H, dd, 9.6, 1.2)	115.9
3'b	5.13 (1H, dd, 17.0, 1.8)	115.9	5.10 (1H, dd, 17.2, 1.6)	115.9
1-OCH_3	3.87 (6H, s)	56.0	3.95 (9H, s)	55.9
2-OCH_3	3.85 (3H, s)	60.8	3.95 (9H, s)	61.3
3-OCH_3	3.87 (6H, s)	56.0	3.95 (9H, s)	55.9

[a]Data from literature (Luc et al., 2020)

Figure 4.2A

*IR spectrum of elemicin **(60)***

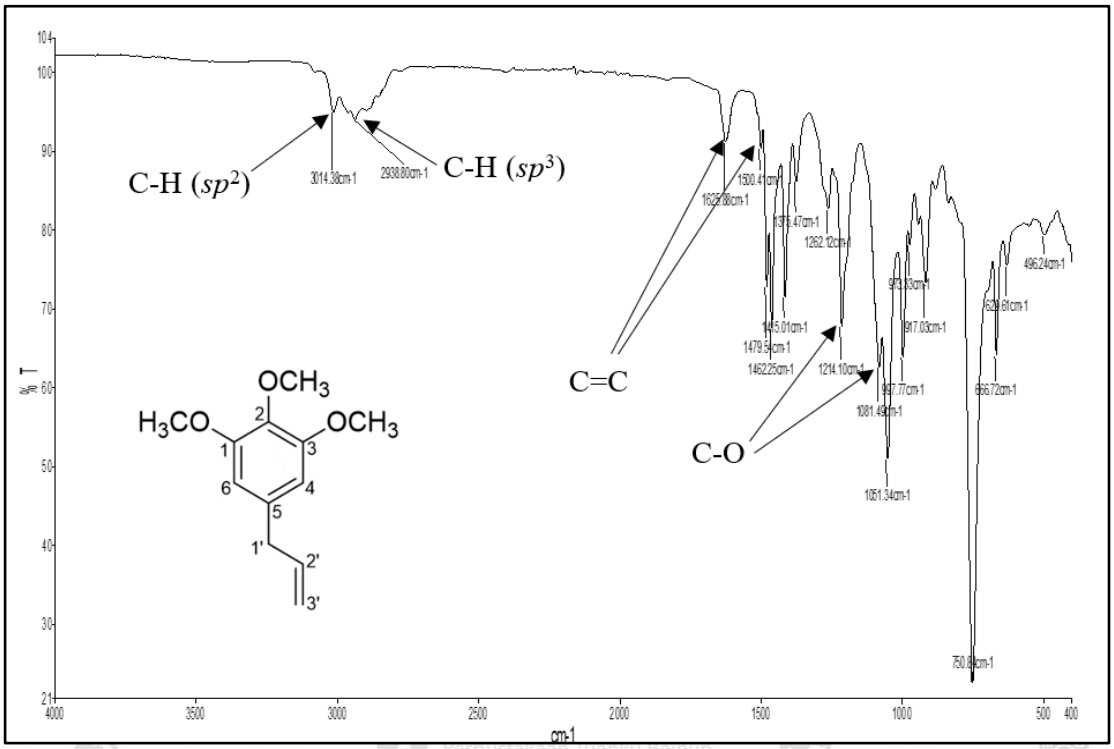


Figure 4.2B

*^{1}H NMR spectrum of elemicin **(60)***

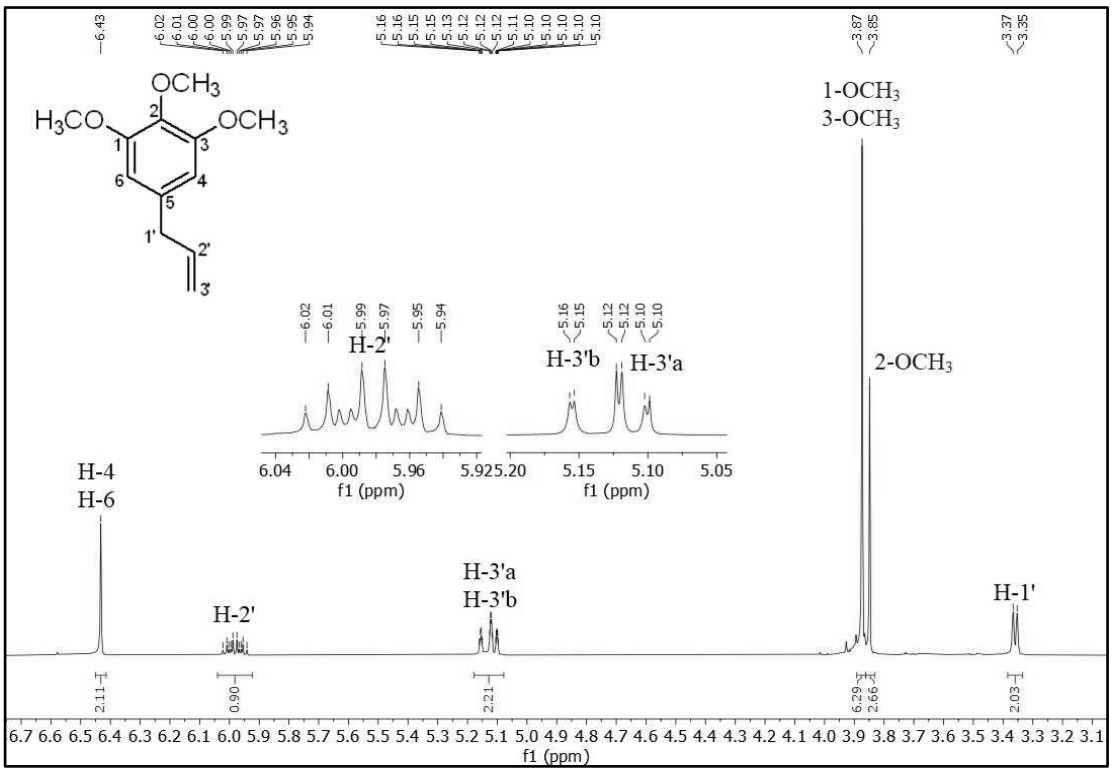


Figure 4.2C

*COSY spectrum of elemicin **(60)***

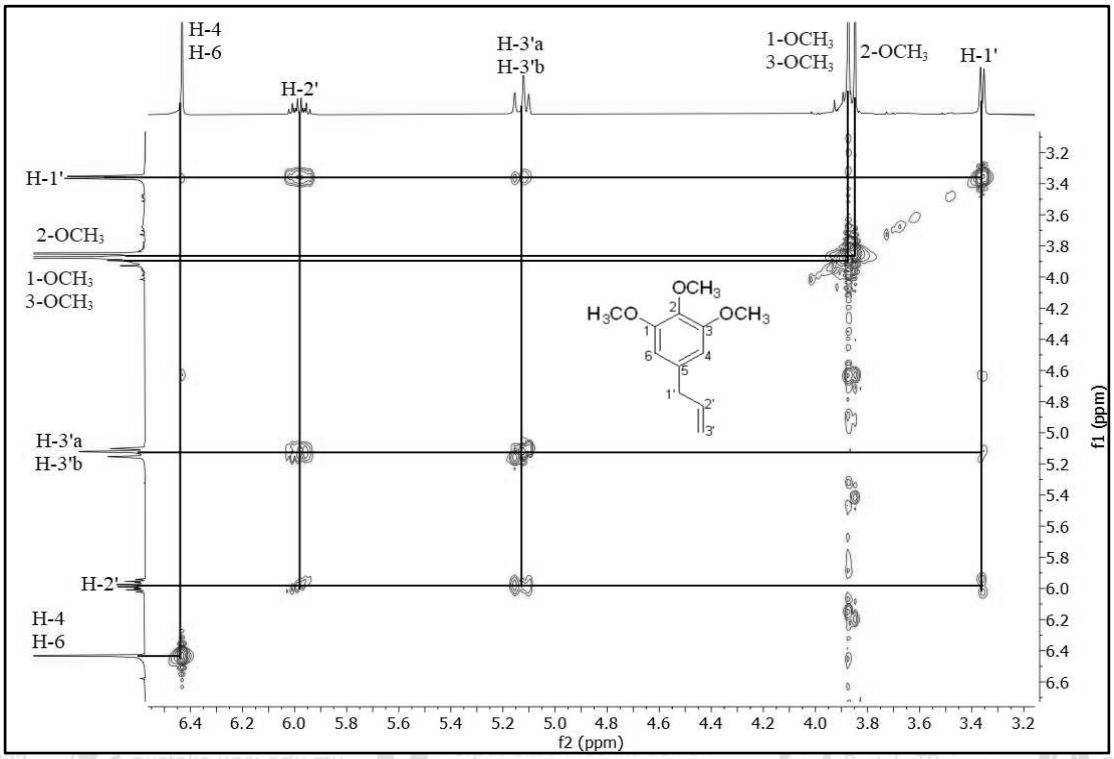


Figure 4.2D

*^{13}C NMR spectrum of elemicin **(60)***

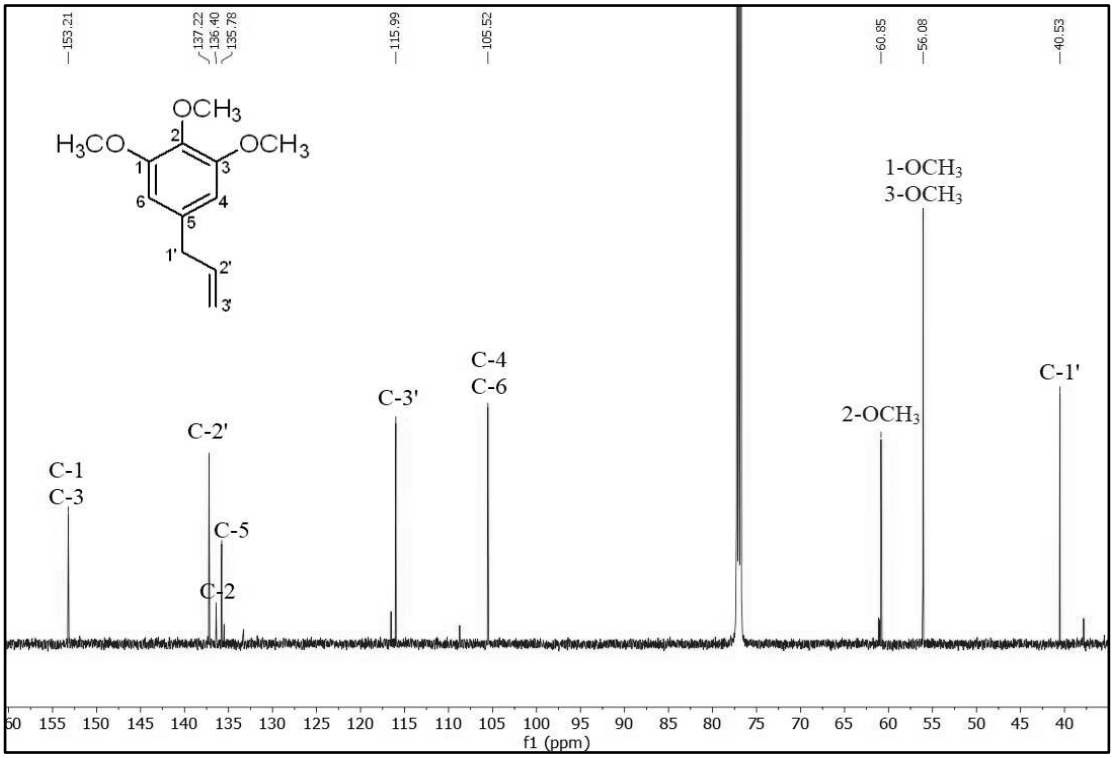


Figure 4.2E

*DEPT spectra of elemicin **(60)***

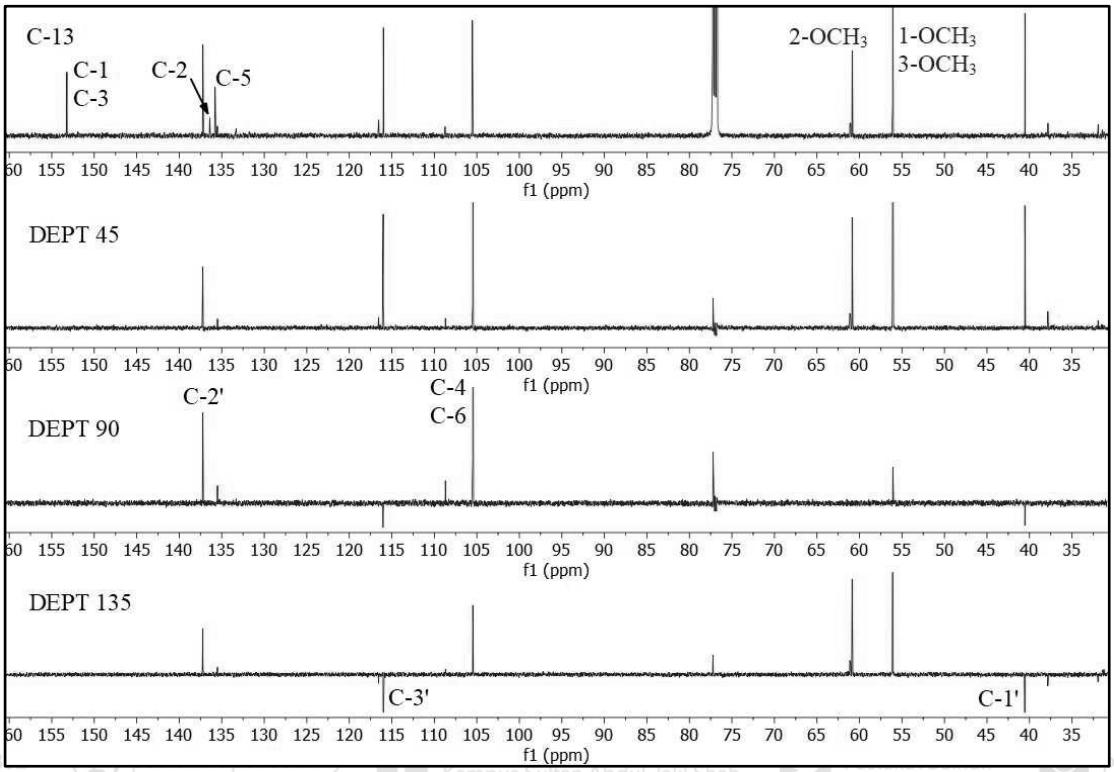


Figure 4.2F

*MS spectrum of elemicin **(60)***

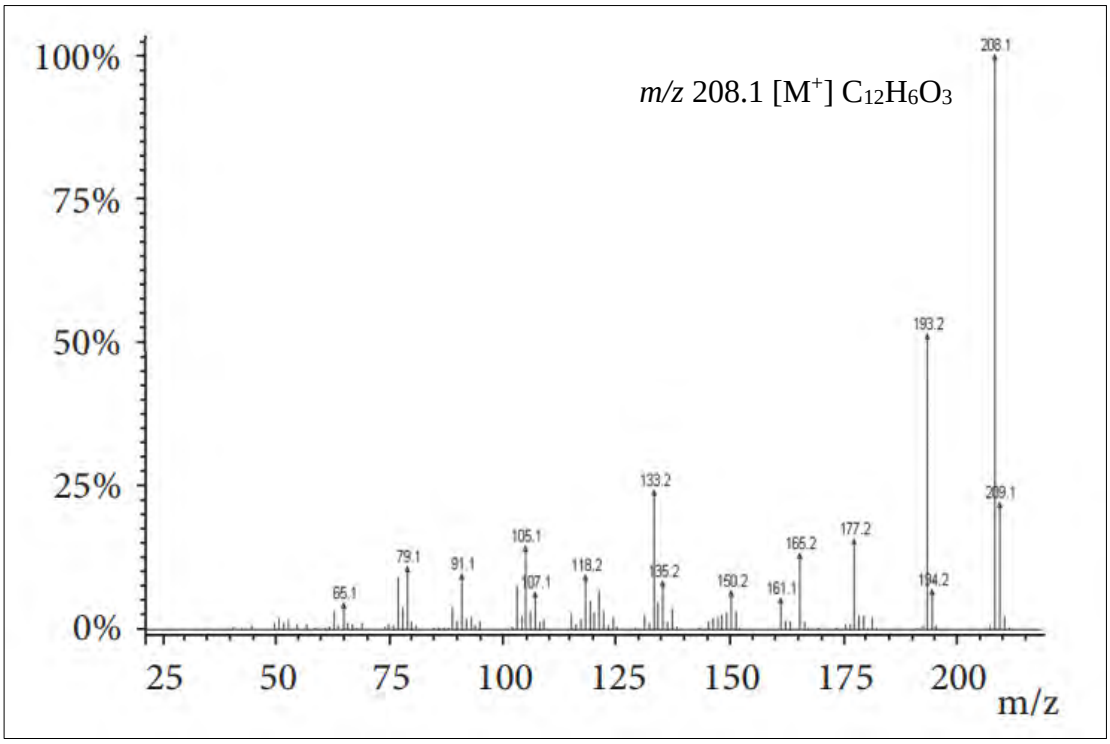


Figure 4.2G

*HMQC spectrum of elemicin **(60)***

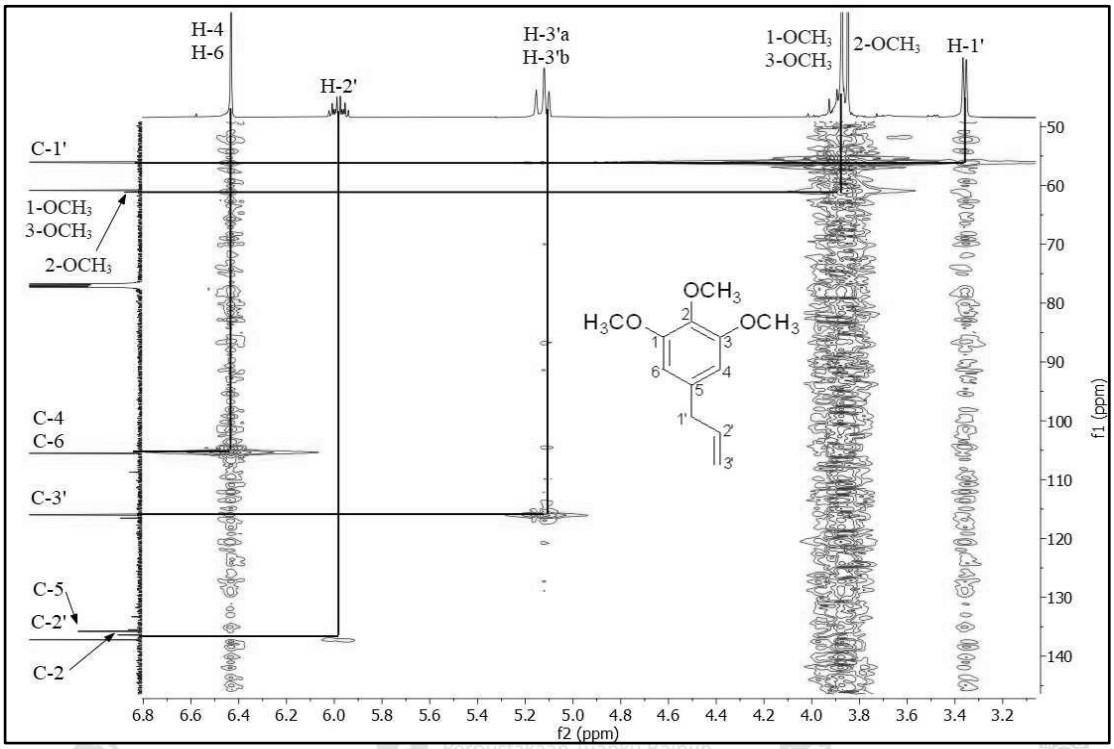
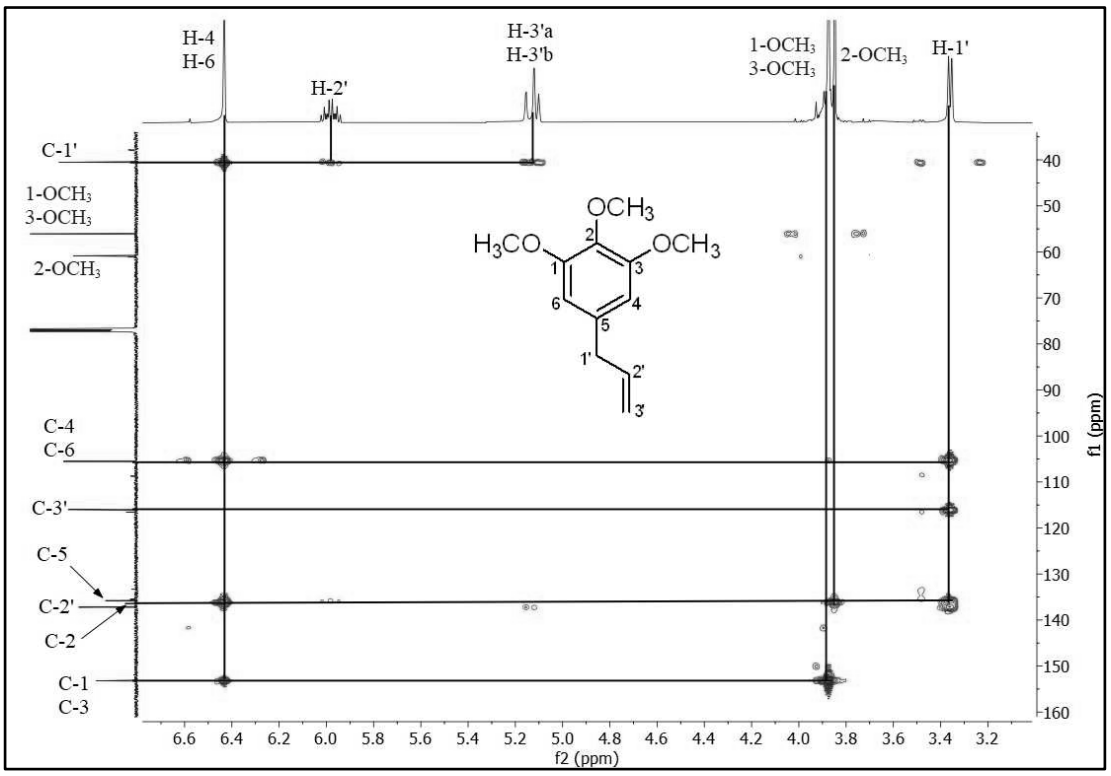


Figure 4.2H

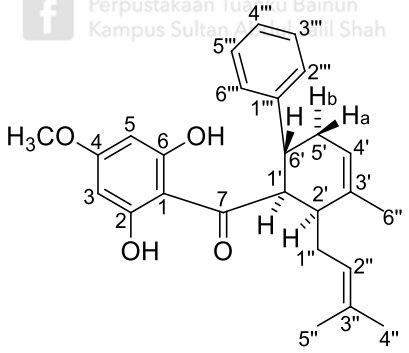
*HMBC spectrum of elemicin **(60)***



4.3 Phytochemical Studies of the Rhizomes of *Boesenbergia albosanguinea*

Isolation and purification from the rhizome extracts of *B. albosanguinea* had successfully afforded seven compounds. They were identified as panduratin A **(72)**, isopanduratin A **(76)**, 5,6-dehydrokawain **(196)**, pinostrobin **(115),** pinocembrin **(114)**, kaempferol **(131)** and luteolin **(138)**. All isolated compounds have been reported previously from various *Boesenbergia* species. To the best of our knowledge, this is the first report on the phytochemical studies of *B. albosanguinea* from Malaysia.

4.3.1 Panduratin A (72)



(72)

Isolation of the *n*-hexane extract by CC using n-hexane:DCM afforded compound **(72)** as a white crystalline needle. The IR spectrum (Figure 4.3A) exhibited the presence of O-H stretching at 3252 cm^{-1}. In addition, it showed the signals of sp^2 C-H stretching at 2963 cm^{-1}. The C=O stretching of the conjugated ketone was observed at 1630 cm^{-1}, and the C=C appeared at 1574 cm^{-1}. The absorption at 1163 cm^{-1} corresponds to the stretching of the C-O bond.

The ^{1}H NMR spectrum (Figure 4.3B) revealed a singlet signals assignable to three methyls at δ 1.51 (H-4" and H-5") and 1.77 (H-6"). Two sets doublet of doublets of doublets appeared at δ 2.05/2.38 (H-5'a/H-5'b) and δ 2.16/2.27 (H-1"a/H-1"b) which attributed to methylene protons. Besides, three methine protons was displayed at δ 2.62 (ddd), 3.42 (ddd), 4.67 (dd) which were corresponded to H-2', H-6', and H-1', respectively. Two tri-substituted olefins were resonated at δ 4.85 (H-2") as triplet (*J* = 6.9 Hz) and 5.42 (H-4') as doublet of doublets (*J* = 3.0 and 5.0 Hz). In addition, seven aromatic protons were discovered at δ 5.87 (H-3/H-5), 7.10 (H-4'"), 7.21 (H-2'"/H-6'"/H-3'"/H-5'"), together with a methoxyl group at δ 3.74 (4-OCH_3). Furthermore, the COSY spectrum (Figure 4.3C) showed the correlation of protons H-1' (δ 4.67) with H-2' (δ 2.62) and H-6' (δ 3.42). In addition, proton H-4' (δ 5.42) was found to correlate with H-5' (δ 2.05/2.38).

The ^{13}C NMR spectrum (Figure 4.3D) exhibited 26 signals of carbon present as one methoxyl carbon at δ 55.0 (4-OCH_3), one carbonyl at δ 207.0 (C-7), two methylene carbons 29.6 (C-1"), 35.8 (C-5'), three methyl carbons at δ 17.3 (C-5"), 22.9 (C-6"), 25.5 (C-4"), seven quaternary carbons δ 41.8 (C-2'), 105.6 (C-1), 137.3 (C-3'), 145.7 (C-1'"), 163.0 (C-2/C-6), 167.6 (C-4), and twelve methine carbons at δ 37.1 (C-6'), 41.8 (C-2'), 54.0 (C-1'), 96.6 (C-3/C-5), 122.6 (C-4'), 124.1 (C-2"), 125.6 (C-4'"), 127.2 (C-2'"/C-6'"), and 127.9 (C-3'"/C-5'"). The relative stereochemistry at C-l', C-2', and C-6' was established by comparing the coupling constants with those of saggenon D and saggenon C (Nomura et al., 1982). These data lead to the conclusion that H-1' and H-2' are *cis*-oriented (*J* = 4.7 Hz) and H-1'/H-6' have a *trans* relationship (*J* = 11.4 Hz). The MS spectrum (Figure 4.3E) indicated the molecular ion peak at *m/z* 407.2, corresponding to the chemical formula $C_{26}H_{31}O_4$.

Table 4.3

*NMR data of panduratin A **(72)** and literature*

C	δ_H (ppm) (Int. mult. *J* in Hz) ($CDCl_3$, 600 MHz)	δ_C (ppm)	δ_H (ppm) (Int. mult. *J* in Hz) ($CDCl_3$, 400 MHz)[a]	δ_C (ppm)[a]
1	-	105.6	-	106.4
2	-	163.0	-	163.2
3	5.87 (2H, s)	96.6	5.86 (2H, s)	94.6
4	-	167.6	-	165.3
5	5.87 (2H, s)	96.6	5.86 (2H, s)	94.6
6	-	163.0	-	163.2
7	-	207.0	-	206.6
1'	4.67 (1H, dd, 4.7, 11.4)	54.0	4.78 (1H, dd, 4.4, 11.0)	54.1
2'	2.62 (1H, ddd, 4.7, 7.4, 7.8)	41.8	2.67 (1H, ddd, 4.4, 7.5, 7.7)	42.8
3'	-	137.3	-	137.3
4'	5.42 (1H, dd, 3.0, 5.0)	122.6	5.43 (1H, dd, 3.0, 5.0)	121.3
5'a	2.38 (1H, ddd, 4.6, 7.0, 17.7)	35.8	2.40 (1H, ddd, 5.0, 6.3, 18.0)	35.9
5'b	2.05 (1H, ddd, 3.0, 11.8, 18.0)	35.8	2.05 (1H, ddd, 3.0, 11.0, 18.0)	35.9
6'	3.42 (1H, ddd, 6.2, 10.8, 10.9)	37.1	3.45 (1H, ddd, 6.3, 11.0, 11.0)	37.2
1"a	2.16 (1H, ddd, , 7.0, 7.5, 15.0)	29.6	2.15 (1H, ddd, 7.0, 7.5, 15.0)	28.9

C	δ_H (ppm) (Int. mult. *J* in Hz) ($CDCl_3$, 500 MHz)	δ_C (ppm)	δ_H (ppm) (Int. mult. *J* in Hz) ($CDCl_3$, 400 MHz)[a]	δ_C (ppm)[a]
1"b	2.27 (1H, ddd, 7.3, 7.3, 14.9)	29.6	2.30 (1H, ddd, 7.0, 7.7, 15.0)	28.9
2"	4.85 (1H, t, 6.9)	124.1	4.89 (1H, t, 7.0)	124.4
3"	-	132.4	-	132.0
4"	1.51 (6H, s)	25.5	1.52 (6H, s)	25.7
5"	1.51 (6H, s)	17.3	1.52 (6H, s)	17.9
6"	1.77 (3H, s)	22.9	1.73 (3H, s)	22.8
1"'	-	145.7	-	147.2
2"'	7.21 (4H, m)	127.2	7.21 (4H, m)	127.3
3"'	7.21 (4H, m)	127.9	7.21 (4H, m)	128.0
4"'	7.10 (1H, m)	125.6	7.09 (1H, m)	125.7
5"'	7.21 (4H, m)	127.9	7.21 (4H, m)	128.0
6"'	7.21 (4H, m)	127.2	7.21 (4H, m)	127.3
4-OCH_3	3.74 (3H, s)	55.0	3.67 (3H, s)	55.5

[a]Data from literature (Tuntiwachwuttikul et al., 1984)

Based on the comparison of spectral data, the structure of compound **(72)** was identified as panduratin A. This compound was reported previously from the rhizome of *B. rotunda* (Sanguansermsri et al., 2024) and *B. pandurata* (Cheenpracha et al., 2006).

Figure 4.3A

IR spectrum of panduratin A ***(72)***

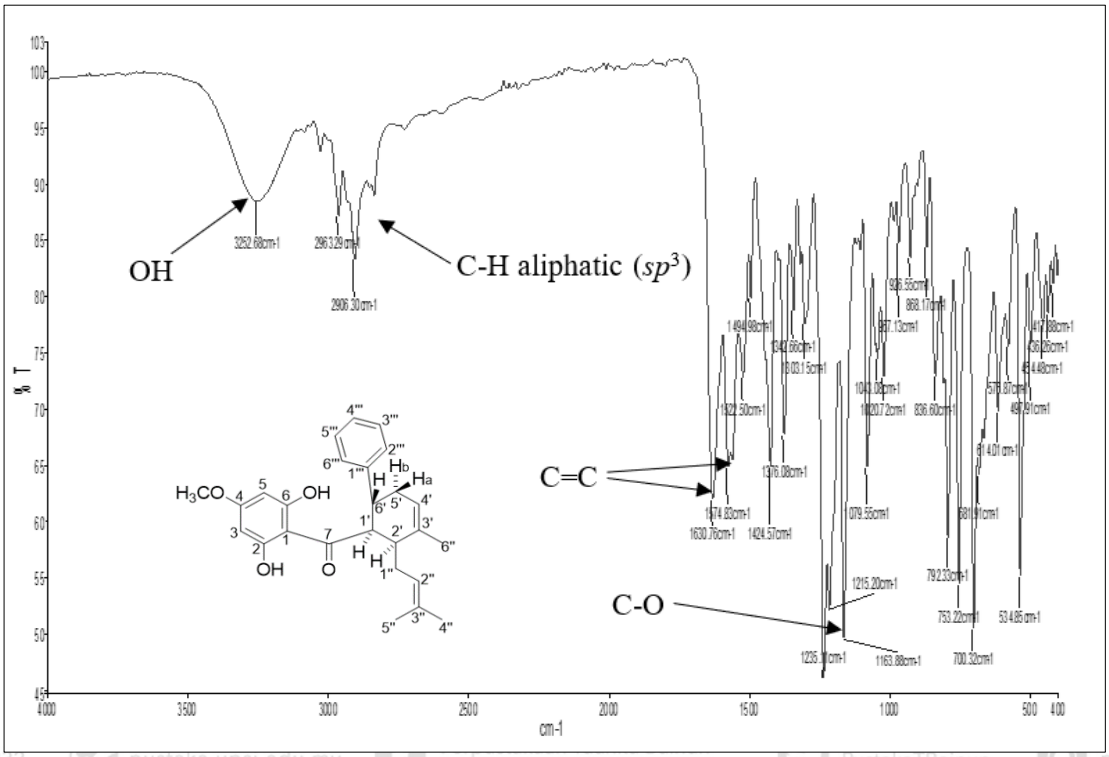


Figure 4.3B

^{1}H NMR spectrum of panduratin A ***(72)***

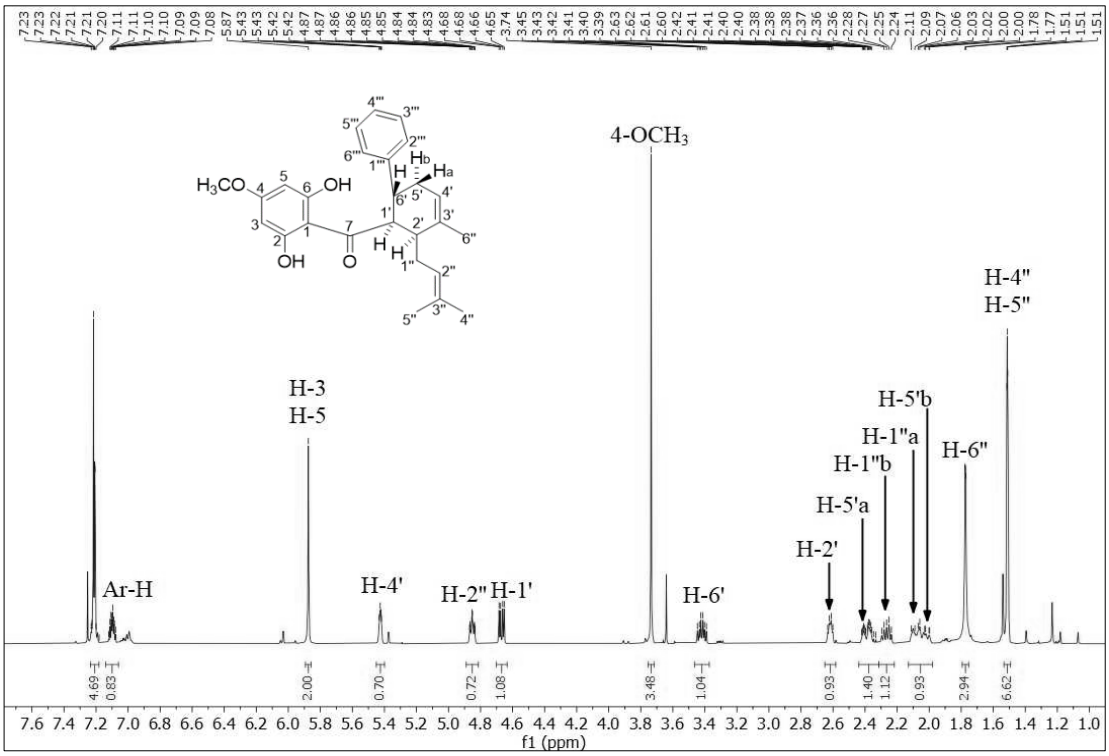


Figure 4.3C

*COSY spectrum of panduratin A **(72)***

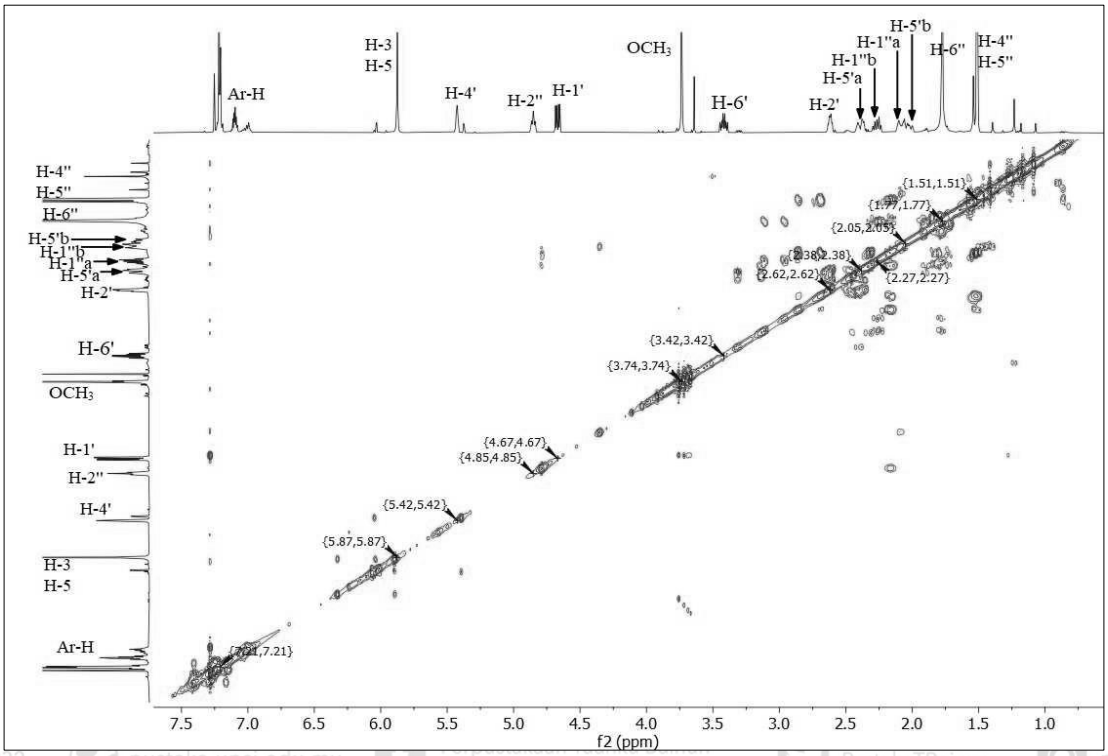


Figure 4.3D

*^{13}C NMR spectrum of panduratin A **(72)***

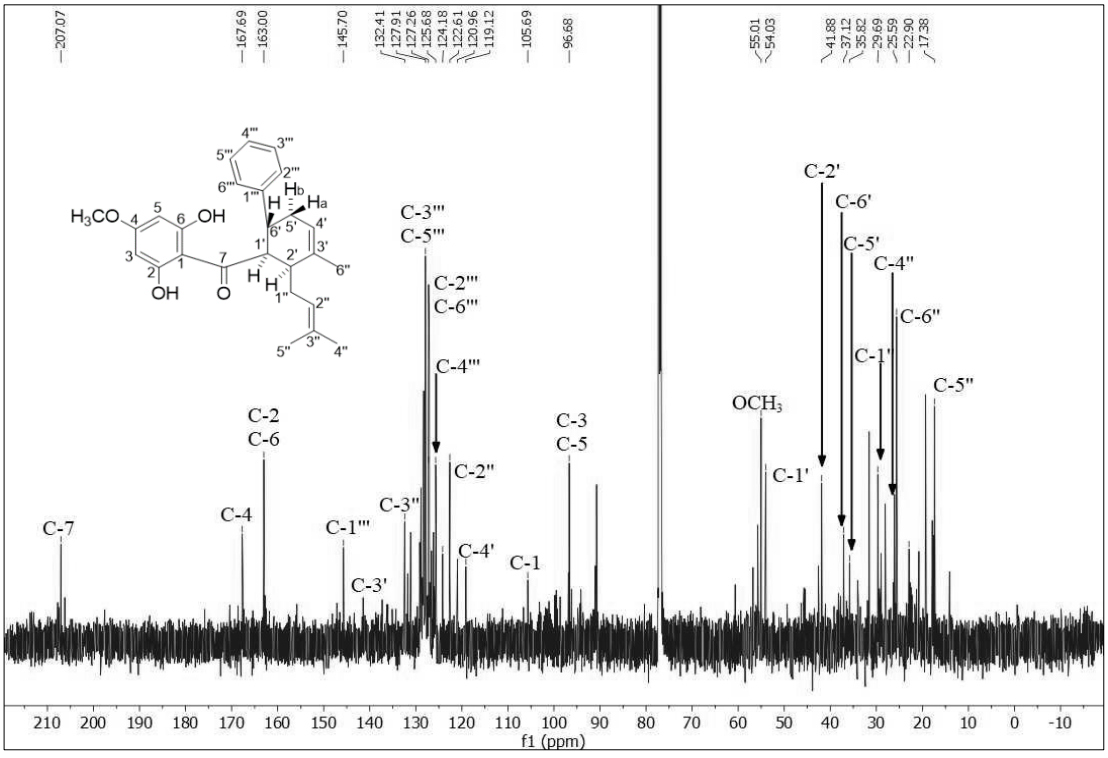
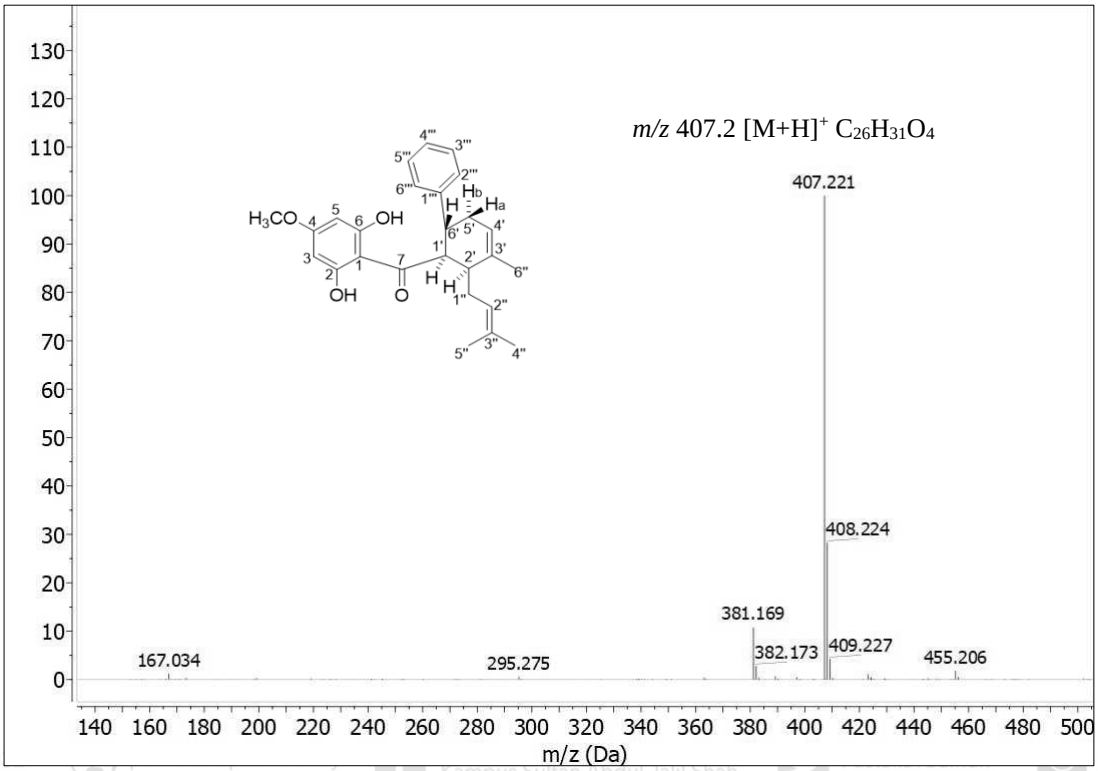
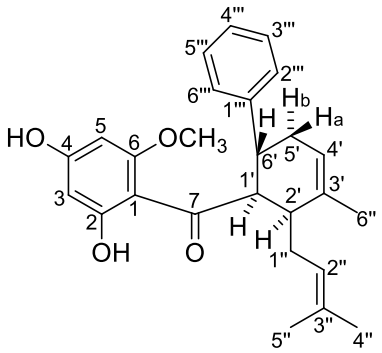


Figure 4.3E

MS spectrum of panduratin A ***(72)***



4.3.2 Isopanduratin A (76)



(76)

Isolation of the DCM extract by CC using *n*-hexane:DCM had afforded compound **(76)** as a yellow oil. The IR spectrum (Figure 4.4A) showed a broad absorption band at 3288 cm^{-1} corresponding to OH group. The absorption at 3021 and 2923 cm^{-1} represent sp^2 C-H and sp^3 C-H stretching, respectively. The peak at 1622 cm^{-1} was associated with C=O stretching vibrations. Additionally, the peak at 1593 cm^{-1} was attributed to C=C stretching. Meanwhile, 1164 cm^{-1} was attributed to C-O stretching vibrations, which are indicative of the presence of oxygenated functional groups of alcohols within the molecule.

The ^{1}H NMR (Figure 4.4B) was closely resemble to the ^{1}H NMR spectrum of panduratin A **(72)** except the position of methoxyl group. Compound **(76)** features a methoxy group at C-6 position, while panduratin A **(72)** features a methoxy group at C-4 position. The HMBC spectrum (Figure 4.4C) confirmed this position by showing the correlation of 6-OCH_3 (δ 55.7) with C-6 (162.4). The ^{13}C NMR spectrum (Figure 4.4D) and DEPT spectra (Figure 4.4E) showed the presence of 26 signals attributed to 26 carbons, which were found similar with that of compound **(72)**. The spectral data was further supported by the MS spectrum (Figure 4.4F), which gave a molecular ion peak at *m/z* 405.2 consistent with a molecular formula $C_{26}H_{29}O_4$. The comparison of the assignments of the ^{1}H NMR and ^{13}C NMR are summarized in Table 4.4.

Through a comparison of spectral data, the structure of compound **(76)** was identified as isopanduratin A. This compound has been previously reported from the rhizome of *B. pandurata* (Morikawa et al., 2008).

Table 4.4

*NMR data of isopanduratin A **(76)** and literature*

C	δ_H (ppm) (Int. mult. *J* in Hz) ($CDCl_3$, 500 MHz)	δ_C (ppm)	δ_H (ppm) (Int. mult. *J* in Hz) ($CDCl_3$, 500 MHz)[a]	δ_C (ppm)[a]
1	-	106.6	-	106.6
2	-	167.5	-	167.5
3	5.94 (1H, s)	96.8	5.90 (1H, s)	96.8
4	-	162.7	-	162.8
5	5.96 (1H, s)	90.9	5.92 (1H, s)	90.9
6	-	162.4	-	162.4
7	-	206.3	-	206.3
1'	4.52 (1H, dd, 4.7, 11.3)	54.1	4.49 (1H, dd, 4.6, 11.3)	54.2
2'	2.52 (1H, m)	42.6	2.49 (1H, m)	42.6
3'	-	137.2	-	137.3
4'	5.45 (1H, br s)	120.9	5.42 (1H, br s)	121.0
5'a	2.43 (1H, m)	35.8	2.40 (1H, m)	35.8
5'b	2.05 (1H, m)	35.8	2.02 (1H, m)	35.8
6'	3.44 (1H, m)	37.1	3.42 (1H, m)	37.1
1''a	2.10 (1H, m)	28.9	2.10 (1H, m)	28.9
1''b	2.26 (1H, m)	28.9	2.23 (1H, m)	28.9
2''	4.88 (1H, t-like)	124.1	4.86 (1H, t-like)	124.2
3''	-	131.8	-	131.8
4''	1.52 (6H, s)	25.6	1.51 (6H, s)	25.7
5''	1.52 (6H, s)	17.8	1.51 (6H, s)	17.9

C	δ_H (ppm) (Int. mult. *J* in Hz) ($CDCl_3$, 500 MHz)	δ_C (ppm)	δ_H (ppm) (Int. mult. *J* in Hz) ($CDCl_3$, 500 MHz)[a]	δ_C (ppm)[a]
6"	1.81 (3H, s)	22.9	1.79 (3H, s)	22.9
1'''	-	147.1	-	147.1
2'''	7.21 (2H, m)	127.0	7.19 (2H, m)	127.0
3'''	7.23 (2H, m)	128.3	7.21 (2H, m)	128.3
4'''	7.11 (1H, m)	125.5	7.09 (1H, m)	125.5
5'''	7.23 (2H, m)	128.3	7.21 (2H, m)	128.3
6'''	7.21 (2H, m)	127.0	7.19 (2H, m)	127.0
6-OCH_3	3.92 (3H, s)	55.7	3.90 (3H, s)	55.7

[a]Data from literature (Yoshikawa et al., 2008)

Figure 4.4A

*IR spectrum of isopanduratin A **(76)***

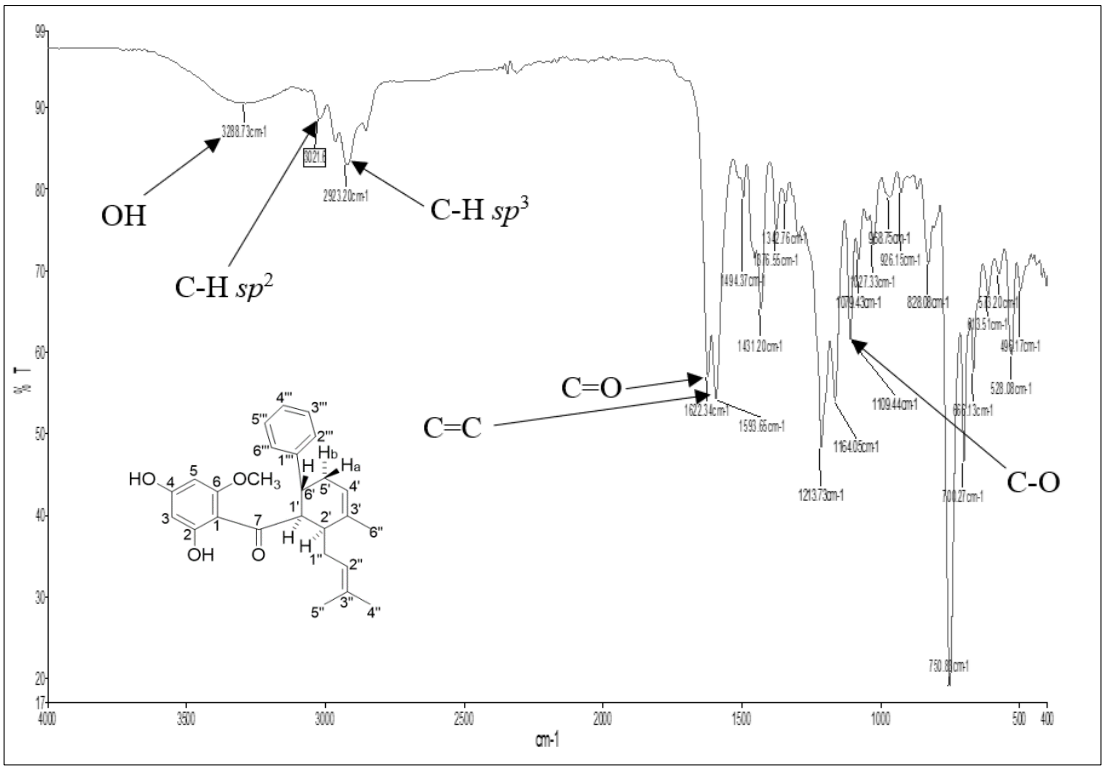


Figure 4.4B

*^{1}H NMR spectrum of isopanduratin A **(76)***

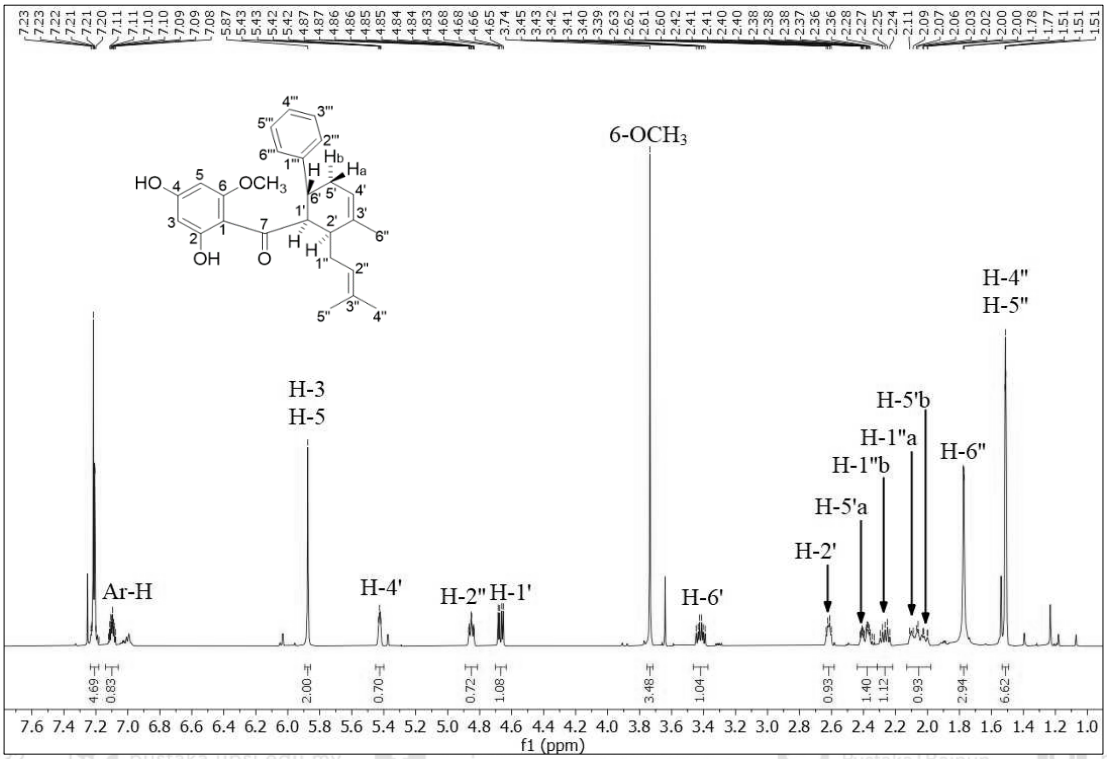


Figure 4.4C

*HMBC spectrum of isopanduratin A **(76)***

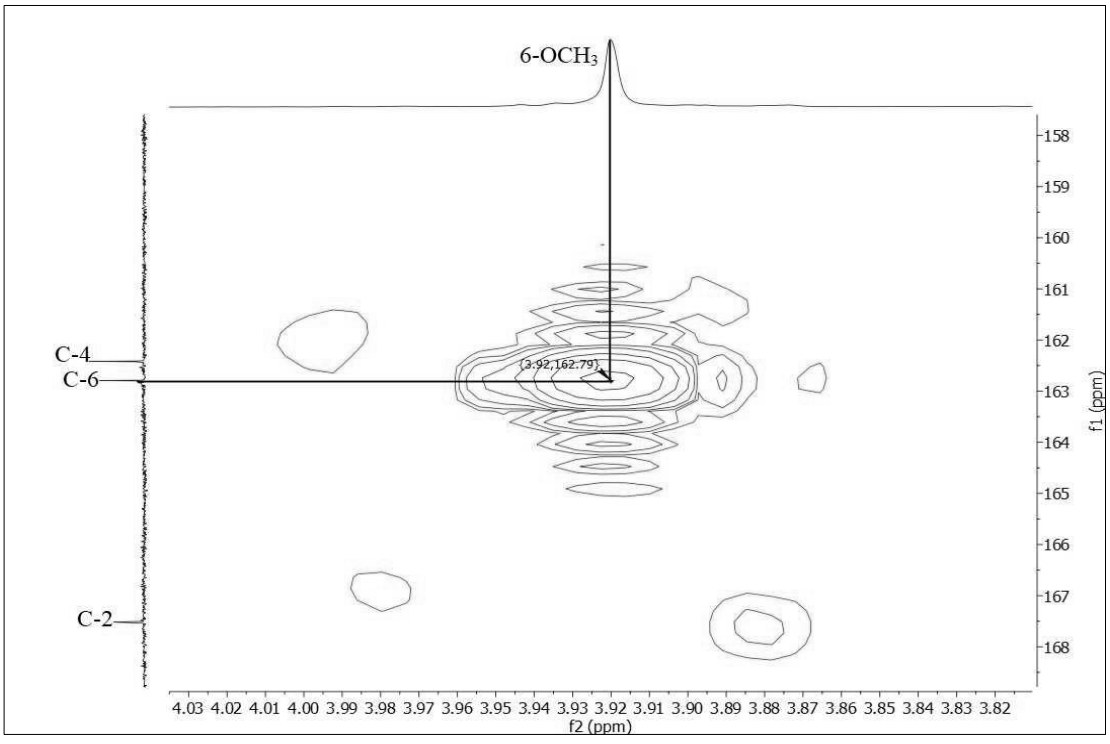


Figure 4.4D

*^{13}C NMR spectrum of isopanduratin A **(76)***

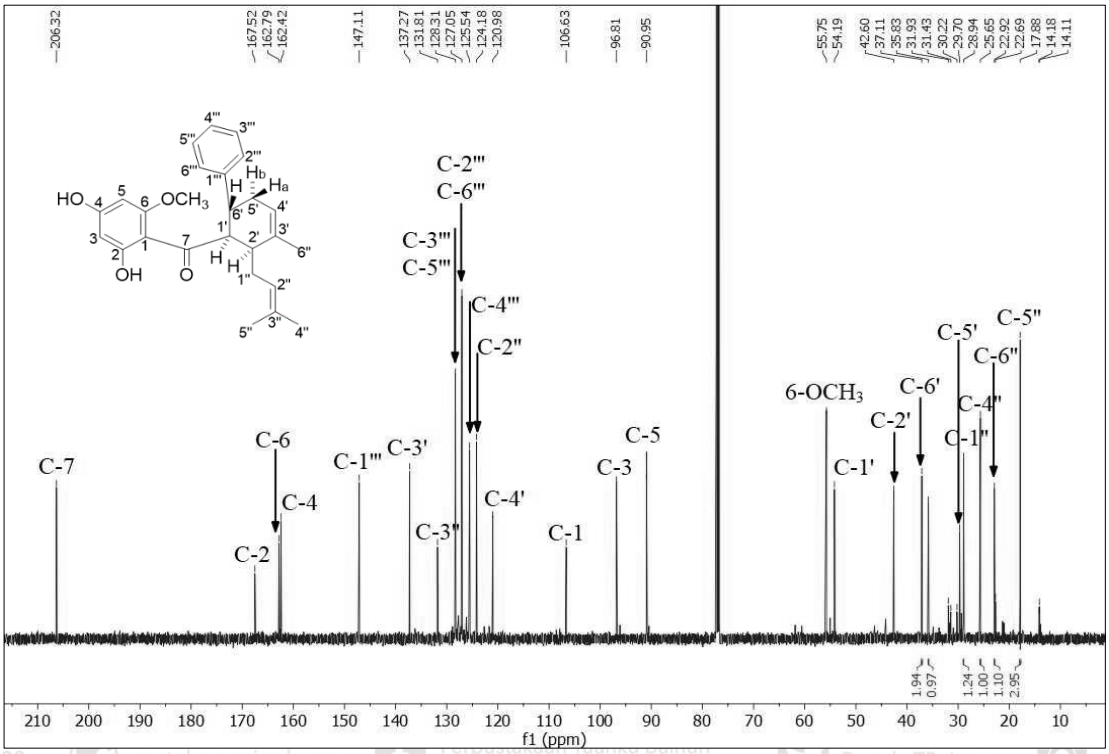


Figure 4.4E

*DEPT spectra of isopanduratin A **(76)***

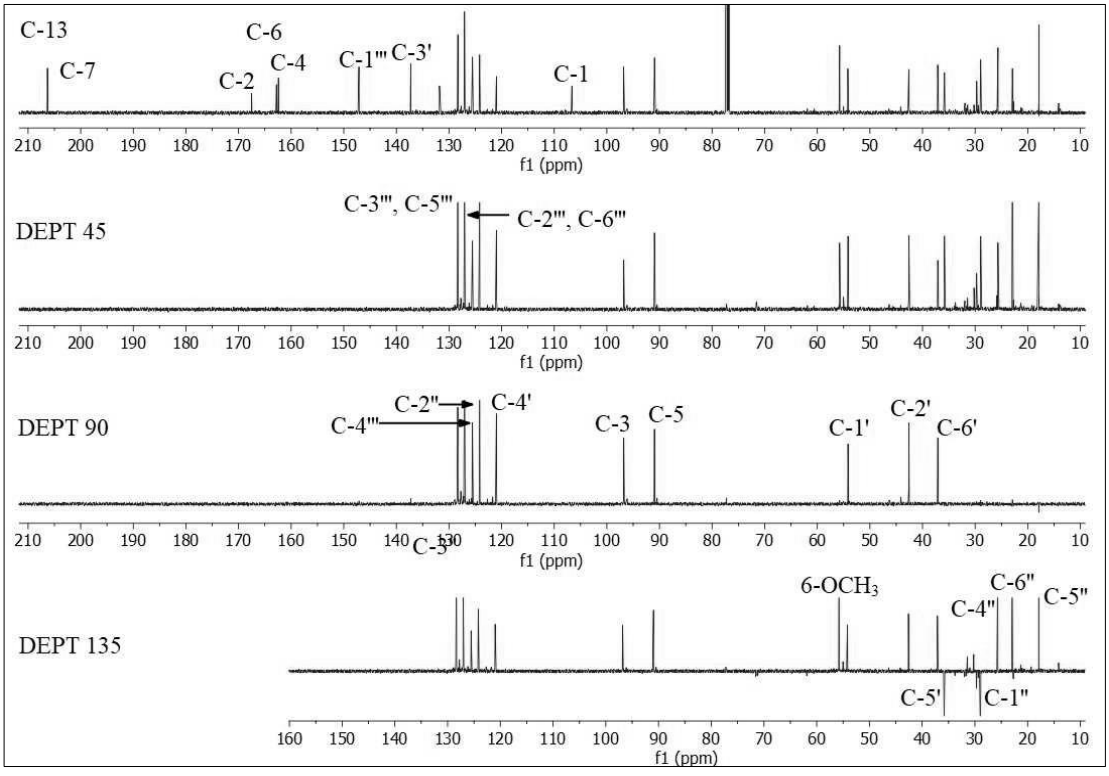
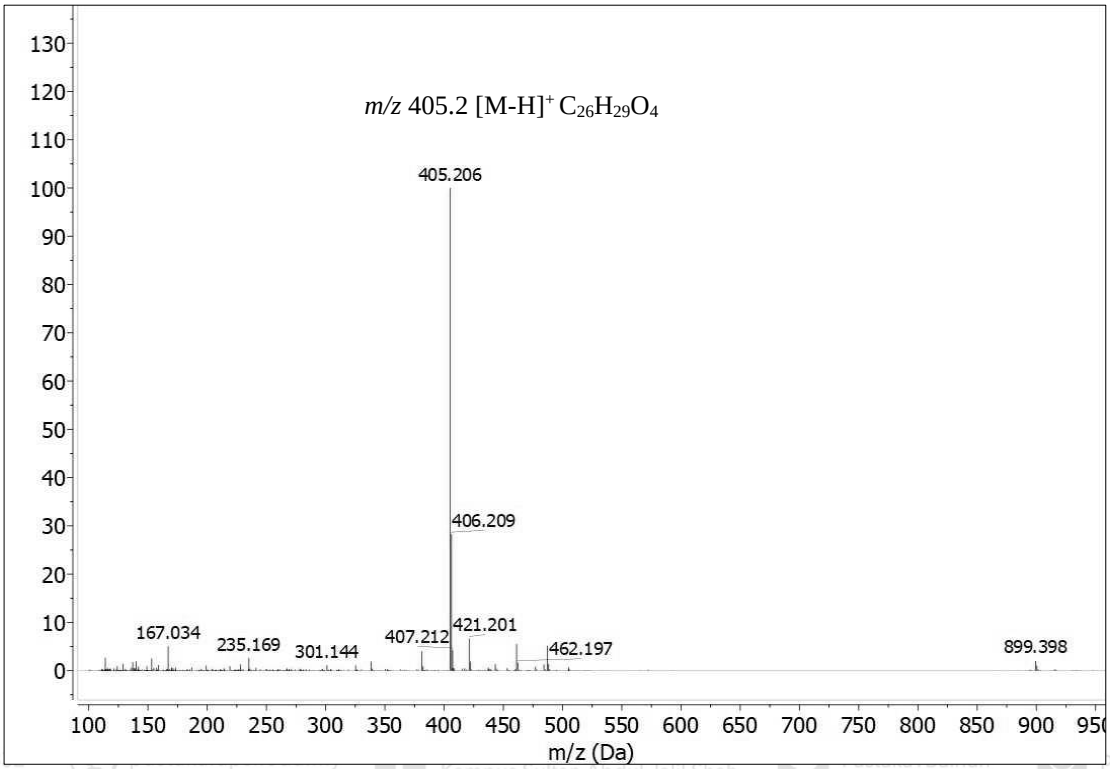
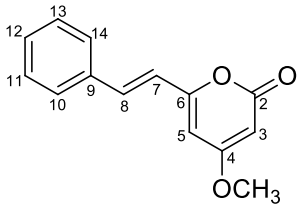


Figure 4.4F

*MS spectrum of isopanduratin A **(76)***



4.3.3 5,6-Dehydrokawain (196)



(**196**)

Compound **(196)** was obtained from DCM extract by CC using *n*-hexane:DCM as a white solid. The IR spectrum (Figure 4.5A) reveals characteristic absorption bands of

sp^2 C-H (3076 cm^{-1}), *sp*^{3} C-H stretching (2951 cm^{-1}), carbonyl (1720 cm^{-1}), C=C (1554 and 1447 cm^{-1}), and C-O (1256 cm^{-1}) stretching.

The ^{1}H NMR spectrum (Figure 4.5B) revealed the presence of a methoxyl group at 4-OCH_3 were detected at δ 3.85. Two doublets observed at δ 6.61 (*J* = 16.0 Hz) and 7.54 (*J* = 16.0 Hz) were assigned to olefinic protons H-7 and H-8, respectively. The large coupling constant, *J* = 16.0 Hz suggested these protons in a *trans* orientation. Another two sets of doublets appeared at δ 5.52 and 5.97 (*J* = 2.2 Hz) were attributed to olefinic protons H-3 and H-5, of pyran-2-one respectively. The aromatic protons (H-10 to H-14) were observed as multiplet signals at δ 7.36-7.42. The COSY spectrum (Figure 4.5C) supported the correlations between proton H-7 (δ 6.61) with H-8 (δ 7.54), while proton H-3 (δ 5.52) was correlated with proton H-5 (δ 5.97). The assignments of the protons and carbons correlations are summarized in Table 4.5.

The ^{13}C NMR (Figure 4.5D) and DEPT spectra (Figure 4.5E) revealed the presence of fourteen carbons; one methoxy carbon at δ 55.9 (4-OCH_3), nine methine carbons at δ 88.8 (C-3), 101.3 (C-5), 118.6 (C-7), 127.4 (C-10/C-14), 128.9 (C-11/C-13), 129.4 (C-12), and 135.2 (C-8), three quaternary carbons at δ 135.8 (C-9), 163.9 (C-6), and 158.6 (C-4), and a carbonyl carbon at δ 171.0 (C-2). The MS spectrum (Figure 4.5F) gave a molecular ion peak at *m/z* 229, corresponding to a molecular formula $C_{14}H_{12}O_3$.

The HMQC spectrum (Figure 4.5G) showed the methoxy carbon at δ 55.9 were directly attached to the protons at δ 3.85 (4-OCH_3). Besides, four methine carbons, H-8 (δ 7.54), H-7 (δ 6.61), H-5 (δ 5.97) and H-3 (δ 5.52) were confirmed directly attached

to carbon C-8 (δ 135.2), C-7 (δ 118.6), C-5 (δ 101.3), and C-3 (δ 88.8), respectively. Meanwhile, the HMBC correlation (Figure 4.5H) showed the correlation between H-5 (δ 5.97) with C-3 (δ 88.8), C-7 (δ 118.6), C-2 (δ 171.0), and C-4 (δ 158.6). Besides, it also showed the correlation between H-7 (δ 6.61) with C-5 (δ 101.3), C-9 (δ 135.8) and, C-4 (δ 158.6) and H-3 (δ 5.97) with C-5 (δ 101.3) and C-2 (δ 171.0).

Table 4.5

*NMR data of 5,6-dehydrokawain **(196)** and literature*

C	δ_H (ppm) (Int. mult. *J* in Hz) ($CDCl_3$, 500 MHz)	δ_C (ppm)	δ_H (ppm) (Int. mult. *J* in Hz) ($CDCl_3$, 400 MHz)[a]	δ_C (ppm)[a]
2	-	171.0	-	171.1
3	5.52 (1H, d, 2.2)	88.8	5.50 (1H, d, 2.0)	88.8
4	-	158.6	-	158.6
5	5.97 (1H, d, 2.1)	101.3	5.95 (1H, d, 2.4)	101.3
6	-	163.9	-	164.0
7	6.61 (1H, d, 16.0)	118.6	6.59 (1H, d, 16.0)	118.6
8	7.54 (1H, m)	135.2	7.52 (1H, m)	135.2
9	-	135.8	-	135.8
10	7.36 – 7.42 (1H, m)	127.4	7.35 – 7.52 (1H, m)	127.4
11	7.36 – 7.42 (1H, m)	128.9	7.35 – 7.52 (1H, m)	128.9
12	7.36 – 7.42 (1H, m)	129.4	7.35 – 7.52 (1H, m)	129.4
13	7.36 – 7.42 (1H, m)	128.9	7.35 – 7.52 (1H, m)	128.9
14	7.36 – 7.42 (1H, m)	127.4	7.35 – 7.52 (1H, m)	127.4
4-OCH_3	3.85 (3H, s)	55.9	3.83 (3H, s)	55.9

[a]Data from literature (Kumagai et al., 2016)

Figure 4.5A

IR spectrum of 5,6-dehydrokawain ***(196)***

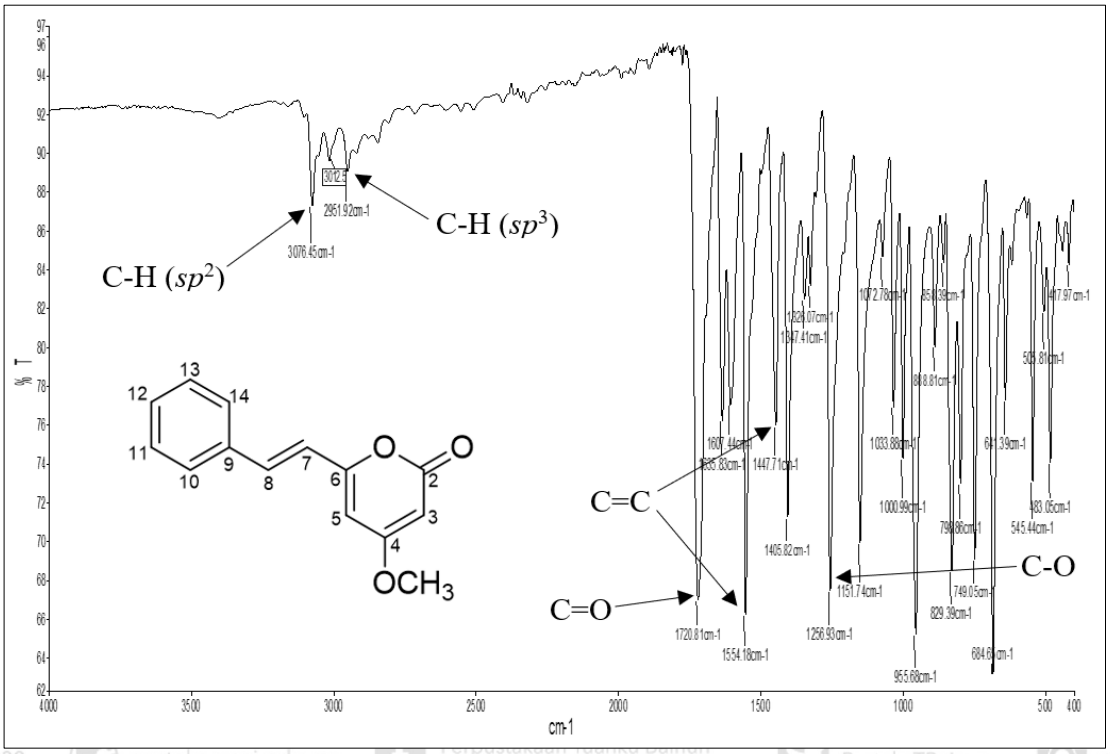


Figure 4.5B

^{1}H NMR spectrum of 5,6-dehydrokawain ***(196)***

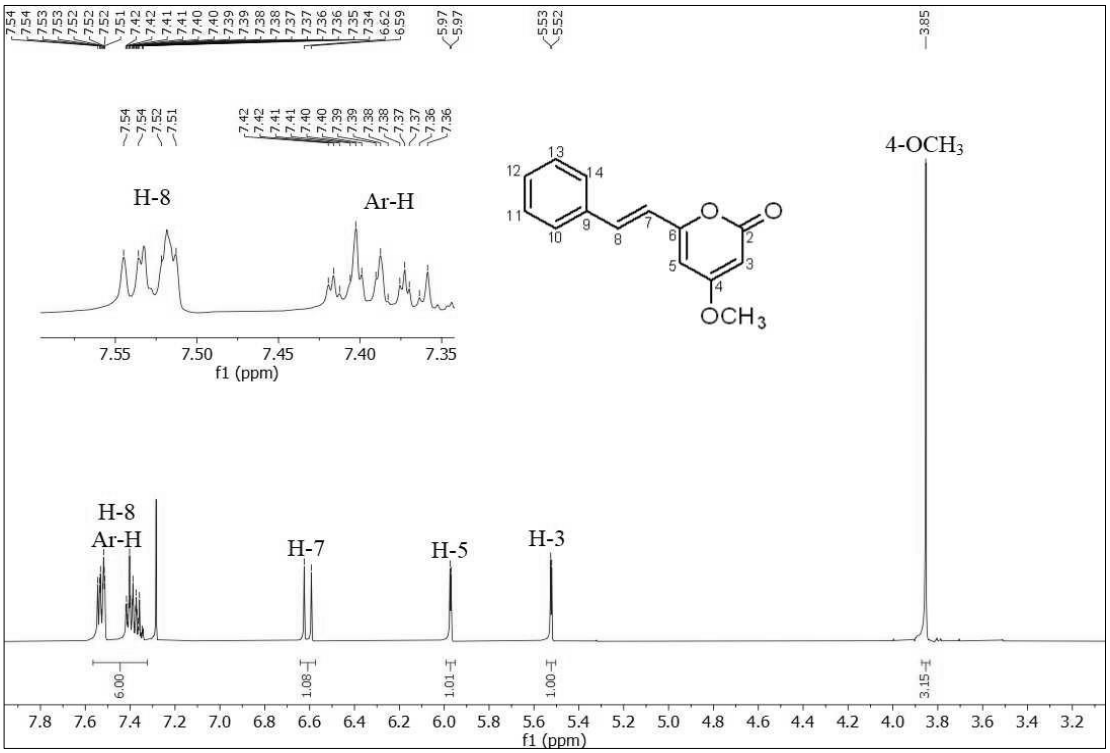


Figure 4.5C

*COSY spectrum of 5,6-dehydrokawain **(196)***

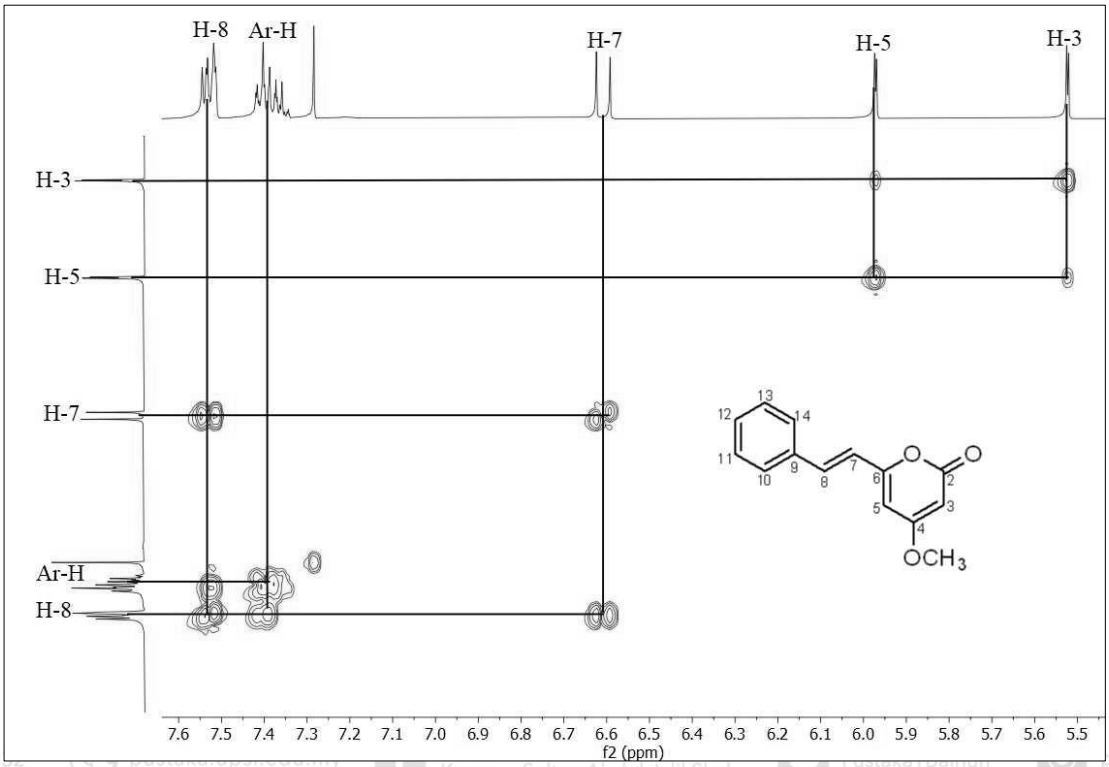


Figure 4.5D

*^{13}C NMR spectrum of 5,6-dehydrokawain **(196)***

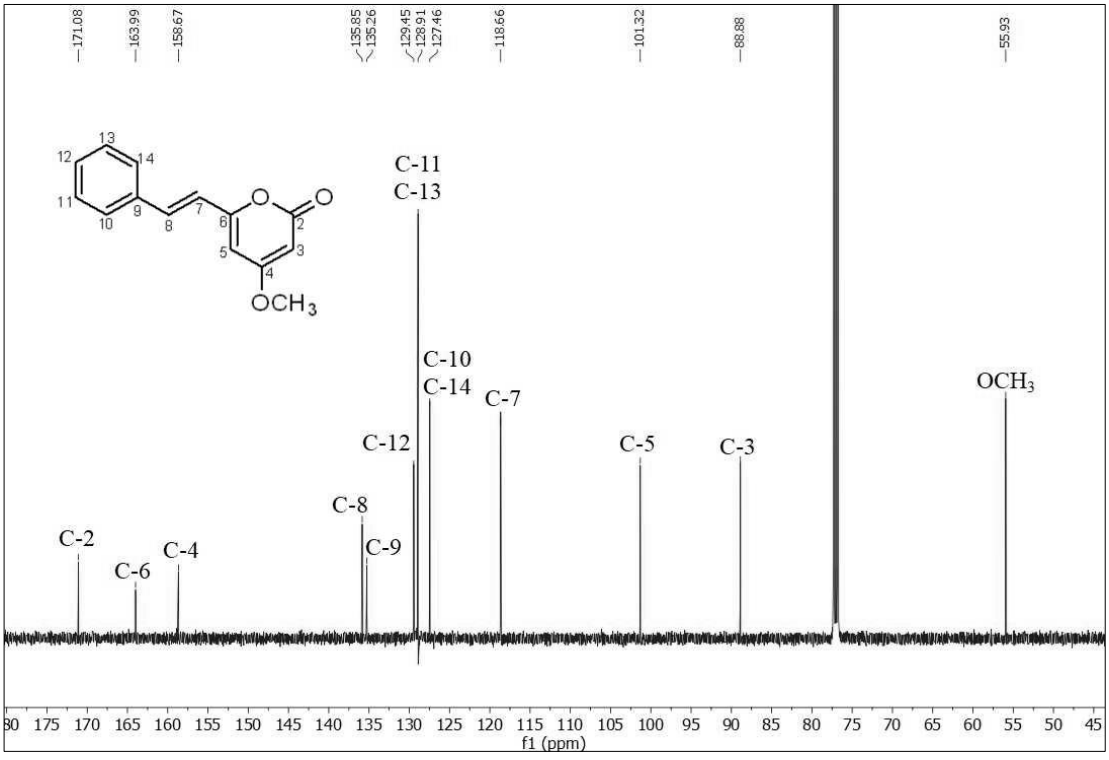


Figure 4.5E

*DEPT spectra of 5,6-dehydrokawain **(196)***

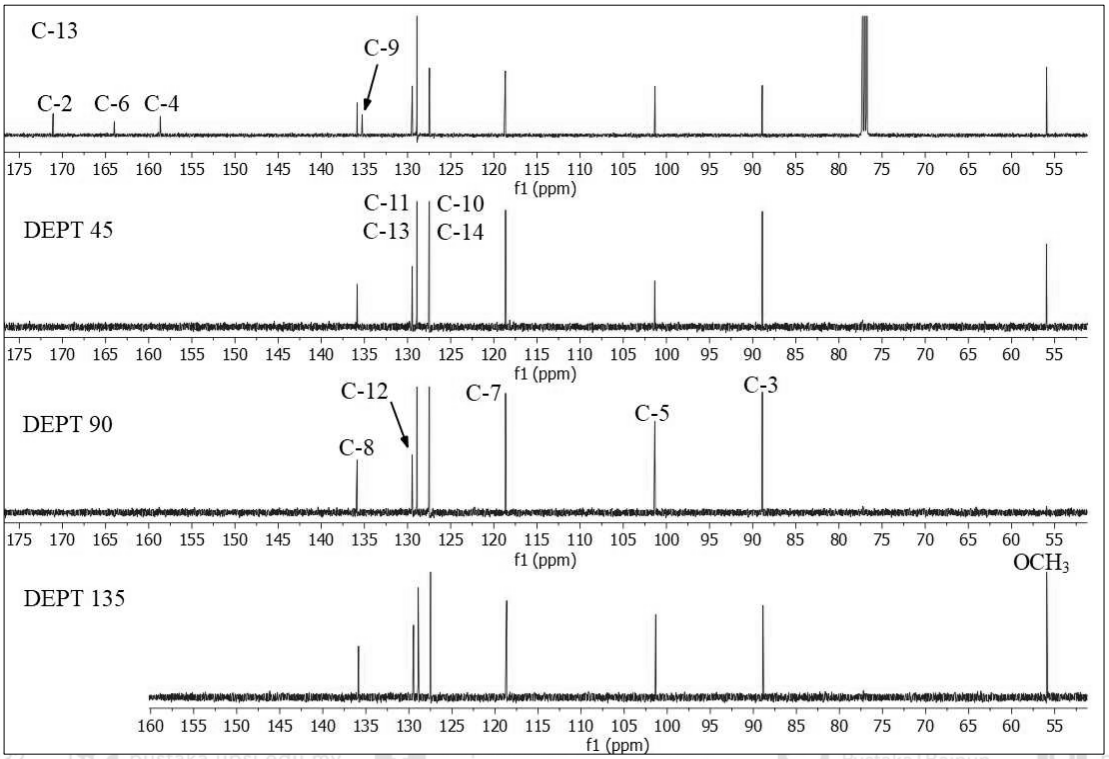


Figure 4.5F

*MS spectrum of 5,6-dehydrokawain **(196)***

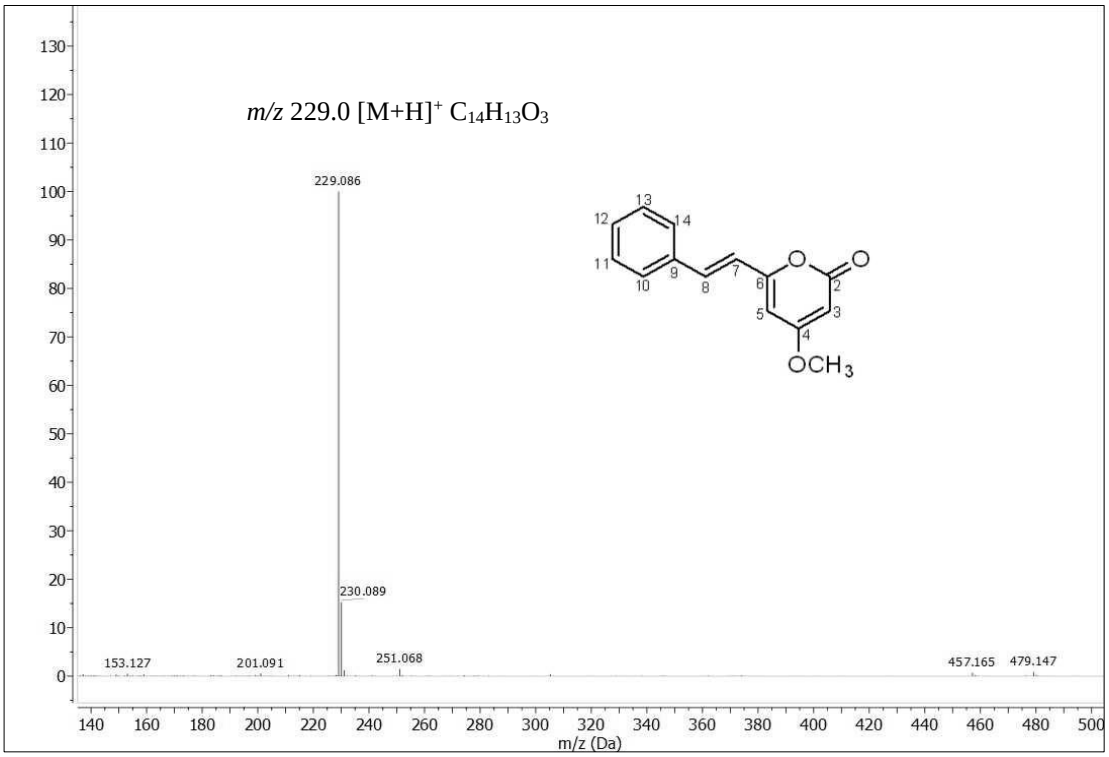


Figure 4.5G

*HMQC spectrum of 5,6-dehydrokawain **(196)***

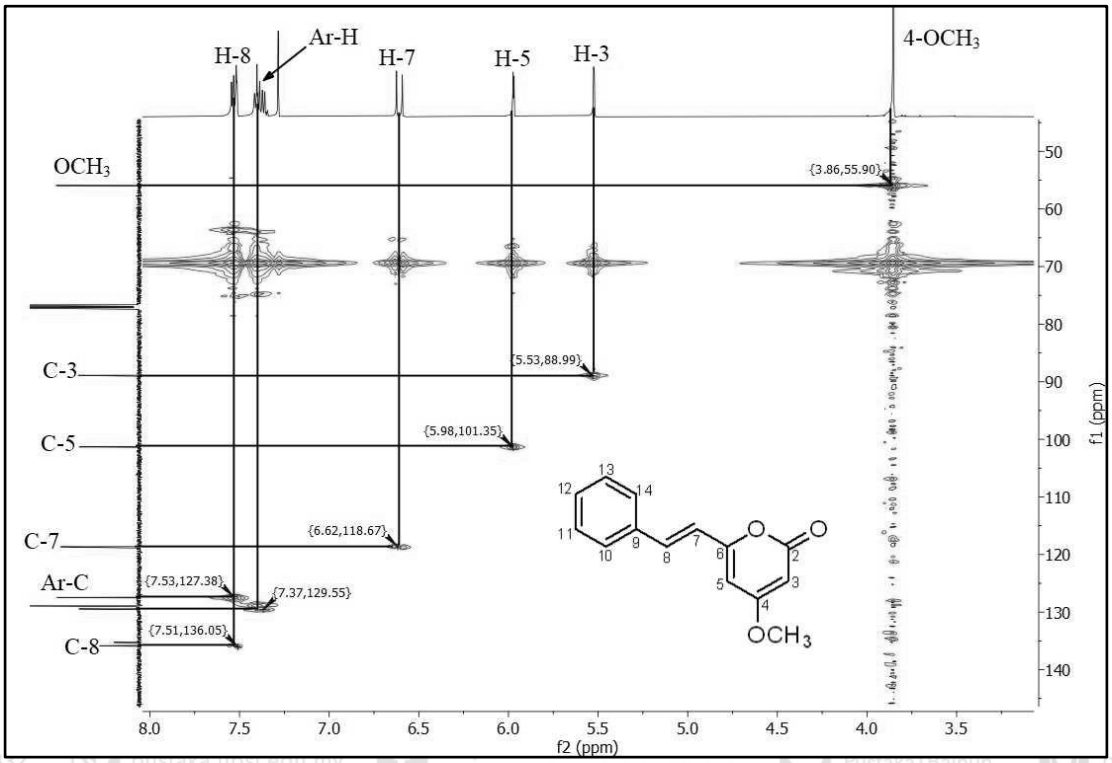
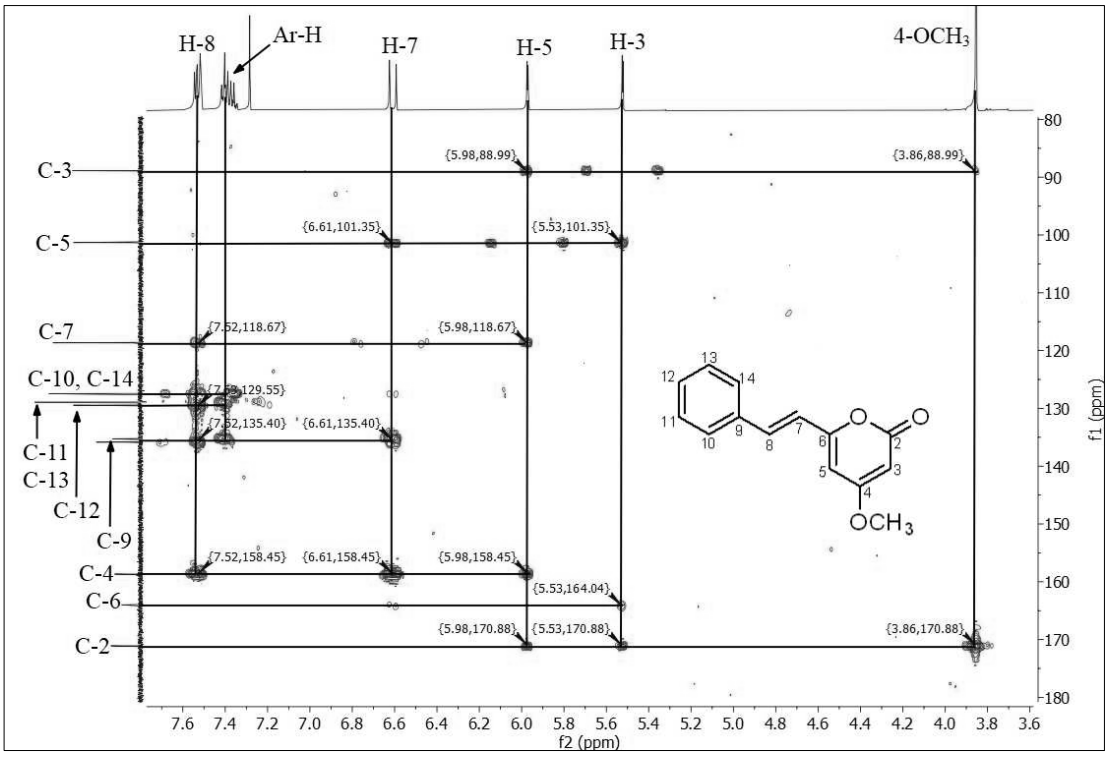
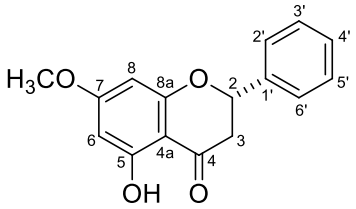


Figure 4.5H

*HMBC spectrum of 5,6-dehydrokawain **(196)***



4.3.4 Pinostrobin (115)



(115)

Purification of the *n*-hexane rhizomes extract by CC using *n*-hexane:DCM had yielded compound **(115)** as a white crystalline solid. The IR spectrum (Figure 4.6A) displayed an absorption band at 3227 cm^{-1} corresponding to OH stretching, whereas 2975 cm^{-1} for sp^3 C-H stretching. Besides, absorption band at 1618 cm^{-1} and 1576 cm^{-1} were corresponded to C=O and C=C stretching. The absorption at 1284 cm^{-1} corresponds to the stretching of the C-O bond.

The ^{1}H NMR spectrum (Figure 4.6B) supported the presence of hydroxyl group which was represented by a singlet signal at δ 12.05. Another singlet peak was observed at δ 3.84, assigned to a methoxy group (7-OCH_3). The *meta*-coupled signals appeared at δ 6.19 and 6.11 (J = 2.2 Hz), which were attributed to aromatic protons H-6 and H-8, respectively. In addition, three set doublet of doublets signal, each integrated for one proton were observed at δ 2.85 (J = 3.0 Hz and 17.1 Hz), 3.12 (J = 13.0 Hz and 17.1 Hz) and 5.45 (J = 3.0 Hz and 13.1 Hz,) were assigned for H-3a, H-3b and H-2, respectively. In addition, a multiplet signal resonated at δ 7.42-7.47 integrating for five protons of aromatic protons, H-2'–H-6' of ring B. Besides, based on chemical shift pattern comparison of H-2, H-3a and H-3b, the configuration at H-3 was deduced as *R* configuration (Yang et al., 2022b).

The COSY spectrum (Figure 4.6C) showed correlation between H-2 (δ 5.45) with H-3a (δ 2.85) and H-3b (δ 3.12), as well as between H-6 (δ 6.11) with H-8 (δ 6.09). The ^{13}C NMR (Figure 4.6D) and DEPT spectra (Figure 4.6E) indicated the presence of sixteen carbons including one methyl, one methylene, one carbonyl carbon, eight methine and five quaternary carbon atoms. The carbon signal of methoxy group (7-OCH_3) was clearly assigned at δ 55.7, while the carbonyl carbon (C-4) was also observed at δ 195.8. The single signal position of methylene carbon (C-3a/C-3b) was observed at δ 43.4. Eight methine carbons were detected at δ 79.3 (C-2), 94.3 (C-8), 95.2 (C-6), 126.2 (C-2'/C-6') and 128.9 (C-3'/C-4'/C-5') as well as five quaternary carbons at δ 103.2 (C-4a), 138.4 (C-1'), 162.8 (C-8a), 164.2 (C-5) and 168.0 (C-7). The ^{1}H NMR and ^{13}C NMR data as tabulated in Table 4.6.

The ^{13}C NMR data were supported by the MS spectrum (Figure 4.6F), which gave a molecular ion peak *m/z* 270 consistent with a molecular formula $C_{16}H_{15}O_4$. In the HMQC spectrum (Figure 4.6G), the aromatic protons at δ 7.42-7.48 (H-2'–H-6') were found directly attached to carbons resonated at δ 126.2 (C-2'/C-6') and 128.9 (C-3'/C-4'/C-5'). Two aromatic protons of the ring A (H-8 and H-6) were found correlated with carbons at δ 94.3 (C-8) and 95.2 (C-6). The methine proton, H-2 (δ 5.45) was confirmed attached to carbon at δ 79.3 (C-2). Meanwhile, two inequivalent methylene protons, H-3 (δ 2.85 and 3.12) showed a cross peak with C-3a/C-3b (δ 43.4).

Table 4.6

*NMR data of pinostrobin **(115)** and literature*

C	δ_H (ppm) (Int. mult. *J* in Hz) ($CDCl_3$, 500 MHz)	δ_C (ppm)	δ_H (ppm) (Int. mult. *J* in Hz) ($CDCl_3$, 400 MHz)[a]	δ_C (ppm)[a]
2	5.45 (1H, dd, 13.1, 3.0)	79.3	5.42 (1H, dd, 12, 3.2)	79.4
3a	3.12 (1H, dd, 17.1, 13.0)	43.4	3.07 (1H, dd, 17.2, 12.8)	43.5
3b	2.85 (1H, dd, 17.1, 3.0)	43.4	2.83 (1H, dd, 17.2, 3.2)	43.5
4	-	195.8	-	195.9
4a	-	103.2	-	103.3
5	-	164.2	-	164.2
6	6.11 (1H, d, 2.2)	95.2	6.06 (1H, d, 2.4)	95.3
7	-	168.0	-	168.1
8	6.09 (1H, d, 2.3)	94.3	6.06 (1H, d, 2.4)	94.4
8a	-	162.8	-	162.9
1'	-	138.4	-	138.5
2'	7.47 (1H, m)	126.2	7.45 (1H, m)	126.3
3'	7.42 (1H, m)	128.9	7.42 (1H, m)	129.0
4'	7.42 (1H, m)	128.9	7.42 (1H, m)	129.0
5'	7.42 (1H, m)	128.9	7.42 (1H, m)	129.0
6'	7.47 (1H, m)	126.2	7.45 (1H, m)	126.3
5-OH	12.05 (1H, s)	-	12.01 (1H, s)	-
7-OCH_3	3.84 (3H, s)	55.7	3.80 (3H, s)	55.9

[a]Data from literature (Fakhrudin et al., 2021)

The HMBC spectrum (Figure 4.5H) exhibited a long-range correlation of hydroxyl proton at δ 12.05 and carbon C-4a (δ 103.2), C-6 (δ 95.2) and C-7 (δ 168.0). The signal proton of H-6 (δ 6.11) was found correlated with C-4a (δ 103.2), C-5 (δ 164.2) and C-7 (δ 168.0), while proton of H-8 (δ 6.09) correlated with C-6 (δ 95.2), C-7 (δ 168.0), C-5 (δ 164.2), and C-4a (δ 103.2). The methine proton of H-2 (δ 5.45) was correlated to C-1' (δ 138.4), C-2' and C-6' (δ 126.2). The signal at δ 3.12 (H-3b) was also correlated to C-2 (δ 79.3), C-4 (δ 195.8), and C-1' (δ 138.4), while for H-3a (δ 2.85) correlated with C-4 (δ 195.8) and C-4a (δ 103.2). Compound **(115)** was identified as 5-hydroxy-7-methoxyflavanone or also known as pinostrobin, based on the comparison of its physical properties and spectral data. Previously, it has been isolated from *B. rotunda* (Sritananuwat et al., 2024).

Figure 4.6A

*IR spectrum of pinostrobin **(115)***

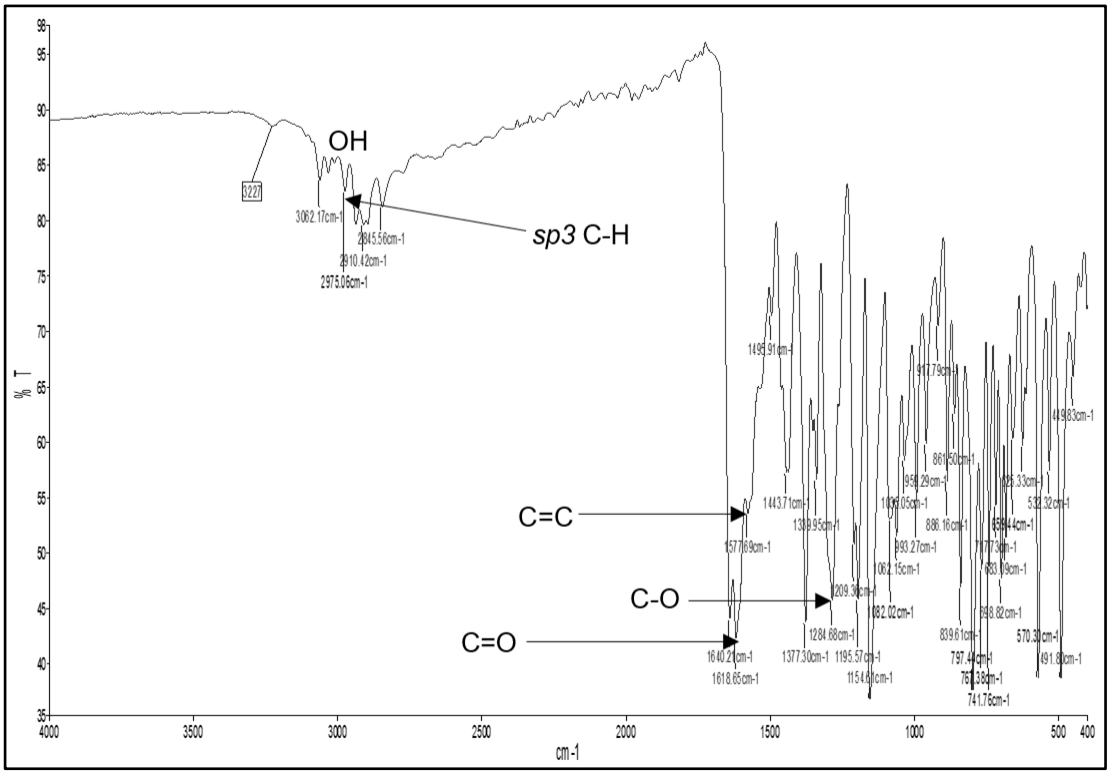


Figure 4.6B

*^{1}H NMR spectrum of pinostrobin **(115)***

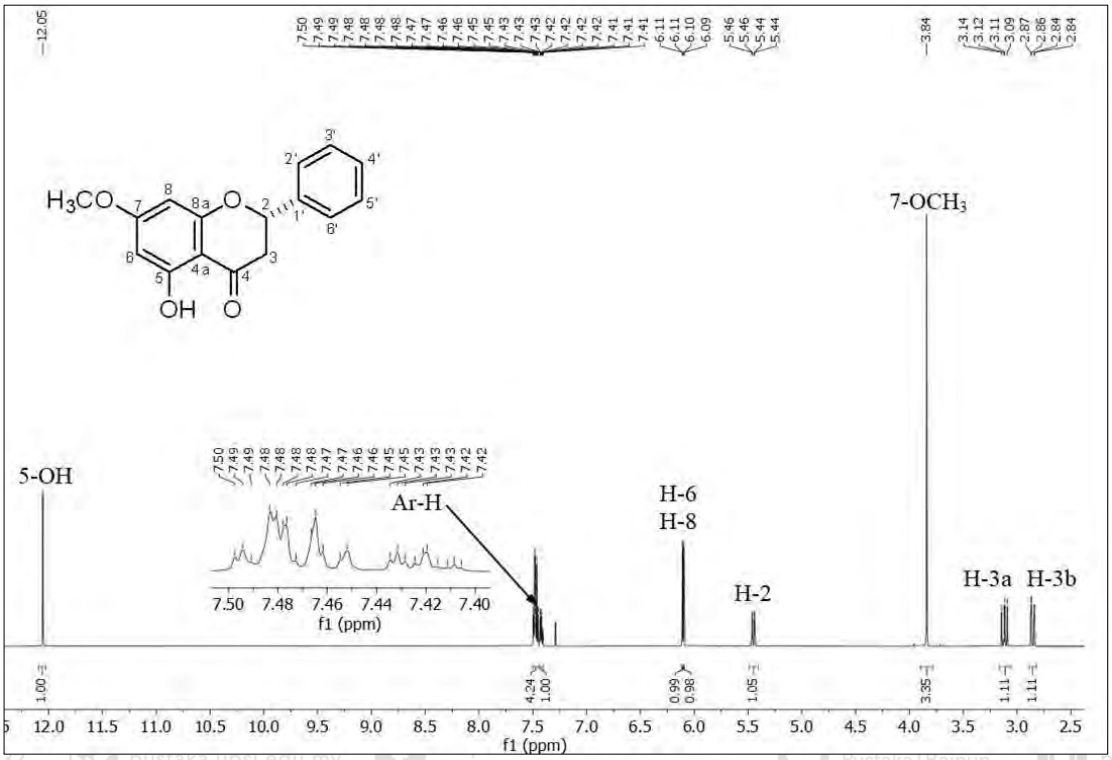


Figure 4.6C

*COSY spectrum of pinostrobin **(115)***

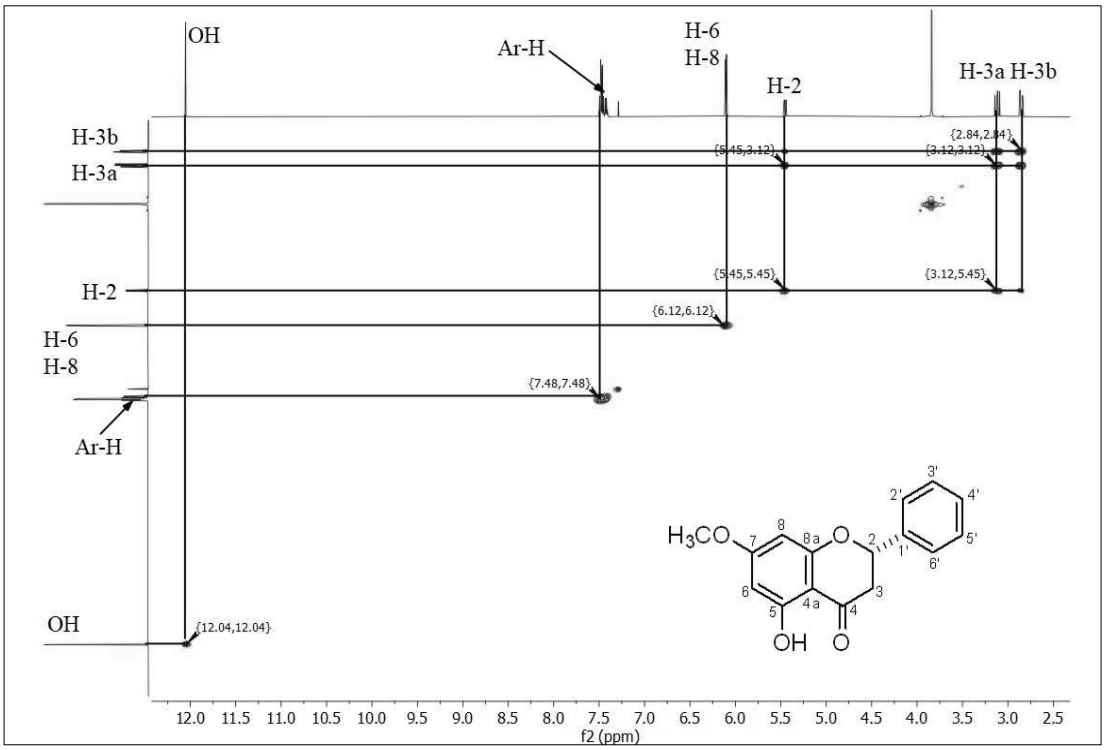


Figure 4.6D

*^{13}C NMR spectrum of pinostrobin **(115)***

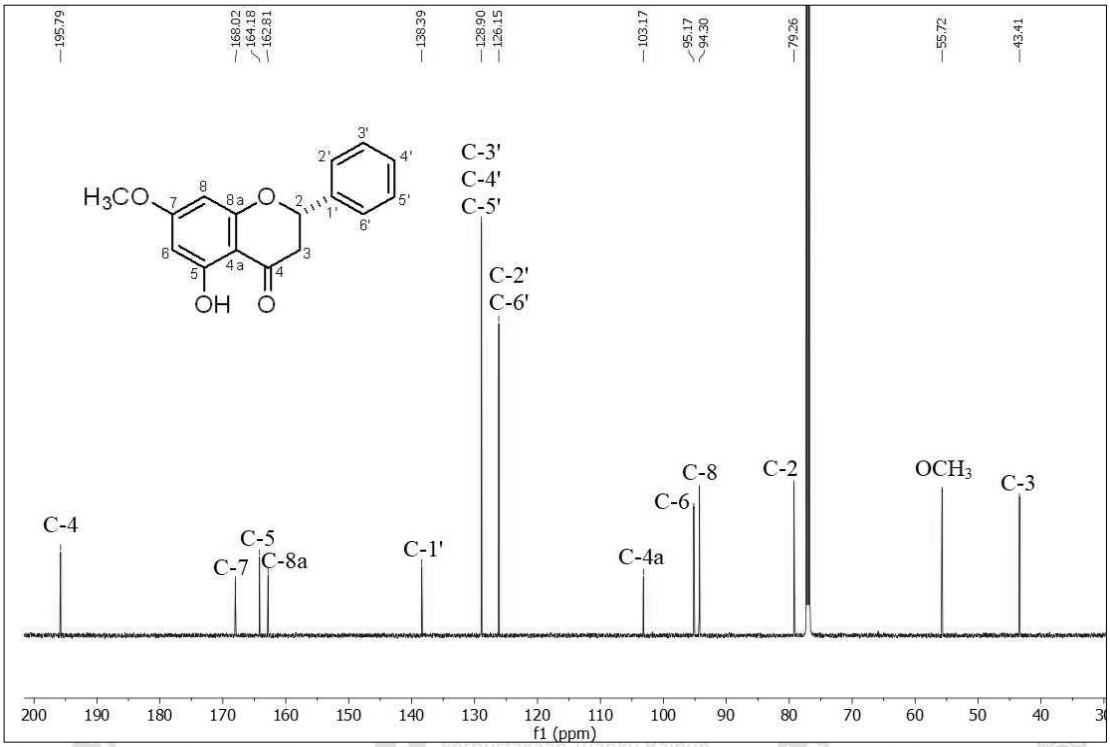


Figure 4.6E

*DEPT spectra of pinostrobin **(115)***

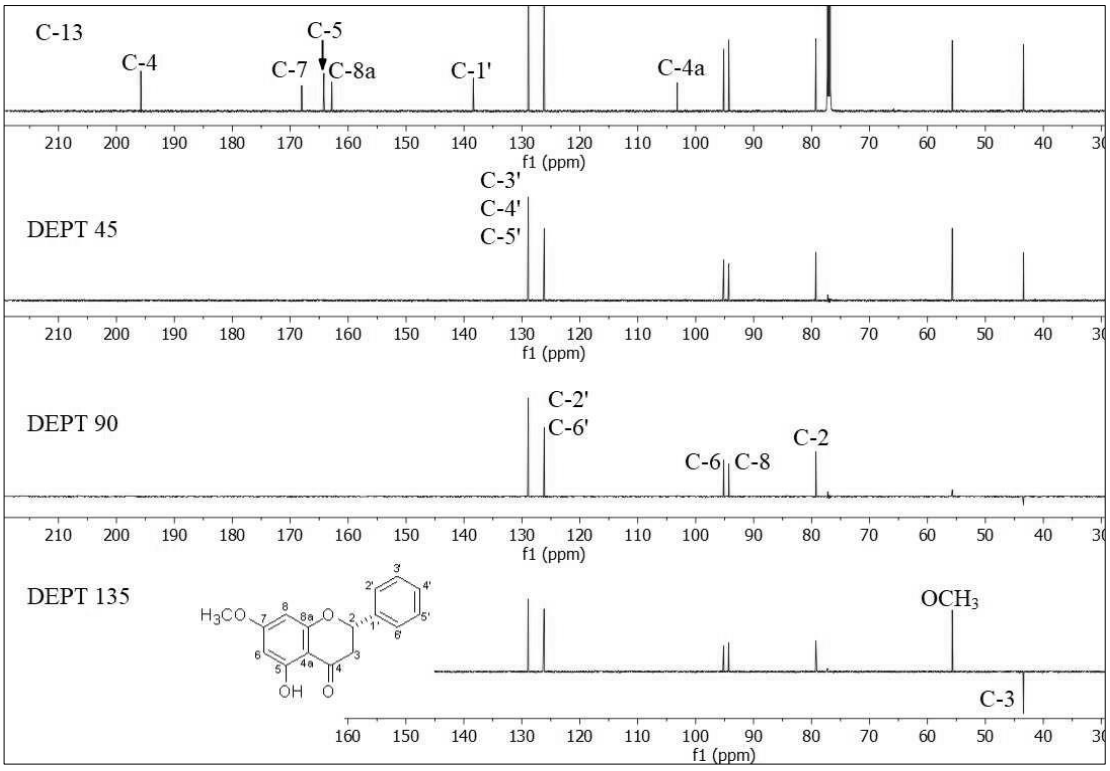


Figure 4.6F

*MS spectrum of pinostrobin **(115)***

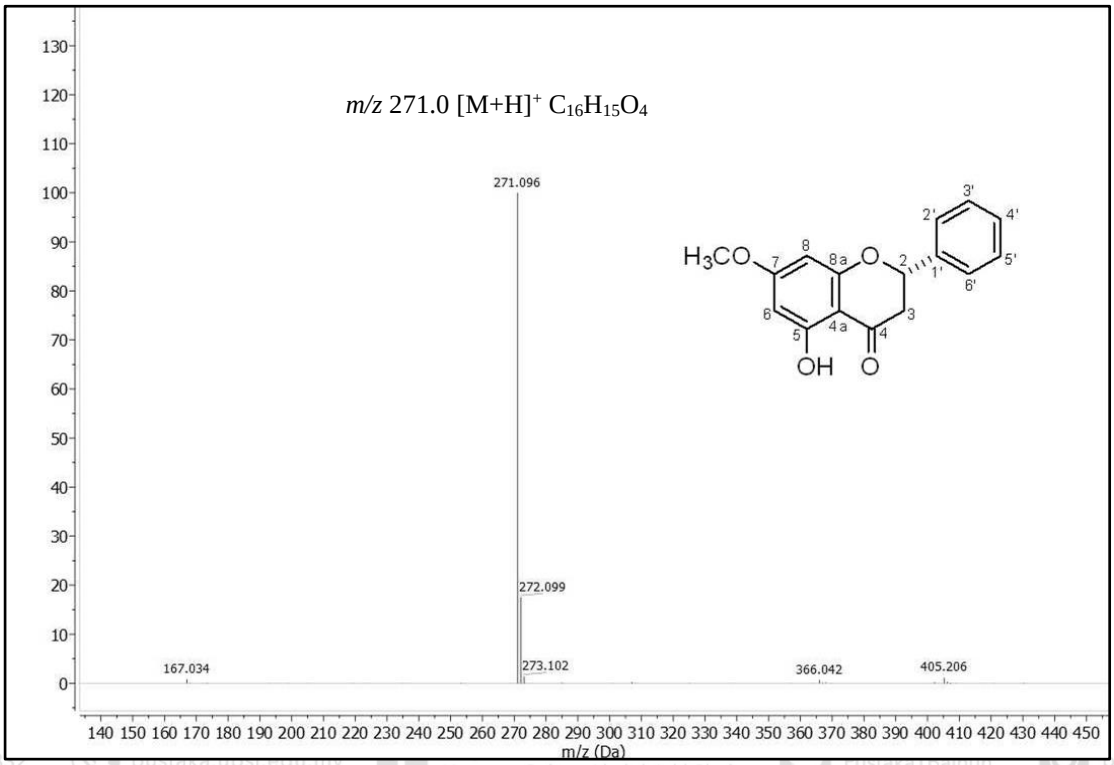


Figure 4.6G

*HMQC spectrum of pinostrobin **(115)***

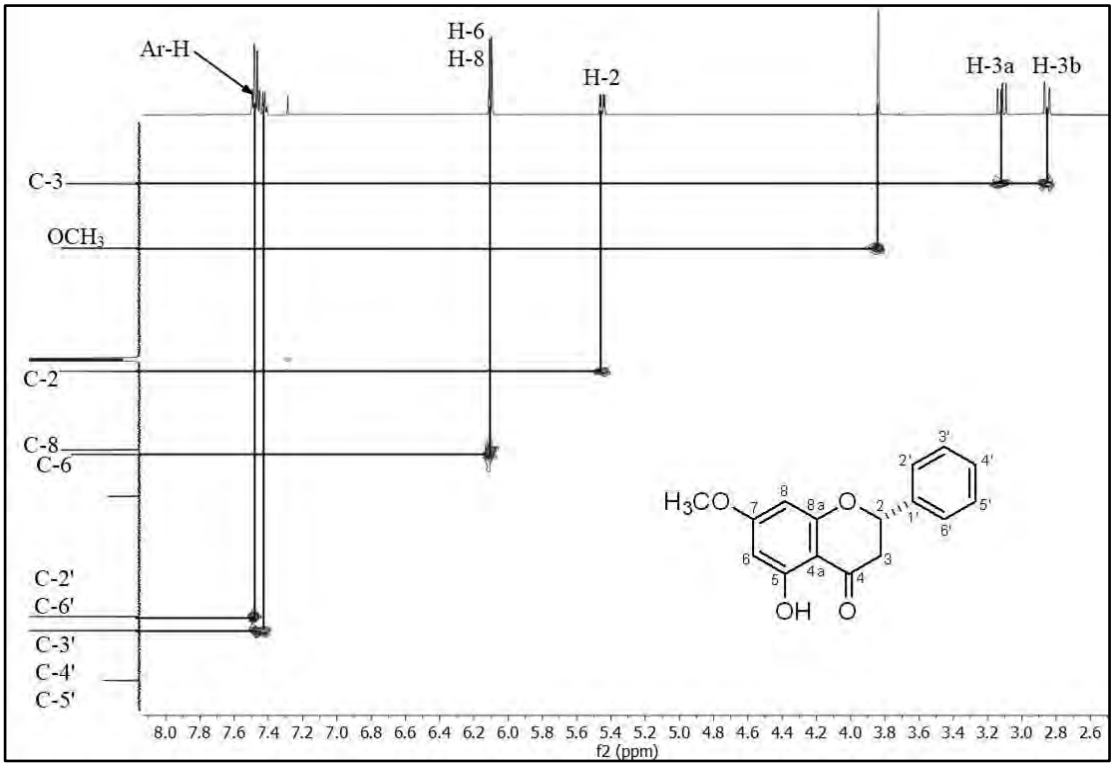
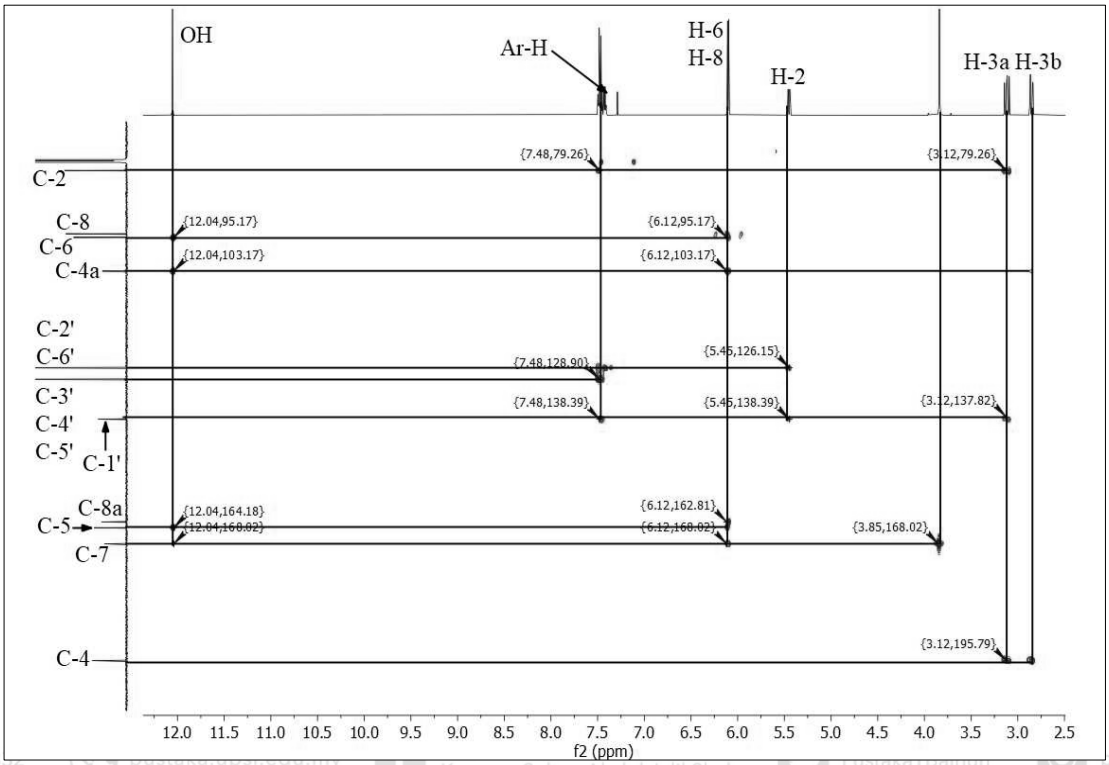
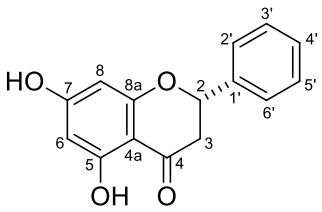


Figure 4.6H

HMBC spectrum of pinostrobin ***(115)***



4.3.5 Pinocembrin (114)



(**114**)

Purification of the DCM extract by CC using *n*-hexane:DCM had afforded compound **(114)** as a yellow solid. The IR spectrum (Figure 4.7A) revealed an absorption band at 3157 cm^{-1} corresponding to OH stretching, whereas 2891 cm^{-1} for sp^3 C-H stretching.

Besides, absorption band at 1630 cm^{-1} and 1582 cm^{-1} were corresponded to C=O and C=C stretching. The absorption at 1166 cm^{-1} corresponds to the stretching of the C-O bond.

The ^{1}H NMR spectrum (Figure 4.7B) of compound **(114)** was almost identical to compound **(115)**. The similarities of those spectra were observed, as the presence of *meta*-coupled signals, the multiplet peak for aromatic protons, and the singlet peak at the downfield region (5-OH). The only difference between both spectra was the absence of a methoxy signal at C-7 with additional proton OH in the ^{1}H NMR spectrum of compound **(114)**. Besides, based on chemical shift pattern comparison of H-2, H-3a and H-3b, the stereochemistry at H-3 was deduced as *R* configuration (Yang et al., 2022). In addition, the COSY spectrum (Figure 4.7C) showed correlation between H-2 (δ 5.45) with H-3a (δ 3.12), H-3b (δ 2.86).

The ^{13}C NMR (Figure 4.7D) and DEPT spectra (Figure 4.7E) exhibited the presence of fifteen carbons, embracing of one methylene carbon, eight methine carbons, five quaternary carbons, and a carbonyl carbon. The ^{13}C NMR data was further supported by the MS spectrum (Figure 4.7F) which gave a molecular ion peak at *m/z* 257 consistent with a molecular formula $C_{15}H_{13}O_4$. The complete ^{1}H NMR and ^{13}C NMR data as tabulated in Table 4.7.

Table 4.7

*NMR data of pinocembrin **(114)** and literature*

C	δ_H (ppm) (Int. mult. *J* in Hz) ($CDCl_3$, 500 MHz)	δ_C (ppm)	δ_H (ppm) (Int. mult. *J* in Hz) ($CDCl_3$, 400 MHz)[a]	δ_C (ppm)[a]
2	5.45 (1H, dd, 3.0, 13.0)	79.3	5.43 (1H, dd, 3.0, 13.2)	79.2
3a	3.12 (1H, dd, 3.0, 17.1)	43.4	3.09 (1H, dd, 3.2, 17.4)	43.3
3b	2.86 (1H, dd, 3.0, 17.1)	43.4	2.84 (1H, dd, 3.0, 17.4)	43.3
4	-	195.8	-	195.7
4a	-	103.3	-	103.2
5	-	164.4	-	164.3
6	6.03 (2H, s)	96.8	6.00 (2H, s)	96.7
7		164.5		164.7
8	6.00 (2H, s)	95.5	6.00 (2H, s)	95.5
8a	-	163.1	-	163.1
1'	-	138.3	-	138.3
2'	7.41-7.49 (1H, m)	126.2	7.37-7.45 (1H, m)	126.1
3'	7.41-7.49 (1H, m)	128.9	7.37-7.45 (1H, m)	128.9
4'	7.41-7.49 (1H, m)	128.9	7.37-7.45 (1H, m)	128.9
5'	7.41-7.49 (1H, m)	128.9	7.37-7.45 (1H, m)	128.9
6'	7.41-7.49 (1H, m)	126.2	7.37-7.45 (1H, m)	126.1
5-OH	12.07 (1H, s)	-	12.07 (1H, s)	-

[a]Data from literature (Tra et al., 2023)

The HMQC spectrum (Figure 4.7G) revealed the aromatic protons at δ 7.41-7.49 (H-2′-H-6′) were found directly attached to carbons resonated at δ 126.2 (C-2′/C-6′) and 128.9 (C-3′/C-4′/C-′5). Two aromatic protons of the ring A which were *meta*-coupled (H-6 and H-8) were correlated with carbons at δ 96.8 (C-6) and 95.5 (C-8). Two methine protons, H-2 (δ 5.55) and H-6 (δ 6.03) were confirmed attached to carbon at δ 79.3 (C-2) and δ 96.8 (C-6), respectively. In addition, the two inequivalent methylene protons δ 3.12 (H-3a) and 2.86 (H-3b) showed a cross peak with a carbon at δ 43.4 (C-3). Based on comparison of all spectral data with the literature, the structure of compound **(114)** was elucidated as 5,7-dihydroxyflavanone or known as pinocembrin. This compound was reported to be present in the rhizomes of *B. rotunda* (Widyananda et al., 2023) and *B. pandurata* (Trakoontivakorn et al., 2001).

Figure 4.7A

*IR spectrum of pinocembrin **(114)***

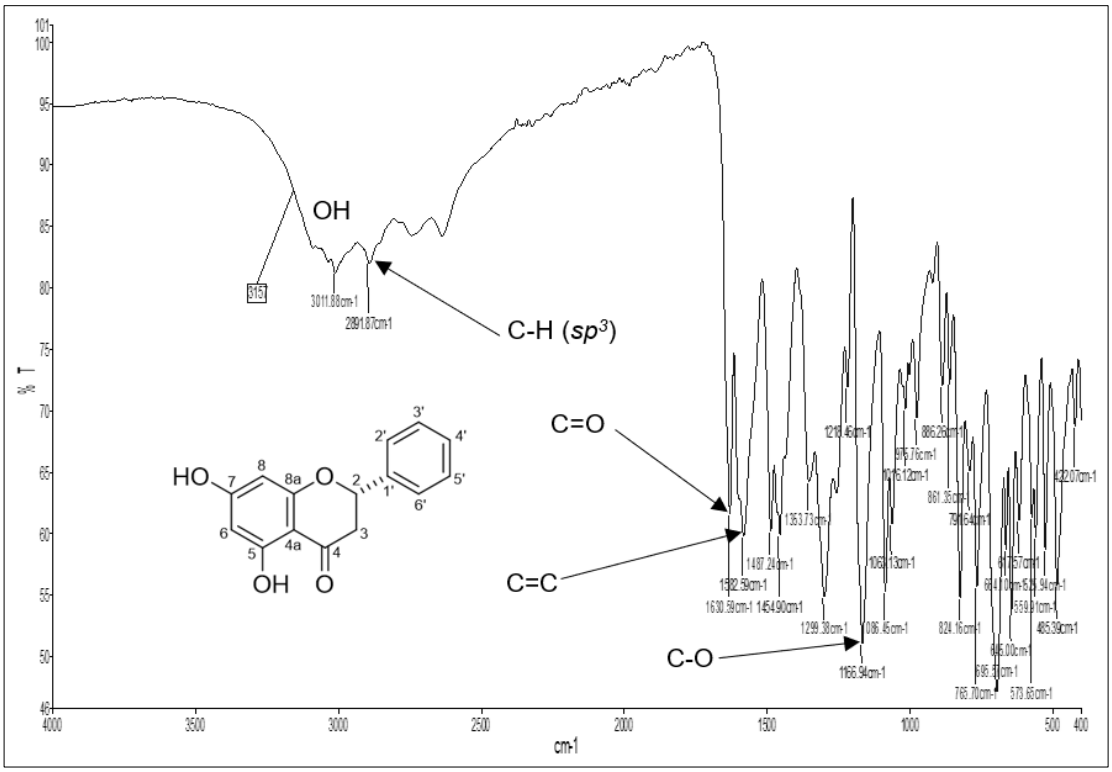


Figure 4.7B

*^{1}H NMR spectrum of pinocembrin **(114)***

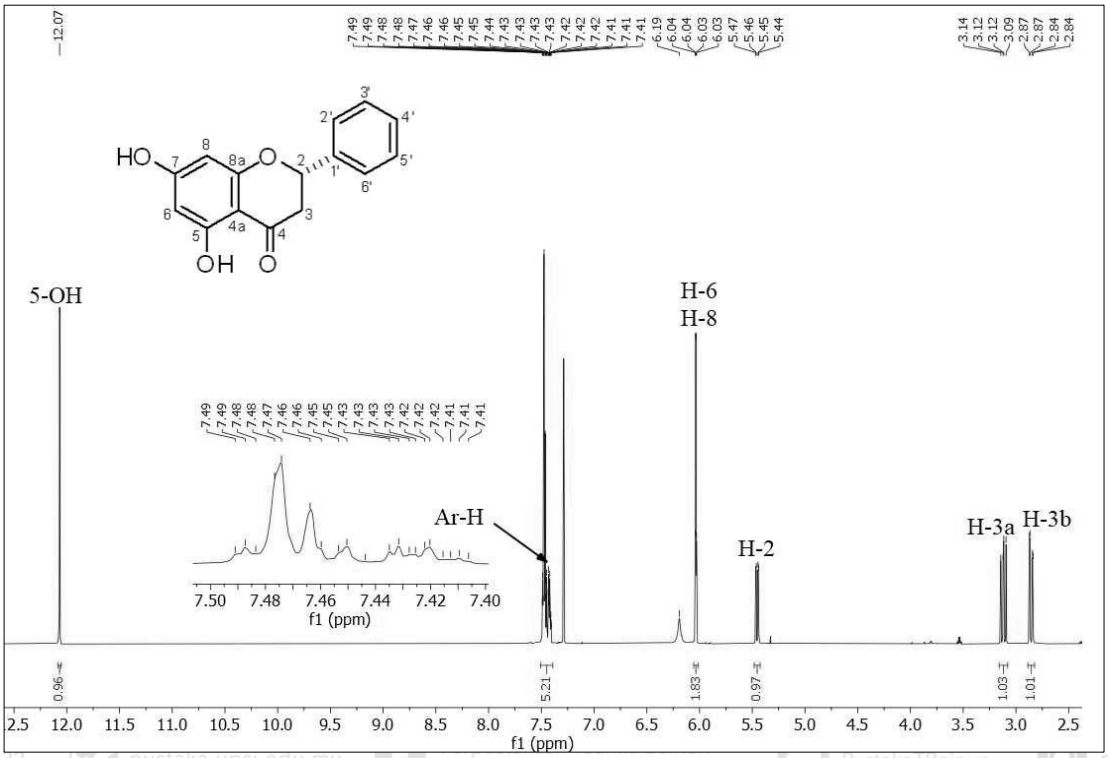


Figure 4.7C

*COSY spectrum of pinocembrin **(114)***

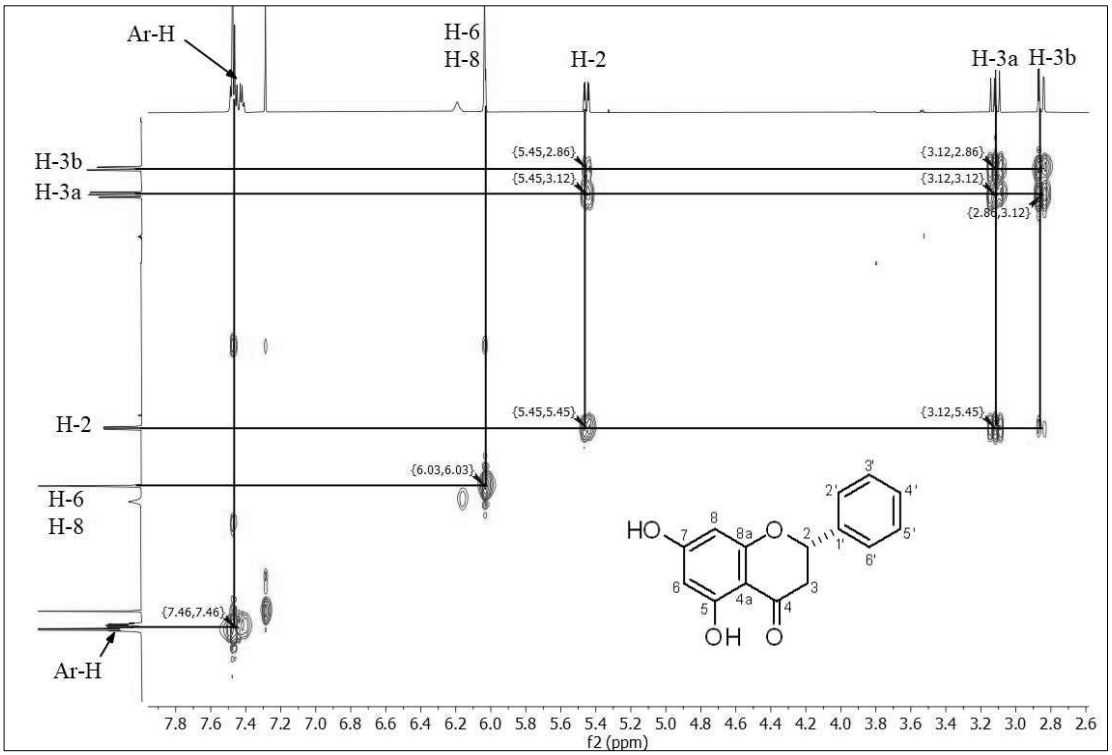


Figure 4.7D

*^{13}C NMR spectrum of pinocembrin **(114)***

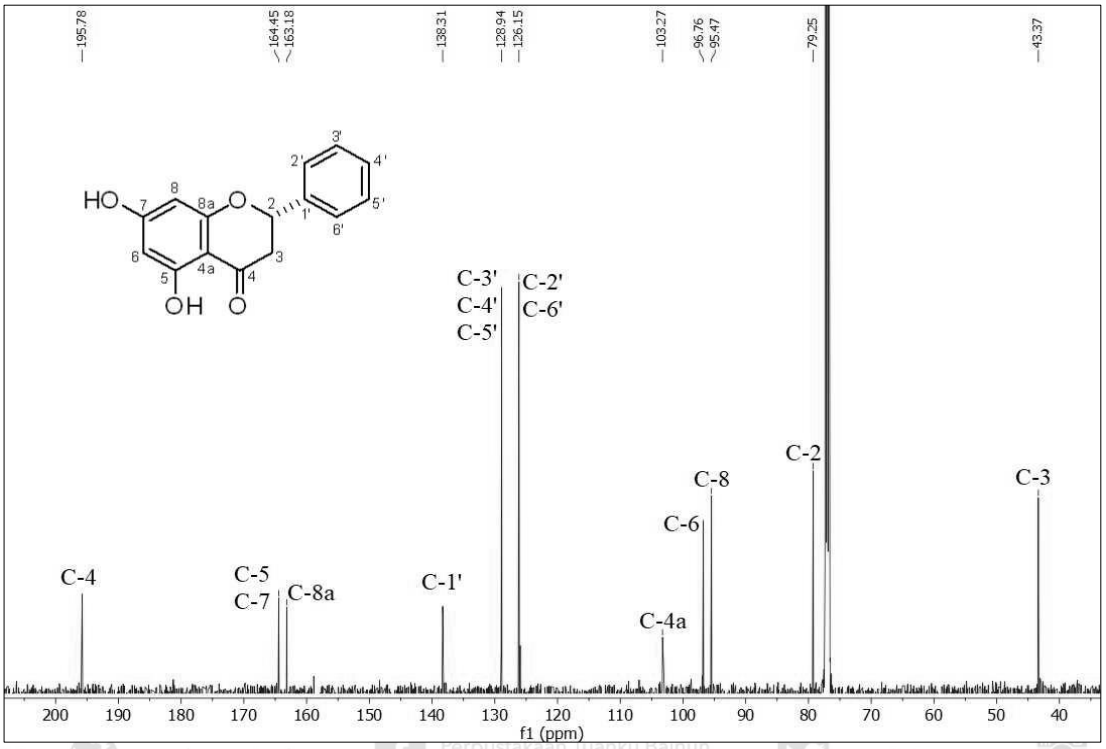


Figure 4.7E

*DEPT spectra of pinocembrin **(114)***

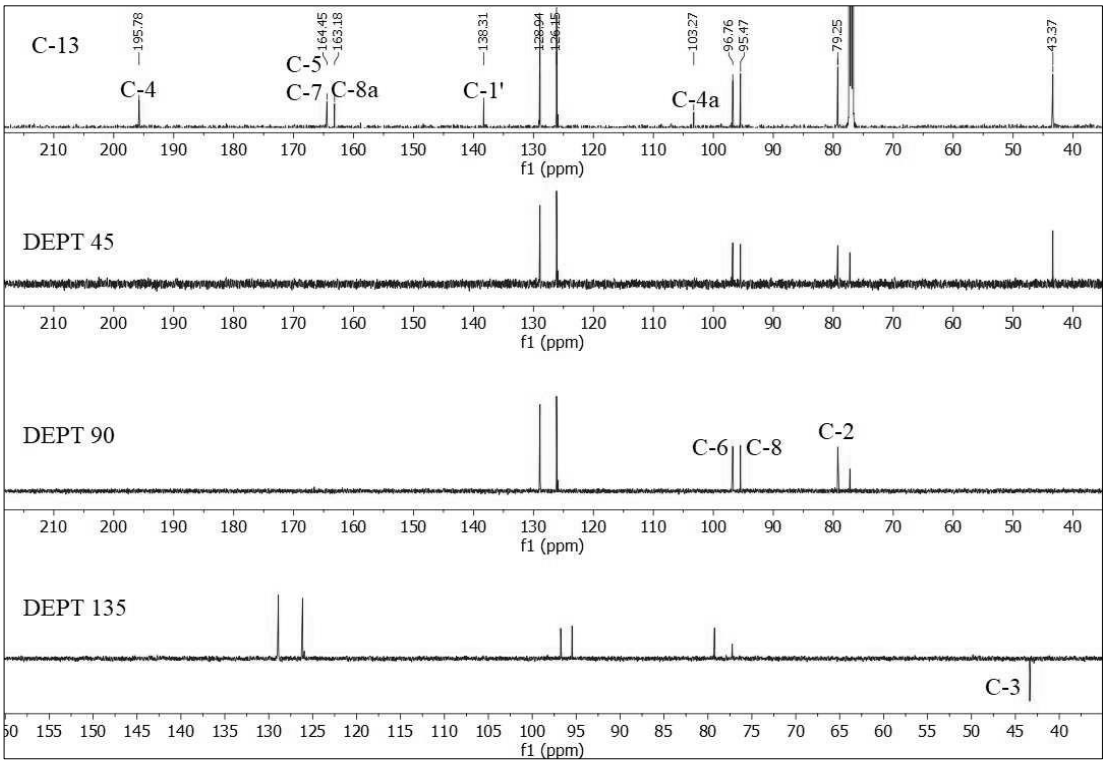


Figure 4.7F

*MS spectrum of pinocembrin **(114)***

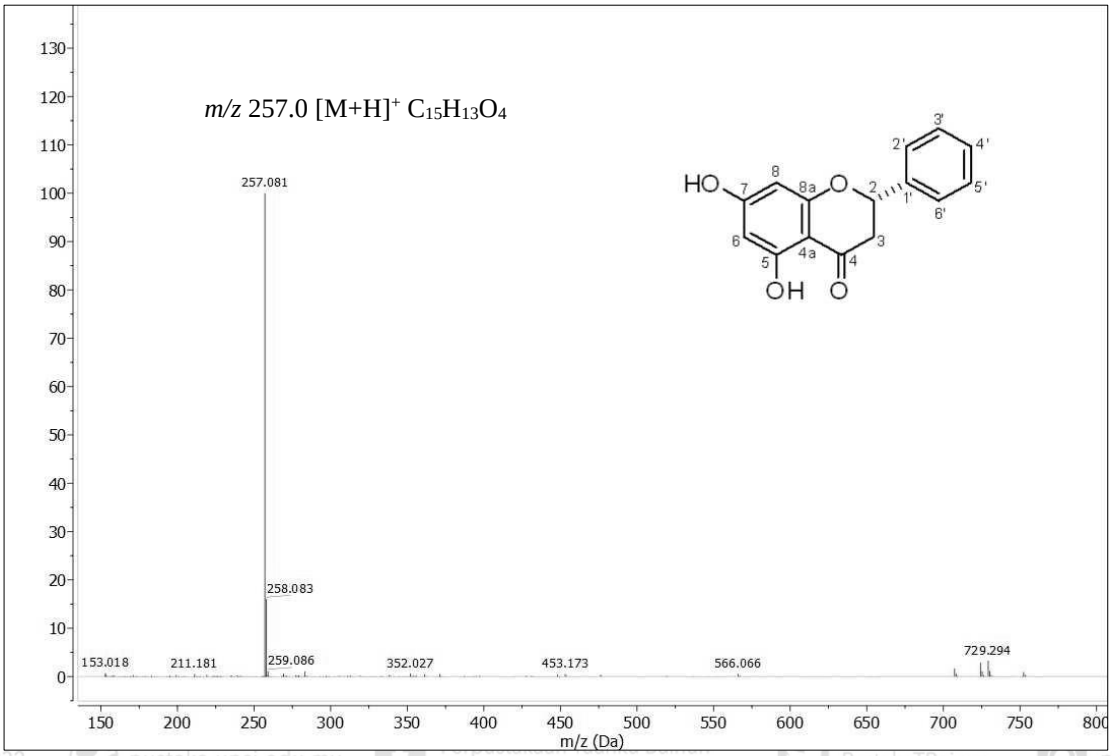
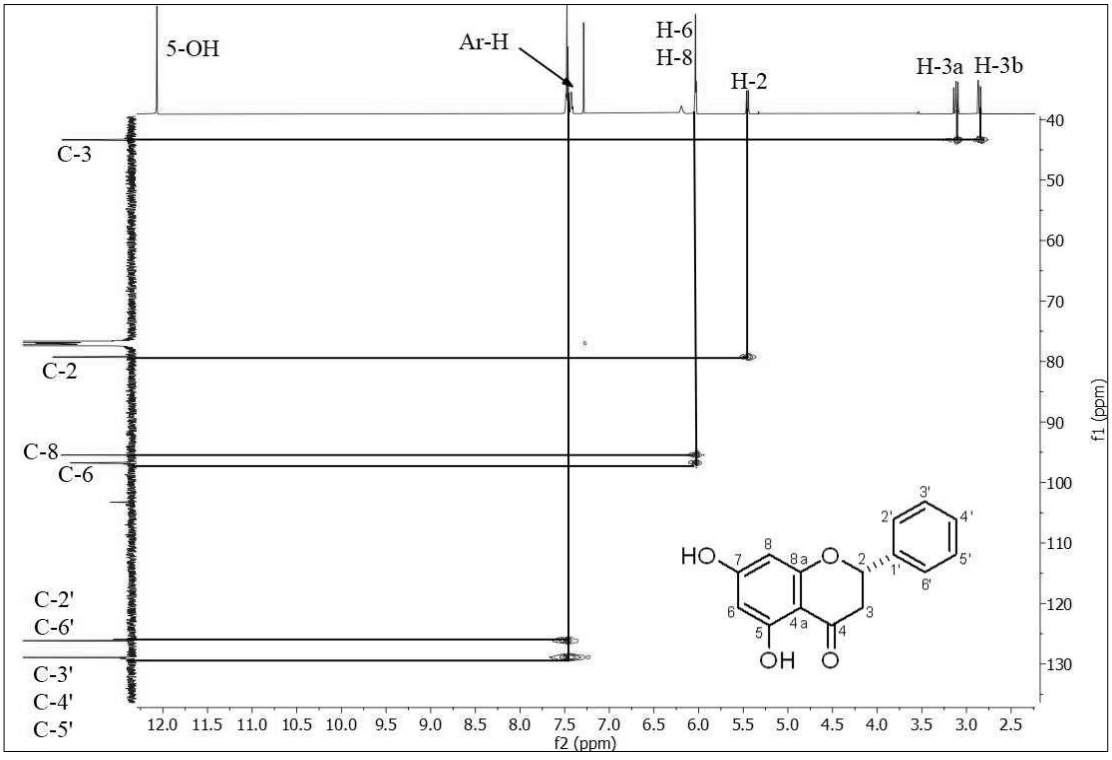
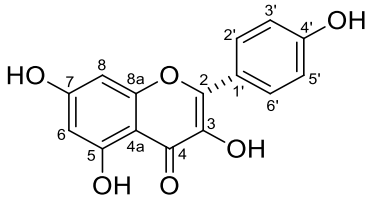


Figure 4.7G

*HMQC spectrum of pinocembrin **(114)***



4.3.6 Kaempferol (131)



(131)

Purification of the MeOH extract by CC using *n*-hexane:DCM had afforded compound **(131)** as a white solid. The IR spectrum (Figure 4.8A) showed absorption bands at 3421 cm^{-1} indicated the presence of hydroxyl group and 1661 cm^{-1} for carbonyl group stretching.

The ^{1}H NMR spectrum (Figure 4.8B) showed a one proton singlet at δ 6.15 which was assigned for aromatic proton at H-6 and one proton singlet δ 6.40 for aromatic proton at H-8. The peak with two protons at δ 8.00 appeared as doublet of doublets was assigned to H-2' and H-6'. Another doublet of doublets peak with two protons at δ 6.89 was assigned for H-5' and H-3'. The COSY spectrum (Figure 4.8C) showed the proton-proton correlation for H-2'/H-3' and H-5'/H-6'.

The ^{13}C NMR spectrum (Figure 4.8D) established the resonances of fifteen carbon atoms. Characterization of these signals as six methines appeared at δ 98.7 (C-6), 94.0 (C-8), 115.9 (C-3'/C-5'), 130.0 (C-2'/C-6') and nine quaternary carbons resonated at δ 103.5 (C-4a), 122.1 (C-1'), 136.2 (C-3), 147. 3 (C-2), 159.7 (C-4'), 156.7 (C-8a), 161.2 (C-5), 161.2 (C-7), and 176.4 (C-4). The MS spectrum (Figure 4.8E)

showed a molecular ion peak at *m/z* 287.1 corresponding to the molecular formula of $C_{15}H_{11}O_6$. Table 4.8 shows the complete assignments of the NMR data.

Table 4.8

*NMR data of kaempferol **(131)***

C	δ_H (ppm) (Int. mult. *J* in Hz) (DMSO, 500 MHz)	δ_C (ppm)	COSY	HMQC
2	-	147.3	-	-
3	-	136.2	-	-
4	-	176.4	-	-
4a	-	103.5	-	-
5	-	161.2	-	-
6	6.15 (1H, d, 2.0)	98.7	H-8	C-6
7	-	164.4	-	-
8	6.40 (1H, d, 2.0)	94.0	H-6	C-8
8a	-	156.7	-	-
1'	-	122.2	-	-
2'	8.00 (1H, d, 8.8)	130.0	H-3′, H-6′	C-2′
3'	6.89 (1H, d, 8.8)	115.9	H-2′, H-5′	C-3′
4'	-	159.7	-	-
5'	6.89 (1H, d, 8.8)	115.9	H-6′, H-2′	C-5′
6'	8.00 (1H, d, 8.8)	130.0	H-5′, H-3′	C-6′
5-OH	12.44 (1H, s)	-	-	-

The ^{13}C NMR data was further supported by the HMQC spectrum (Figure 4.8F) which exhibited direct attachment of *para*-disubstituted aromatic protons δ 8.00 (H-6′) and 6.89 (H-3') to carbon at δ 130.0 (C-2') and 115.9 (C-3'), respectively. Two aromatic protons which appeared at δ 6.40 (H-8) and 6.15 (H-6) were attached to carbons at δ 98.7 (C-6) and 94.0 (C-8), respectively.

Based on the spectroscopic data and comparison with the literature, it was confirmed that compound **(131)** was identified as 3,4',5,7-tetrahydroxyflavone or known as kaempferol. It has been reported previously from the rhizomes of *B. rotunda* (Eng-Chong et al., 2011) and the tuberous root of *B. pandurata* (Chahyadi et al., 2014).

Figure 4.8A

*IR spectrum of kaempferol **(131)***

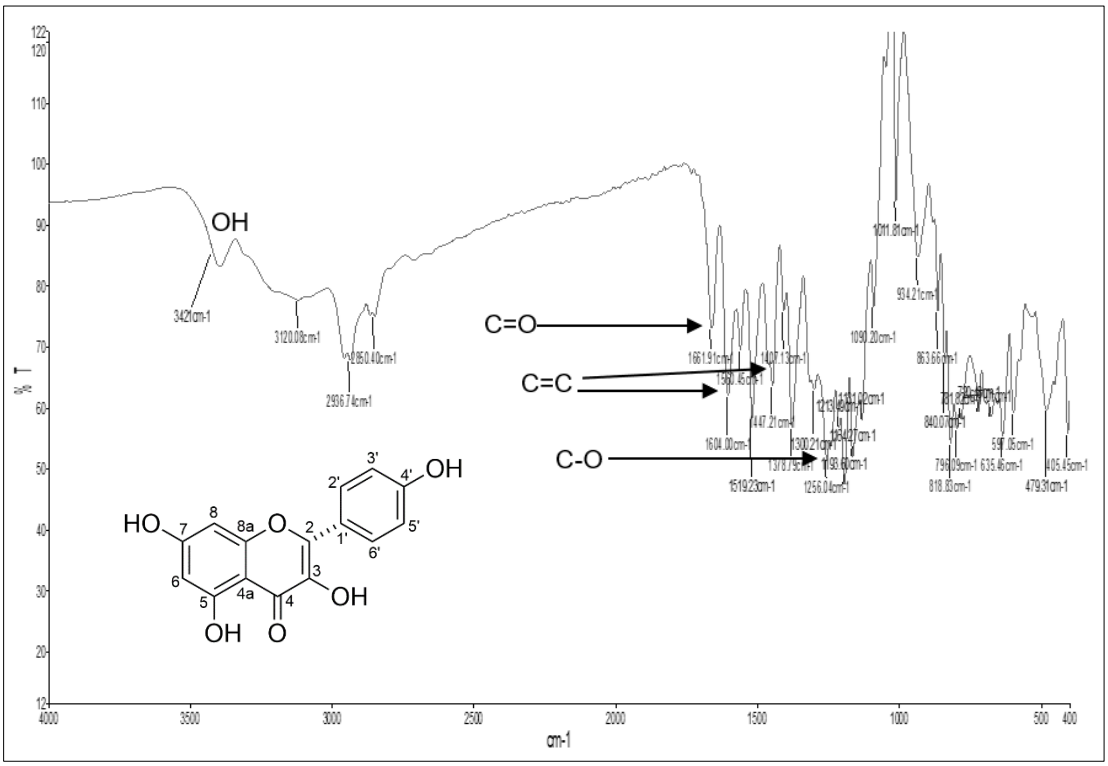


Figure 4.8B

*^{1}H NMR spectrum of kaempferol **(131)***

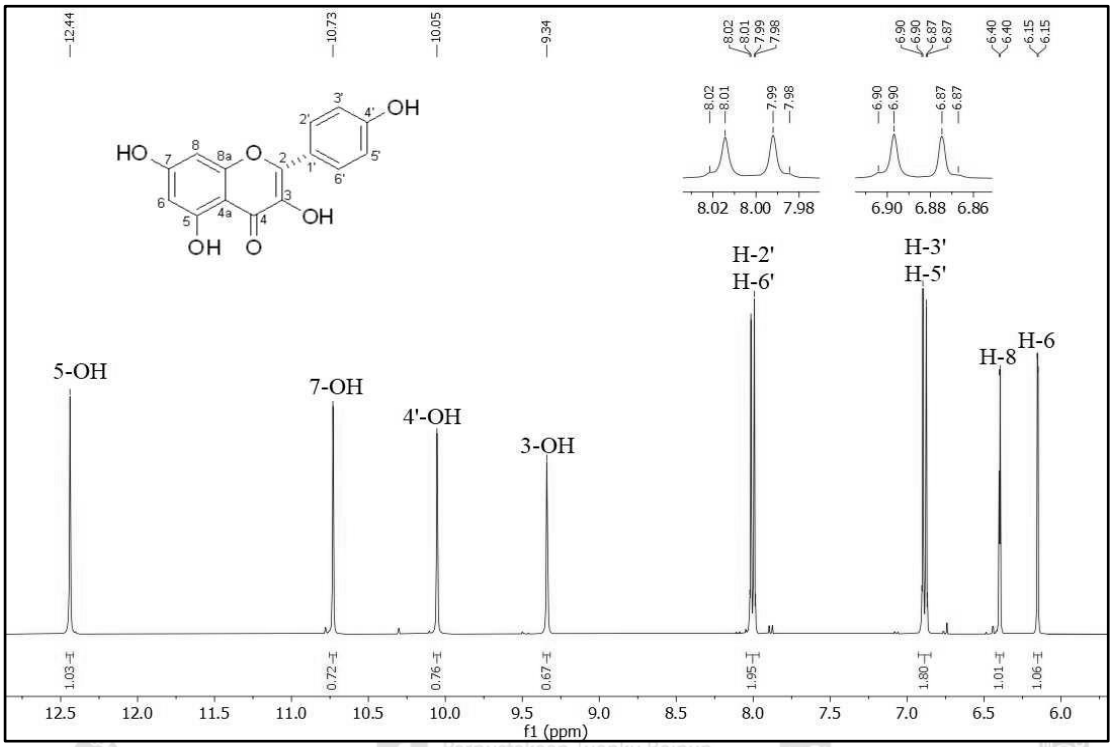


Figure 4.8C

*COSY spectrum of kaempferol **(131)***

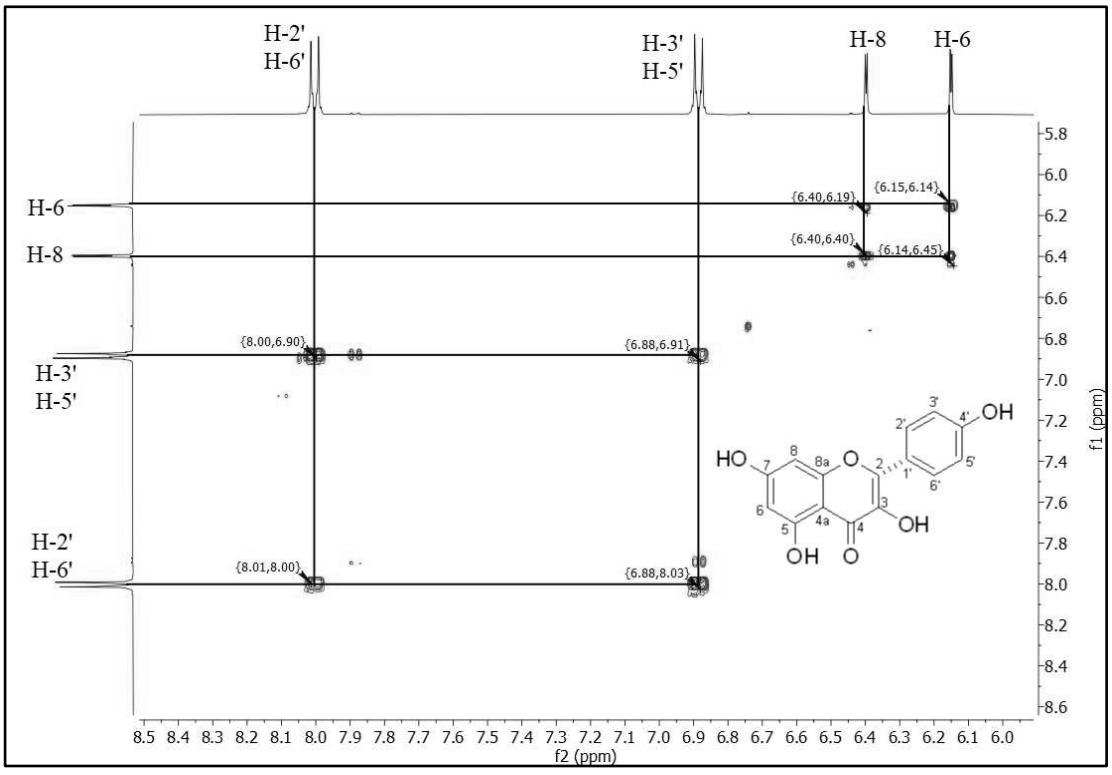


Figure 4.8D

^{13}C NMR spectrum of kaempferol ***(131)***

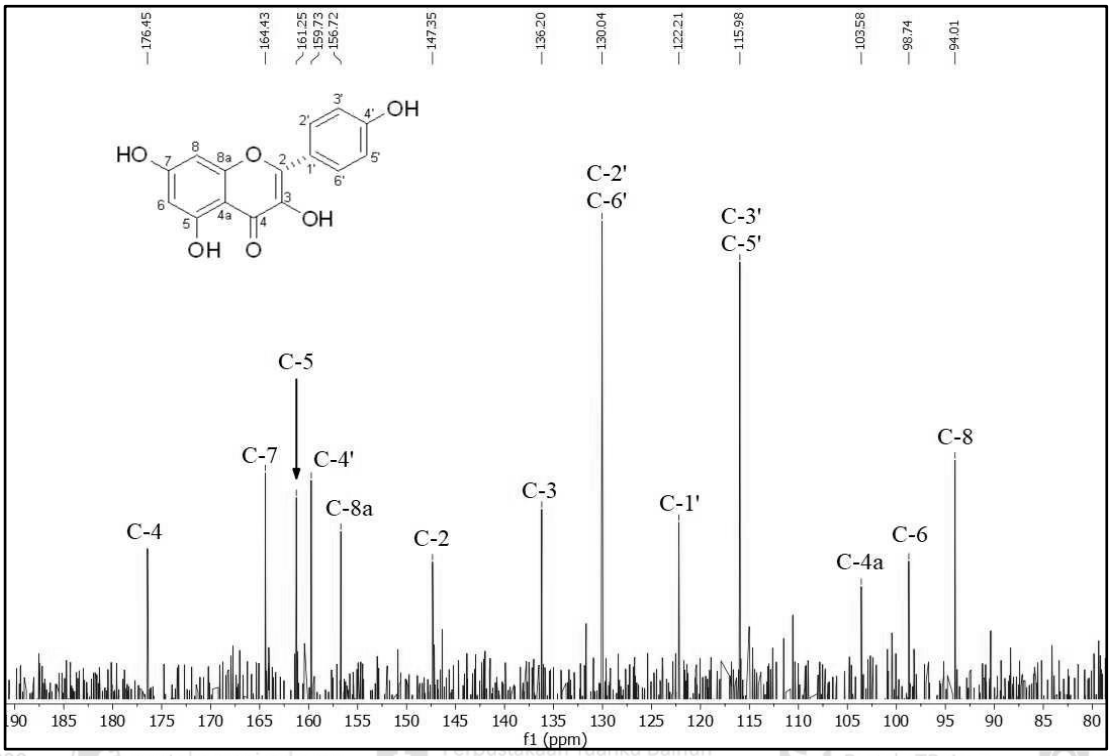


Figure 4.8E

MS spectrum of kaempferol ***(131)***

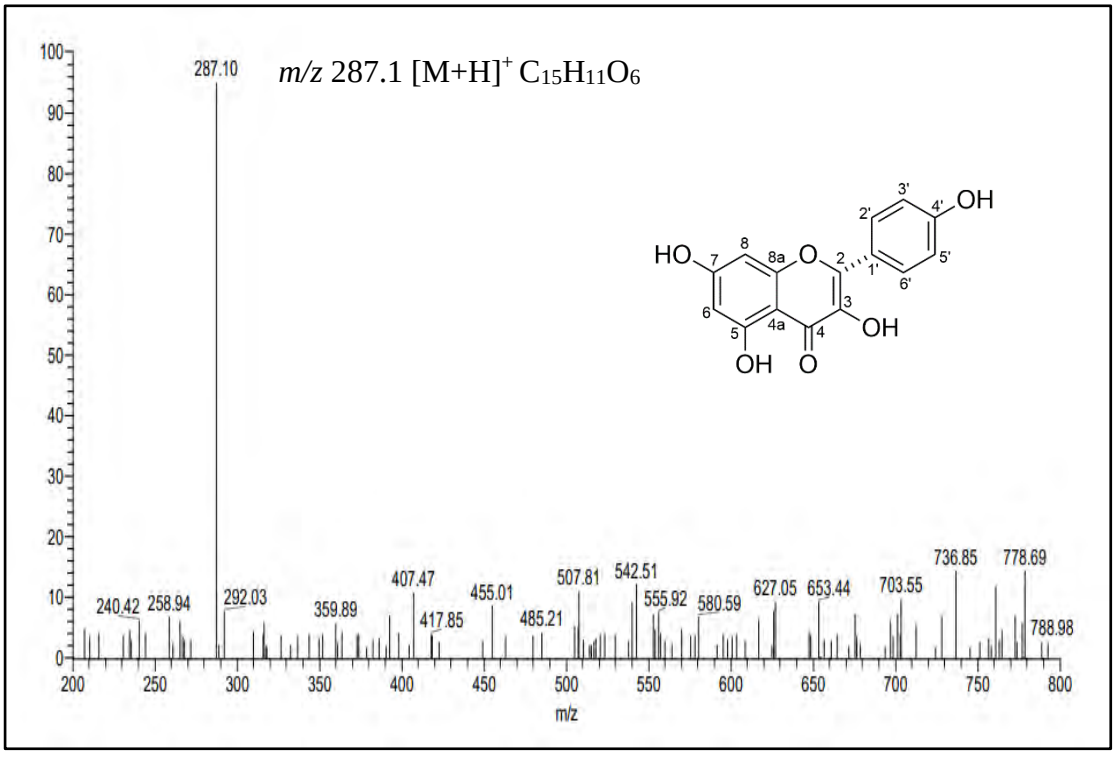
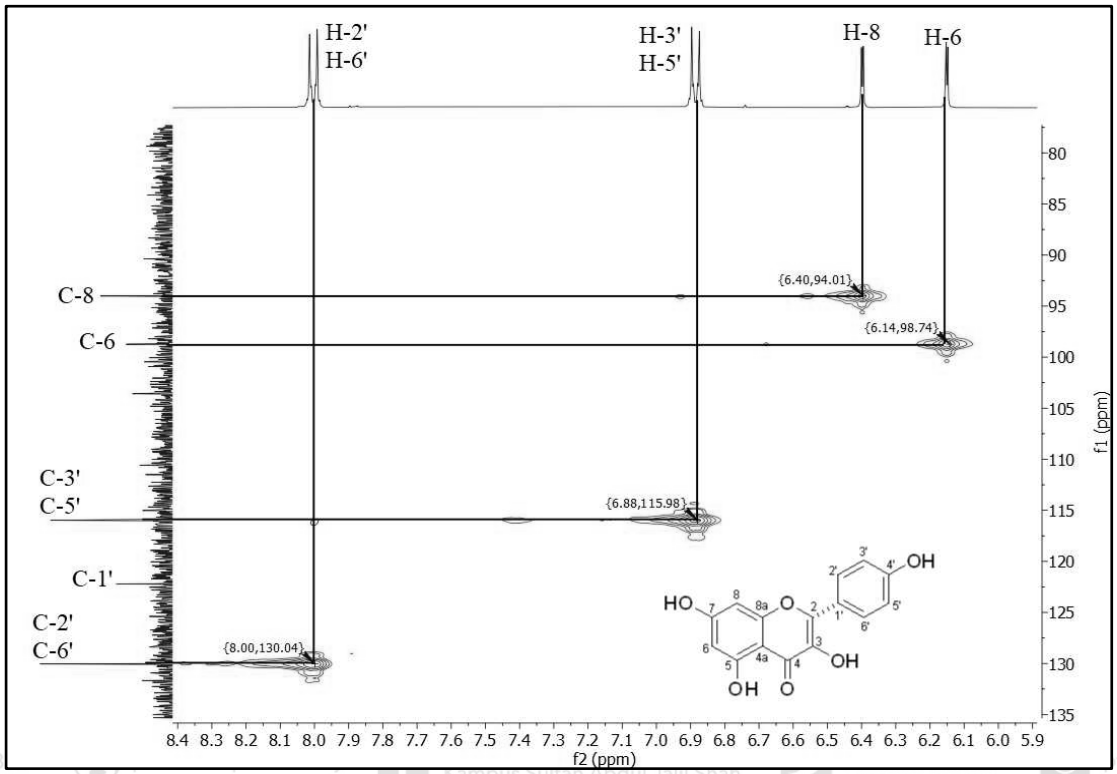
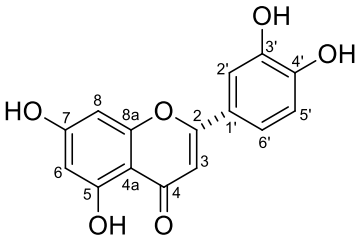


Figure 4.8F

HMQC spectrum of kaempferol ***(131)***



4.3.7 Luteolin (138)



(138)

Purification of the MeOH extract by CC using *n*-hexane:DCM had afforded compound **(138)** as a white solid. The IR spectrum (Figure 4.9A) was identical to that of

kaempferol **(131)** with the presence of hydroxyl and chelated carbonyl stretching absorption bands at 3222 and 1661 cm^{-1} respectively.

The ^{1}H NMR spectrum (Figure 4.9B) was found very similar to kaempferol **(131)** except for an additional singlet signal at δ 6.47 which was attributed to H-3. In addition, the ABX aromatic spin system signals which were observed at δ 7.31 (1H, d, *J* = 2.3), 7.28 (1H, d, *J* = 1.7 Hz), and 6.74 (1H, d, *J* = 8.1 Hz), and were assigned to methine protons of H-2′, H-6′, and H-5′ in B ring, respectively. The COSY spectrum (Figure 4.9C) supported the ortho coupling in B ring between H-5′ and H-6′ through correlations between signals at δ 6.74 and 7.28. Furthermore, signal at δ 6.25 correlated with signal at δ 6.00, which supported the meta coupling between H-8 and H-6 in B ring.

The fifteen carbons established from the ^{13}C NMR spectrum (Figure 4.9D) were categorised into six methines, nine quaternary carbons and one carbonyl. It was consistent with the molecular formula $C_{15}H_9O_6$ determined from the molecular base peak at *m/z* 285, in the MS spectrum (Figure 4.9E). Table 4.9 shows the complete assignments of the NMR data.

Based on the spectroscopic data and comparison with the literature values, it was confirmed that compound **(138)** was identified as 5,7,3′,4′-tetrahydroxyflavone or known as luteolin. It has been reported previously from the rhizomes of *B. armeniaca* (Jing et al., 2010).

Table 4.9

*NMR data of luteolin **(138)***

C	δ_H (ppm) (Int. mult. *J* in Hz) (DMSO, 500 MHz)	δ_C (ppm)	COSY
2	-	164.1	
3	6.47 (1H, s)	102.3	
4	-	181.5	
4a	-	102.8	
5	-	161.8	
6	6.00 (1H, d, 2.0)	100.1	H-8
7	-	161.8	
8	6.25 (1H, d, 2.0)	94.8	H-6
8a	-	158.0	
1′	-	120.4	
2′	7.31 (1H, d, 2.3)	112.8	H-6′
3′	-	146.9	
4′	-	152.4	
5′	6.74 (1H, d, 8.1)	116.3	H-6′
6′	7.28 (1H, d, 1.7)	119.4	H-5′
5-OH	12.89 (1H, s)	-	

Figure 4.9A

*IR spectrum of luteolin **(138)***

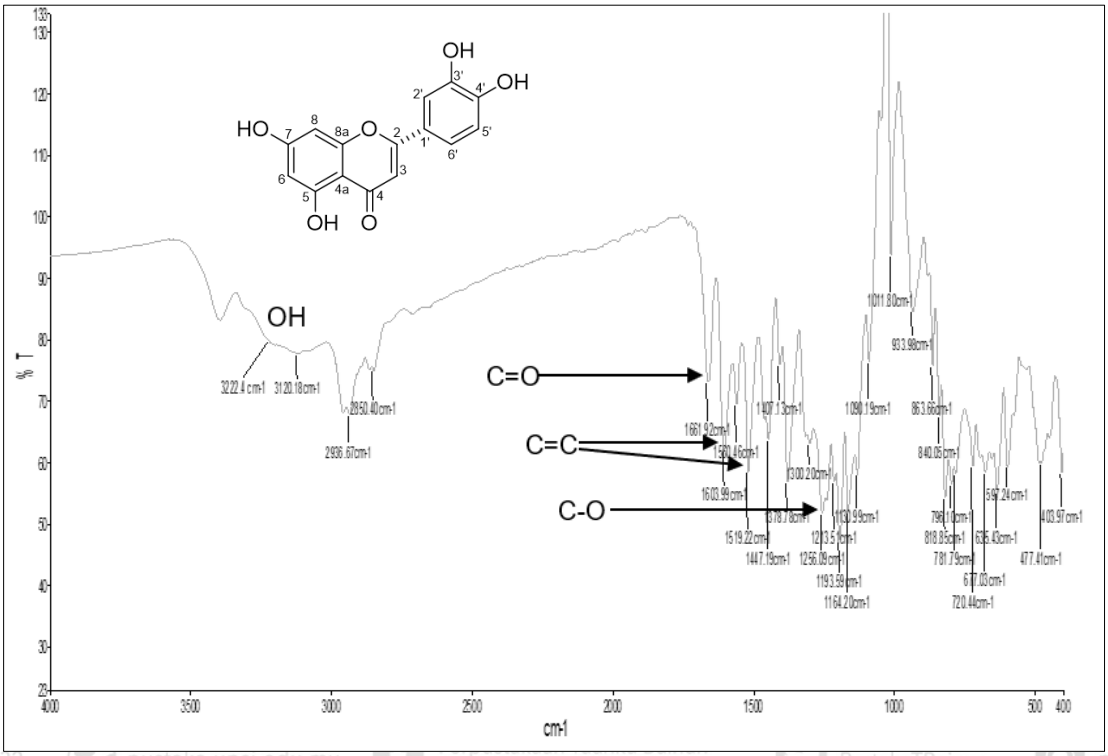


Figure 4.9B

*^{1}H NMR spectrum of luteolin **(138)***

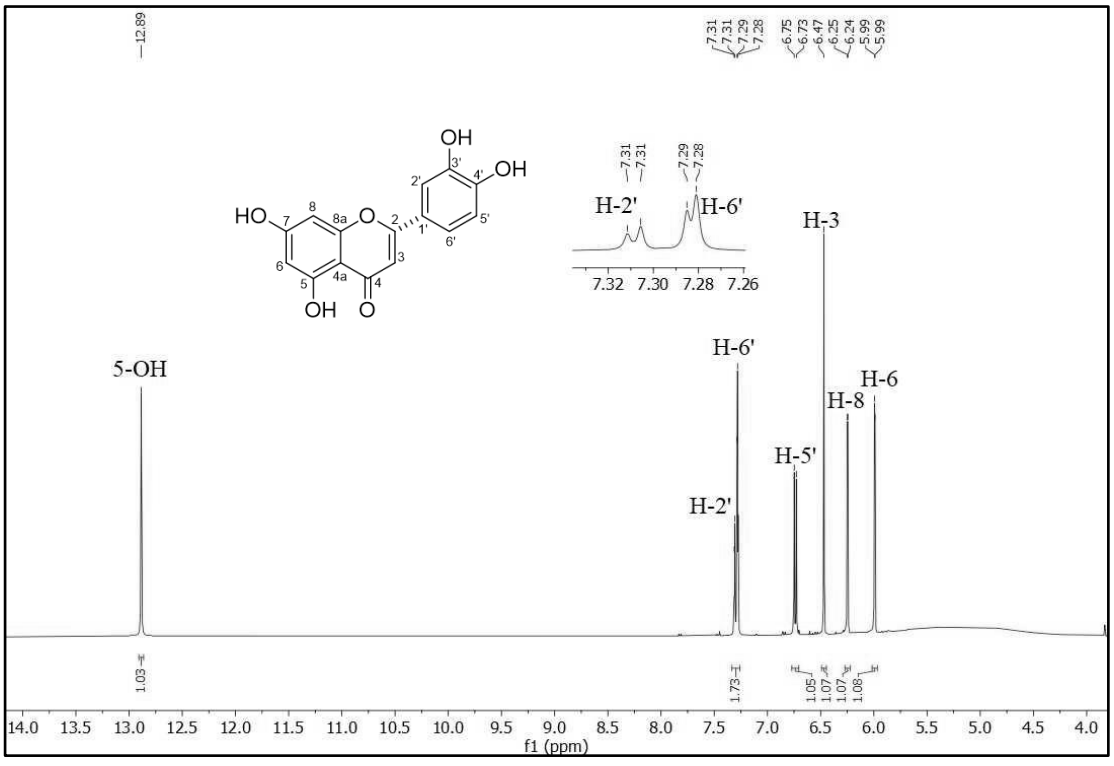


Figure 4.9C

*COSY spectrum of luteolin **(138)***

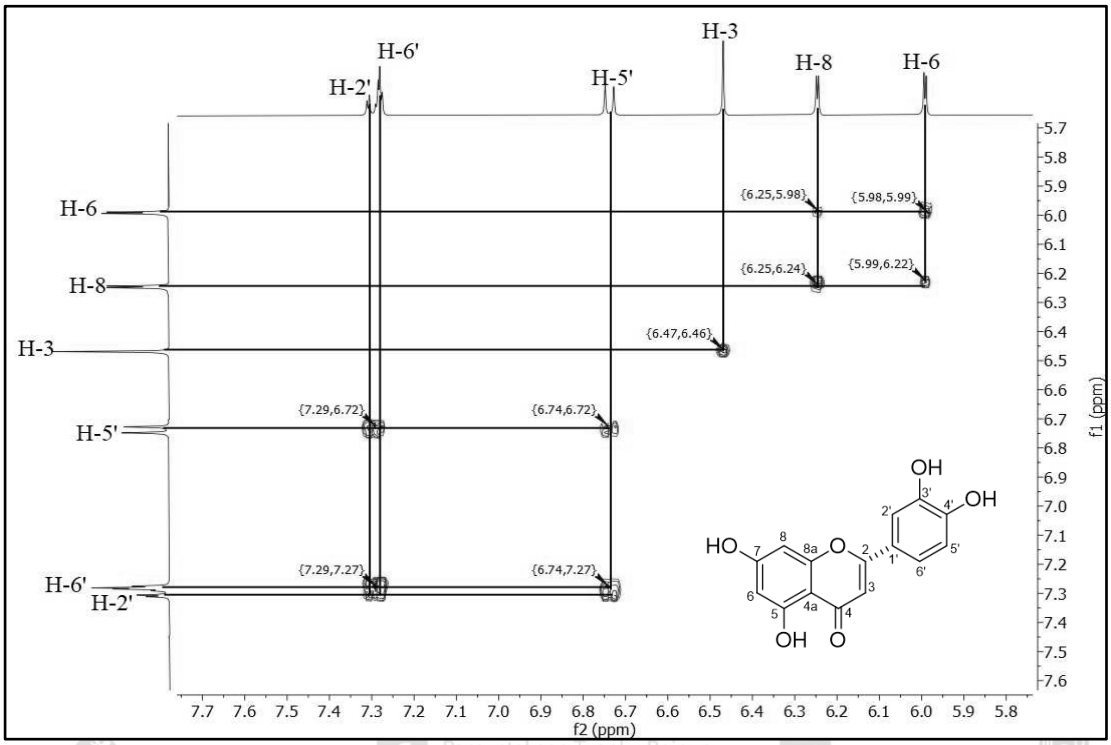


Figure 4.9D

*^{13}C NMR spectrum of luteolin **(138)***

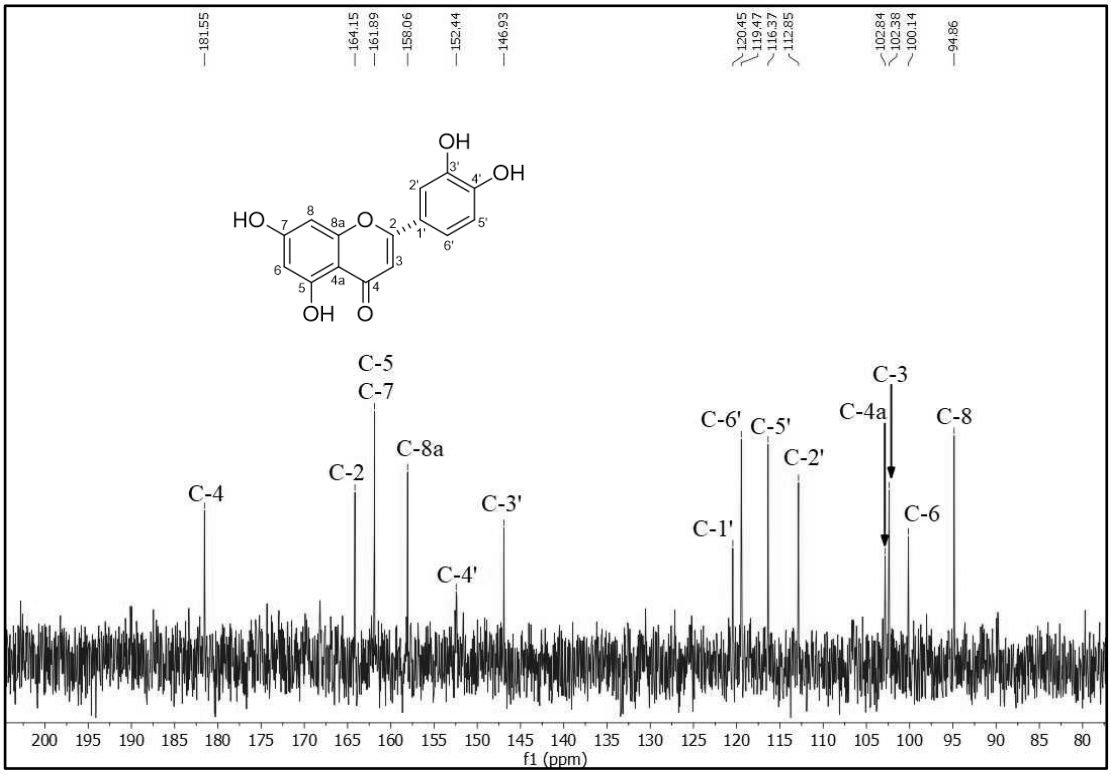
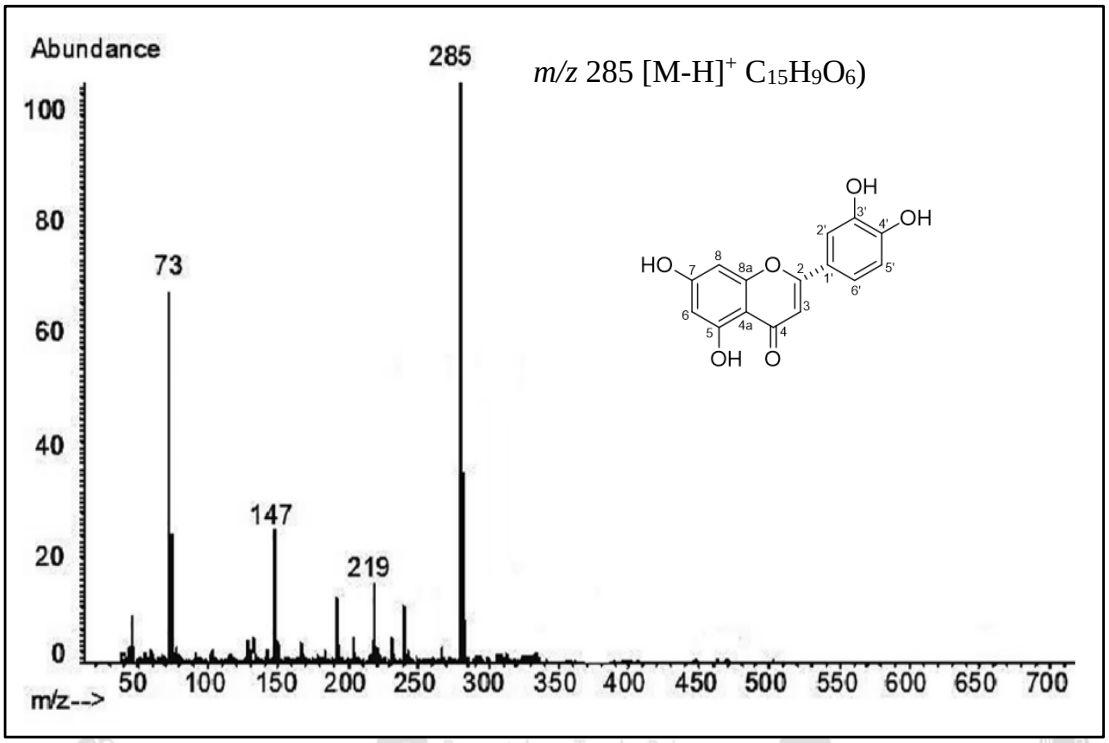


Figure 4.9E

MS spectrum of luteolin ***(138)***



4.4 Antioxidant Activity

The term “antioxidant” refers to substances or molecules that are capable of delaying or even preventing the irreversible damage of other substances or macromolecules due to some metabolites’ instability present in a living system and therefore promoting health benefits. Antioxidant activity tests require different approaches, since different classes of substances may present such activity. For instance, measuring antioxidant activity implies measuring the reaction rate or how antioxidants may affect the autoxidation rate of the substrate which they are known to protect (Neha et al., 2019).

Exogenous natural antioxidants may be considered as bioactive compounds and are specially derived from food and medicinal plants, such as cereals, traditional herbs, fruits, vegetables, and spices. Natural antioxidants present in foods, such as phenolic phytochemicals, seem to provide metabolic benefits and are linked with a lower risk of developing several health complications. In addition, the protective effects of antioxidants present in fruits and vegetables are related to three main groups: phenolic compounds, carotenoids, and vitamins (Alkadi, 2020).

The course of different diseases can lead to increased oxidative stress, which triggers the development of metabolic disorders such as the increased production of reactive oxygen species (ROS) and the glycosylation of non-enzymatic proteins. All these factors can damage DNA molecules, lipids and proteins, leading to the development of diseases such as diabetes, cancer, rheumatism, coronary heart disease, inflammatory bowel diseases, and degenerative diseases. In this circumstance, medicinal plants have been widely used over time to treat different diseases, as they have natural phytochemicals that can be existing in bark, flowers, roots, stems, fruits, and leaves that embody a vital source of antioxidant compounds such as tannins, flavonoids, anthocyanins, phenolic acids and other phenolic compounds, which can cure or avoid oxidation effects (Mendonca et al., 2022).

The antioxidant activity of plant extracts can be revealed by several in vitro methods. There are two common types of assays widely used for different antioxidant studies. The first group includes assays related to electron or radical scavenging, such as the DPPH assay, Trolox Equivalent Antioxidant Capacity (TEAC) assay, and the Ferric Reducing Antioxidant Power (FRAP) assay. These assays are based on reduction

reactions. The other group consists of assays related to lipid peroxidation, including the thiobarbituric acid assay and the β-carotene bleaching assay (Skrovankova et al., 2012).

One of the most popular colorimetric assays to estimate the radical scavenging capacity of plants and extracts is the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. This method is precise, easy to operate, and reasonable, providing a screening of the general activity of the antioxidants and is based in a stable and synthetic radical, DPPH. When DPPH reacts with an antioxidant compound, its free radical property is lost and its colour changes from violet to yellow.

In this study, the antioxidant activity of the crude extracts and isolated phytochemicals of *B. albosanguinea* were performed using DPPH free radical scavenging assay, and the results are summarized in Table 4.10. The percentage inhibition at different concentrations of the crude extracts and isolated phytochemicals are summarized in Figure 4.10 and 4.11, respectively.

All extracts showed significant differences compared to the positive control, ascorbic acid (AA) and butylated hydroxytoluene (BHT). Notably, all extracts displayed the strongest activity with IC_{50} values in the range of 11.2 to 14.5 µg/mL, indicating its notably DPPH radical scavenging capability. This potency is attributed to its richness in antioxidants, such as phenolic compounds, flavonoids, and polyphenols., known for their ability to neutralize free radicals (Salleh et al., 2016).

Table 4.10

DPPH radical scavenging of the crude extracts and isolated phytochemicals

Samples	Inhibition at 200 µg/mL (%)	IC_{50} (µg/mL)
Crude extracts		
BARH	84.7	14.5
BARD	86.0	13.6
BARM	85.7	11.2
Compounds		
Elemicin (60)	73.2	9.3
Panduratin A (72)	73.2	25.8
Isopanduratin A (76)	84.9	12.8
5,6-Dehydrokawain (196)	73.2	25.8
Pinocembrin (114)	76.0	14.4
Pinostrobin (115)	73.2	27.4
Ascorbic acid	88.3	13.3
Butylated hydroxytoluene	79.1	14.9

BARH = *B. albosanguinea* rhizome hexane extract; BARD = *B. albosanguinea* rhizome DCM extract; BARM = *B. albosanguinea* rhizome MeOH extract

In a previous study, the antioxidant activity (IC_{50}) of the ethanol extract from the rhizome of *B. rotunda* collected at Beringharjo Market, Yogyakarta, Indonesia, was found to be 92.6 µg/mL, as determined using the DPPH method at a concentration of 100 µg/mL. This result, being below 100 µg/mL, indicates a high level of antioxidant activity (Atun et al., 2017). Another study on the ethanol extract of *B. rotunda* rhizome from Pathumthani reported an IC_{50} of 76.3 µg/mL (Jitvaropas et al., 2012). Previous study also revealed *B. rotunda* rhizome hexane extracts have high DPPH radical

scavenging activity, with IC_{50} values of 557.97 µg/mL at concentration > 5000 µg/mL. Moreover, antioxidant test using the DPPH method on the ethanol extract of *B. pandurata* rhizome from Bogor, Indonesia, revealed an IC_{50} of 37.05 µg/mL. According to Blois, if the IC_{50} of a substance is below 50 ppm, it is said to have strong antioxidant activity (Yuniarto et al., 2024).

Among the isolated compounds, elemicin **(60)** showed the highest DPPH free radical scavenging assay with IC_{50} values of 9.3 µg/mL. The radical scavenging activity of this compound might be due to the presence of allylic groups which have the capacity to donate electrons and function as reductones (Kitryte et al., 2012). Meanwhile, other compounds also found to have shown strong antioxidant activity. It could be due to the presence of hydroxyl group in the structure. The OH radicals have been reported to be a key player in the physiological regulation and control of cell functions. The reaction rate constant for OH radicals is extremely high, wherein they react broadly with almost every type of biomolecule within the cell and may deviate the normal physiological functions of the cell (Charlton et al., 2023). A higher number of OH groups present in the structure of phenolic compounds results in higher antioxidant properties, as long as there is no steric hindrance (Platzer et al., 2022).

In a previous study, panduratin A **(72)** isolated from the rhizomes of *Boesenbergia* species has garnered attention for its diverse pharmacological activities. Studies suggest that panduratin A **(72)** isolated from ethanol extract of *B. rotunda* acquired an acceptable DPPH inhibition activity percentage when compared to silymarin (Salama et al., 2013). This aligns with the proved characteristic of chalcones, which is their significant free radical scavenging activity (Gacche et al., 2008). It was

also revealed that panduratin A **(72)** was a potent inhibitor with lipid peroxidation activity IC_{50} of 15 μM on rat brain homogenate model. The side chain structures of panduratin A **(72)** are likely causes for the higher inhibitory activity of this compound (Shindo et al., 2006).

Moreover, the ferric-reducing antioxidant power (FRAP) assay determined the antioxidant activity of *B. rotunda* rhizome extracts and compounds using a modified version of the Pandey and Rizvi protocol (Pandey & Rizvi, 2012). The FRAP assay data show that flavonoids such as pinostrobin **(115)** and pinocembrin **(114)** resulted in moderate antioxidant activity at 1 mg/mL with FRAP values of 8.74 and 9.03, respectively. Isopanduratin A **(76)** showed high antioxidant activity at 1 mg/mL with a FRAP value of 14.53 μM/μg (Han et al., 2023). These results are comparable with the findings of literature that compounds containing more methoxy, and phenolic hydroxyl groups exhibit more antioxidant activity (Chen et al., 2020).

Figure 4.10

Percentage inhibition of B. albosanguinea crude extracts at various concentrations

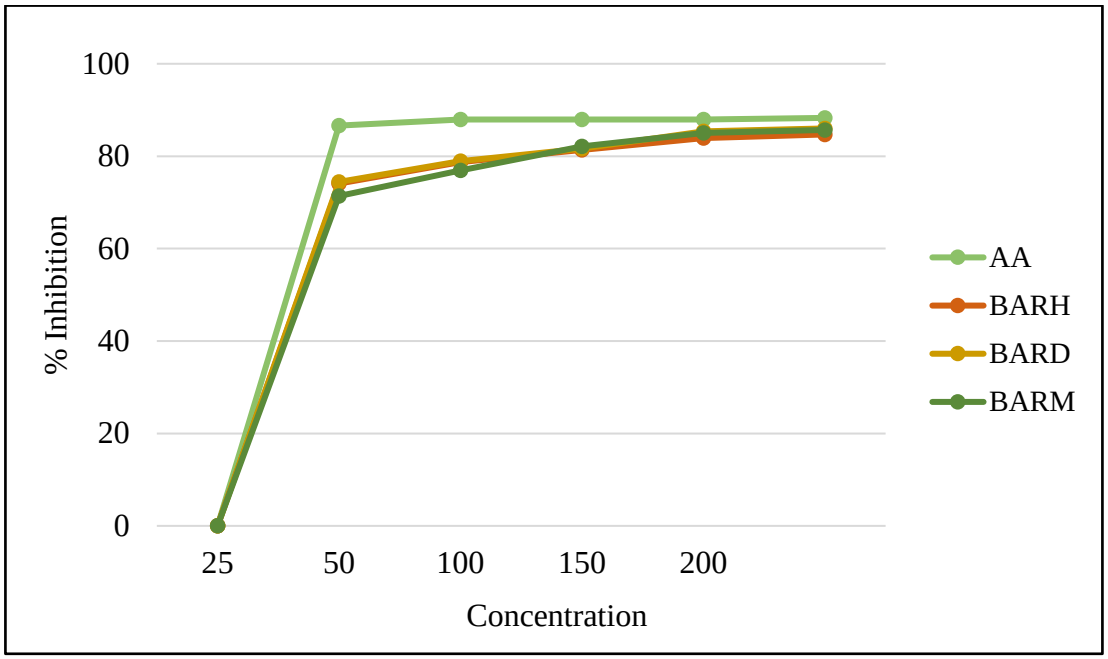
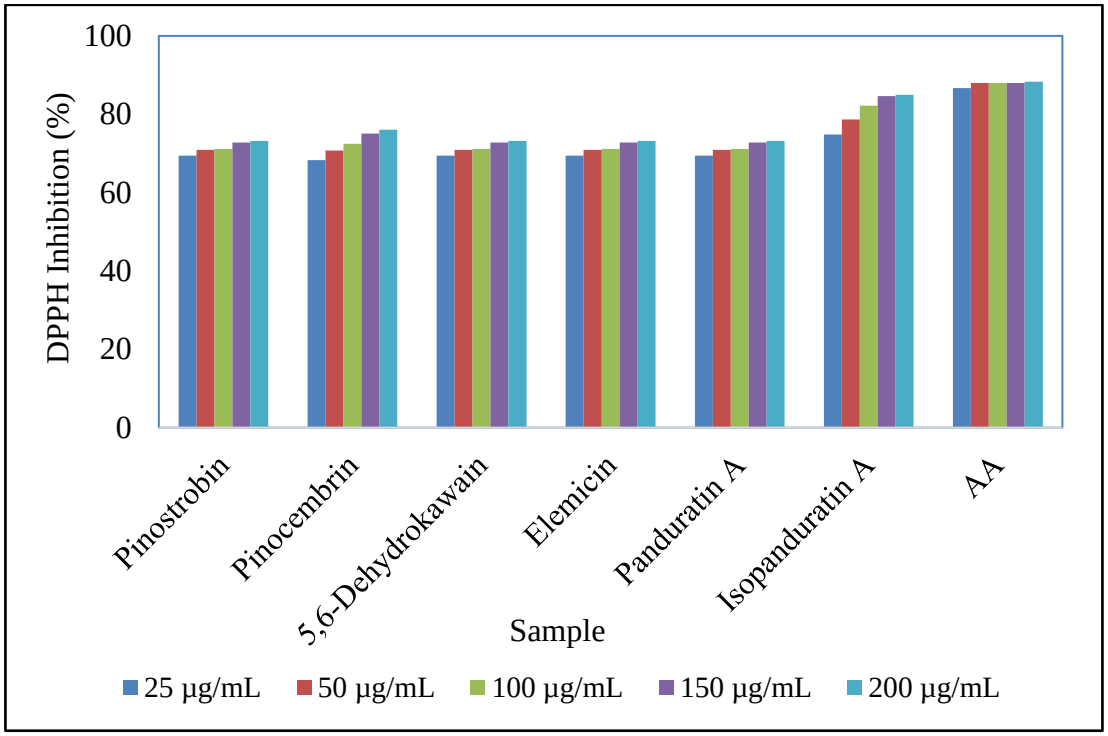


Figure 4.11

Percentage inhibition of B. albosanguinea phytochemicals at various concentrations



4.5 Anti-inflammatory Activity

Inflammation is a protective response caused by a variety of factors such as tissue damage, physical and chemical factors, immunological reactions, and microbial infections. Especially bacteria can release damage-associated molecular patterns (DAMPs) or molecular-associated microorganism patterns (MAMPs), which are detected by tissue resident macrophages. These immune cells differentiate and initiate an inflammatory response. Oxidative stress and excessive production of reactive oxygen species (ROS) are associated with inflammation, leading to the synthesis and release of pro-inflammatory cytokines. Besides, it is an underlying complex mechanism in many chronic disorders and diseases, including hepatic, diabetes, Alzheimer's, cancer, renal, cardiac, and Parkinson's (Dinarello, 2010).

The discovery of a new generation of therapeutic agents for the resolution of inflammation is highly desirable. Treating inflammation involves various mechanisms that can serve as therapeutic targets. Medicinal plants play a crucial role in the development of new and potent drugs due to their production of secondary metabolites with clinically beneficial effects. Medicinal plants are used instead of non-steroidal anti-inflammatory drugs (NSAIDs) as the use of non-steroidal anti-inflammatory drugs is associated with several side effects, among which are undesirable effects on the gastrointestinal tract and the renal system. The biggest hardship of recently available potent synthetic drugs is concerning their toxicity and the reappearance of symptoms after discontinuation. Therefore, the screening and development of drugs with anti-inflammatory activity are needed and there are many attempts to find anti-inflammatory drugs from medicinal plants (Virshette et al., 2019).

Lipoxygenase (LOX) can catalyze fatty acid to produce a number of active metabolic products that are involved in a lot of vital diseases. For instance, Hypertension, type 2 diabetes, type 1 diabetes, and renal diseases, neurological disorders such as Alzheimer's disease and Parkinson's disease. In particular, LOX is a kind of rate-limiting enzyme in the process of arachidonic acid (AA) metabolism into leukotriene which facilitates the occurrence of inflammation. There are huge interest in the development of inhibitors of lipoxygenases. In mammals, hydroperoxides play an important role in immune response and in the inflammatory process, and they have been recently implicated in the formation of atherosclerotic lesions. As a result, there are many pharmacological interest in the study of compounds that inhibit lipoxygenase activity (Hu et al., 2017). In this study, evaluation of LOX assay of the isolated phytochemicals of *B. albosanguinea* are tabulated in Table 4.11.

Table 4.11

LOX inhibitory activity of the isolated phytochemicals

Samples	Percentage inhibition at 1 mg/mL (I%)
Elemicin (60)	73.2
Panduratin A (72)	76.2
Isopanduratin A (76)	84.9
5,6-Dehydrokawain (196)	73.2
Pinocembrin (117)	76.0
Pinostrobin (118)	73.2
Standard - Quercetin	81.9

Several tested phytochemicals showed significant effects on LOX compared to the positive control, quercetin with percentage inhibition of 81.9%. Among them was isopanduratin A **(76)** which displayed the highest inhibition which gave 84.9%, followed by panduratin A **(72)** with 76.2%. Both compounds are chalcone derivatives, isolated from various *Boesenbergia* and *Kaempferia* species, which have shown anti-inflammatory activities. Previously, panduratin A **(72)** showed strongest anti-inflammatory activity against nitric oxide (NO) and prostaglandin E2 (PGE2) and a moderate effect on tumor necrosis factor-alpha (TNF-α) in RAW264.7 cells (Yun et al., 2003). Panduratin A **(72)** also inhibit aminopeptidase N (APN) activity with 61.4% at 30 µM. In the same study, isopanduratin A **(76)** inhibited (TNF-α) in L929 cells with IC_{50} value 6.1 µg/mL (Morikawa et al., 2008).

Pinostrobin **(115)** has also been extensively studied for its anti-inflammatory properties. Pinostrobin **(115)** was shown to be a potent 5-LOX inhibitor (IC_{50} value 0.499 µM) compared to nordihydroguaiaretic acid (IC_{50} value 5.020 µM) and betamethasone dipropionate (IC_{50} value 2.077 µM) as the first-line of atopic dermatitis treatment. Additionally, pinostrobin **(115)** inhibited COX-2 (IC_{50} value 285.67 µM), which was as effective as diclofenac sodium (IC_{50} value 290.35 µM) and betamethasone dipropionate (IC_{50} value 240.09 µM). This study revealed that bioactive pinostrobin **(115)** have promising properties for anti-inflammatory (Liana et al., 2024).

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Chemical analysis of *B. albosanguinea* rhizome oil revealed 34 components, which represent 96.7% of the total oil. Phenylpropanoid made up 54.3% of the essential oil's composition, making them as major category. The essential oil's composition features a number of distinctive constituents. Elemicin **(60)** constitutes the largest percentage at 44.0%, followed by α-gurjunene **(206)** at 9.3%. Additionally, β-caryophyllene **(3)** and safrole **(200)** are present at 4.5% and 4.1%, respectively. As far as we have discovered, this is the first report on the chemical constituents of the essential oil extracted from *B. albosanguinea* rhizomes.

Seven phytochemicals were successfully isolated from the rhizomes of *Boesenbergia albosanguinea*. These include two chalcones: panduratin A **(72)** and isopanduratin A **(76)**; one kavalactone: 5,6-dehydrokawain **(196)**; two flavones: pinocembrin **(114)** and pinostrobin **(115)**; one flavonol: kaempferol **(131)**; and one additional flavone: luteolin **(138)**. In addition, a major component from the rhizomes of *B. albosanguinea* was also successfully isolated and identified as a phenylpropanoid, elemicin **(60)**. The majority of these chemicals have been reported from various *Boesenbergia* species. The antioxidant and anti-inflammatory properties of the crude extracts and extracted phytochemicals were also investigated. In antioxidant activity, elemicin **(60)** showed the most active with IC_{50} value 9.3 µg/mL, while isopanduratin A **(76)** showed the highest activity in lipoxygenase assay which gave inhibition of 84.9%. Besides, all extracts have shown strong activity in DPPH free radical scavenging assay with IC_{50} values between 11.2 to 14.5 µg/mL.

5.2 Recommendations

Extracting and purifying phytochemicals needs careful selection methods to get high yields and keep the compounds intact. Methods like Soxhlet and ultrasonic extraction can improve efficiency by adjusting temperature, time, and solvent ratios. Isolation techniques like liquid-liquid partitioning, column chromatography, and TLC help separate and check compounds. High-purity compounds can be obtained using RP-HPLC, flash chromatography, or crystallization. Throughout the process, TLC or HPLC monitor purity and identity, ensuring the compounds are ready for biological testing.

Modern, greener extraction methods like microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), and pressurized liquid extraction (PLE) are better than traditional methods. They use less solvents, are faster, and are more eco-friendly. SFE, for example, uses non-toxic carbon dioxide, while MAE and PLE reduce solvent use and time. Future studies should also explore cytotoxic and anti-cancer activities of natural compounds, especially from plants like *Boesenbergia*. Many cancer drugs today have serious side effects, so finding safer, plant-based options is important. It's also crucial to study toxicity, dosage, and stability to develop effective treatments. Lastly, new tools like molecular modeling, virtual screening, and AI are speeding up drug discovery. These methods help find new drug candidates faster and more efficiently, making natural products an even stronger part of future medicine development.

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PUBLICATIONS

1. **Ngalang, M.D**., Salleh, W.M.N.H.W., Salihu, A.S., & Ghani, N.A. (**2024**). Chemical profile, anticholinesterase and molecular docking studies of *Boesenbergia albosanguinea* (Ridl.) Loes. essential oil. *Journal of Essential Oil-Bearing Plants*, 27(3), 693-700.

2. **Ngalang, M.D**., Salleh, W.M.N.H.W., Ghani, N.A., Rasol, N.E., Salihu, A.S., Shah, A.B., & Sungthong, B. (**2025**). Lipoxygenase inhibitory activity of isolated constituents from the rhizomes of *Boesenbergia albosanguinea* (Ridl.) Loes. *Bulletin of the Chemical Society of Ethiopia*, 39(6), 1167-1176.

3. **Ngalang, M.D**, Salleh, W.M.N.H.W., Salihu, A.S., Ghani, N.A., Eshboev, F. (**2025**). Secondary metabolites isolated from the rhizomes of *Boesenbergia albosanguinea* and its acetylcholinesterase inhibitory activity. *Chemistry of Natural Compounds*, 61(3), 542-544.

CONFERENCES

1. 39th International Conference on Natural Products (ICNP)
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Research Article

Chemical profile, anticholinesterase and molecular docking studies of *Boesenbergia albosanguinea* (Ridl.) Loes. essential oil

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Abstract
Boesenbergia albosanguinea is a member of the genus *Boesenbergia*, which traditionally serves a range of purposes, including food, medicinal applications, ornamental use, and ritualistic practices. It is found growing in wet, shady forest areas on sandstone or quartz-derived soils. This study aims to characterize the essential oils from the rhizomes of *B. albosanguinea*, assessing their activity against acetylcholinesterase using *in vitro* methods, along with molecular docking analysis. The essential oil was obtained through hydrodistillation and the volatile components were analysed using gas chromatography (GC-FID) and gas chromatography mass spectrometry (GC-MS) techniques. A total of 34 chemical components, which constitute 96.7% of the oil content, were successfully identified. The most abundant components were elemicin (44.0%), α-gurjunene (9.3%), β-caryophyllene (4.5%), and safrole (4.1%). Anticholinesterase activity was assessed using Ellman method. A moderate inhibitory effect was observed for acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes with IC_{50} values of 90.5 ± 0.2 and 110.8 ± 0.2 µg/mL, respectively. The molecular docking studies of elemicin against the cholinesterase target revealed a robust docking score (-7.0 kcal/mol), but it also showed important interactions with some key amino acid residues, providing crucial insights into its potential as a cholinesterase inhibitor.

Keywords
Boesenbergia albosanguinea, Cholinesterase, Docking, Elemicin, Essential oil, Zingiberaceae.

INTRODUCTION

Zingiberaceae, commonly known as the ginger family, is a family of flowering plants comprising more than 1300 species divided into about 52 genera of aromatic perennial herbs, distributed throughout tropical Africa, Asia, and America[1]. Many species belonging to the ginger family have been widely used as spices or flavoring agents, due to its aromatic odor, pungent, and spicy taste[1]. *Boesenbergia* is a genus of the family Zingiberaceae, which comprises about 80 species ranging from India to China and Southeast Asia[2]. Traditionally, *Boesenbergia* serves a range of purposes, including food, medicinal applications, ornamental use, and ritualistic practices[2]. The aromatic rhizomes of *Boesenbergia* species are frequently incorporated into regional cuisines, adding unique flavours to dishes[2]. A great variety of plants belonging to the genus *Boesenbergia* have been phytochemically investigated and several compounds have been isolated and identified. The phytochemical analysis discovered the isolation of flavonoids, diterpenoids, phenylpropanoids, terpenes, and phenolics, with potential anticancer, anti-inflammatory, antimicrobial, antioxidant, antidiabetic, antiallergic, antiobesity, hepato-protective, neuroprotective, gastroprotective,

skin protective, vasorelaxant, and aphrodisiac activities[3].

Nowadays, researchers are paying attention to herbal medicines because of their fewer side effects; and there are some studies about medicinal plants inhibitory effects on acetylcholinesterase. While much of the research has focused on the culinary and medicinal uses of Zingiberaceae, recent studies have indeed shown promising results indicating AChE inhibition activity in certain species within this family. *Curcuma longa*, commonly known as turmeric, which has been extensively studied for its neuroprotective effects, including AChE inhibition activity, is an example. The bioactive compound responsible for this activity is curcumin, a polyphenolic compound abundant in turmeric[4]. Additionally, other members of the Zingiberaceae family, such as *Alpinia officinarum* (galangal) have also shown potential AChE inhibition activity in preclinical studies[5]. These findings suggest that Zingiberaceous plants may serve as a valuable source of AChE inhibitors with potential therapeutic applications in neurodegenerative disorders such as Alzheimer's disease. Acetylcholine is a neurotransmitter at synapses and within the central nervous system. Alzheimer's disease (AD) is one of the most common forms of dementia. The reduction in acetylcholine synthesis is the main cause of AD. Therefore, increasing the cholinergic levels in the brain by inhibiting the biological activity of acetylcholinesterase (AChE) is one of the potential therapeutic strategies for preventing AD[6].

Essential oils are among the important secondary metabolites of medicinal and aromatic plants. They have many usages in various industries and fields; from pharmaceuticals and cosmetic to food and aromatherapy industries[7-9]. Plant-derived essential oils exhibit pharmacological properties traceable to the presence of various structurally diverse bioactive chemical components and are increasingly harnessed for their anticholinesterase properties. For instance, Aazza *et al*.[10] reported the acetylcholinesterase inhibiting potential of commercial essential oils of *Citrus aurantium*, *Cupressus sempervirens*, and *Eucalyptus globulus*. Considering the importance of a sufficient knowledge base for accurate recommendations on the use of plant essential oils, although the anticholinesterase activities of many plant compounds have been demonstrated, *in vitro* laboratory trials using essential oil of tropical plants are very limited.

Boesenbergia albosanguinea (Ridl.) Loes. is mainly distributed in Malaysia (Langkawi, Pulau Langgun) and Thailand (Satun Province). It can be found on limestone outcrops in shaded habitats in close proximity to the sea. Recently, it has been concluded that *B. albosanguinea* is distinct from *B. prainiana*[11,12]. The literature search did not reveal any report on the essential oil composition of this species. Therefore, this study reports the chemical composition and evaluation of anticholinesterase activity of the essential oil from the rhizomes of *B. albosanguinea*.

Materials and Methods

Plant material

Boesenbergia albosanguinea rhizomes were collected from Langgun Island, Langkawi (August 2023) and were identified by Dr Shamsul Khamis at Universiti Kebangsaan Malaysia (UKM). The voucher specimen (PL-13/23) was deposited at the Herbarium of UKM.

Isolation of essential oil

The fresh rhizomes of *B. albosanguinea* (400 g) were subjected to hydrodistillation for 4 hours in Clevenger-type apparatus. The obtained essential oil was dried over anhydrous magnesium sulphate and stored at 4-6°C[13].

Analysis of essential oil

As reported in our previous work[13], Gas chromatography flame ionisation detection (GC-FID) analysis was performed on a Hewlett Packard 6890 series II A gas chromatograph equipped with HP-5 column (30 m × 0.25 mm × 0.25 μm film thickness). Helium was used as a carrier gas at a flow rate of 0.7 mL/min. Injector and detector temperatures were set at 250 and 280°C, respectively. The oven temperature was kept at 50°C, then gradually increased to 280°C at 5°C/min rate, and finally held isothermally for

15 min. Diluted samples (1.0 µL, 1/100 v/v in diethyl ether) were injected manually (split ratio 50:1). Injection was repeated three times and peak area percentages were reported as means ±SD of triplicate. Peak area percentages were calculated from flame ionisation detection (FID) using GC HP Chemstation software (Agilent Technologies).

Gas chromatography-mass spectrometry (GC-MS) chromatograms were recorded using a gas chromatograph Hewlett Packard Model 5890A and a Hewlett Packard Model 5989A mass spectrometer. The GC was equipped with HP-5 column. Helium was used as the carrier gas at a flow rate of 1 mL/min. The injector temperature was 250°C. The oven temperature was programmed from 50°C (5 min hold) to 250°C at 10°C/min and finally held isothermally for 15 min. For GC-MS detection, an electron ionization system with an ionization energy of 70 eV was used. A scan rate of 0.5 s (cycle time: 0.2 s) was applied, covering a mass range from 50-400 amu.

Identification of oil components

Similar to our previous work[13], the essential oil components were identified by co-injection with standards (major components: elemicin, α-gurjunene, β-caryophyllene, and safrole) and their comparison with reported retention indices and mass spectra found in Adams, NIST 08 and FFNSC2 libraries[14]. Semi-quantification of essential oil components was made by peak area normalization considering the same response factor for all volatile components.

Anticholinesterase activity

Acetylcholinesterase (AChE) and butyryl-cholinesterase (BChE) inhibitory activities of the essential oil were measured according to the reported spectrophotometric method with minor modifications[15]. Electric eel AChE/horse serum BChE (0.22 U/mL) was used, while acetylthiocholine iodide and butyrylthiocholine chloride were employed as substrates of the reaction. DTNB acid was used for measurement of anticholinesterase activity. Essential oil samples were prepared at various concentrations ranging from 125 to 1000 µg/mL, while galantamine served as a positive control. Each experiment was conducted with a minimum of three replicates, and the data presented represent the mean ± standard deviation of these replicates. In the procedure, 140 µL of sodium phosphate buffer (pH 8.0), 20 µL of DTNB, 20 µL of essential oil, and 20 µL of AChE/BChE solution (0.22 U/mL) were added to a 96-well microplate and incubated for 15 min at 25°C. The reaction was initiated by adding 10 µL of acetylthiocholine iodide/ butyrylthiocholine chloride, and the hydrolysis acetylthiocholine iodide/ butyrylthiocholine chloride was monitored by the formation of yellow 5-thio-2-nitrobenzoate anion because of the reaction of DTNB with thiocholines, catalyzed by enzymes at 412 nm utilizing a 96-well microplate reader (Epoch Micro-Volume Spectrophotometer, USA). Percentage of inhibition of AChE/BChE (I%) was determined by comparison of sample reaction rates relative to a blank sample (EtOH in phosphate buffer, pH 8) using the formula:

$$I\% = [E - S / E] \times 100;$$

where E is the activity of the enzyme without test sample and S is the activity of the enzyme with the test sample. IC_{50} values were determined by plotting concentration versus percentage inhibition and fitting the data to a suitable dose-response curve using SPSS, with statistical significance assessed using one-way analysis of variance (ANOVA) and a significance level ($\rho < 0.05$).

Molecular docking study

The Protein Data Bank (PDB) was the source for the crystal structure of acetylcholinesterase with code ID 4EY7 (http://www.rcsb.org). Employing the conjugate gradient algorithm and the AMBER force field with UCSF Chimera 1.10.1, we minimized the retrieved protein structure. Chemical structure of elemicin was acquired from PubChem in sdf format, and energy minimization for the ligand was performed using the openbabel tool embedded in PyRx. The parameters included steepest descent steps of 100 with a step size of 0.02 Å and an update interval fixed at 1[16]. Utilizing the PyRx virtual

screening tool with the AutoDock VINA Wizard approach, a molecular docking experiment was conducted for the major component (elemicin) of acetylcholinesterase receptor. The grid box center values were set to -14.0821627001, -43.4770035843, and 27.1045441861 for X, Y, and Z, respectively, mirroring the coordinates of the native ligand. The grid box size was adjusted adequately to encompass the residue in the binding pocket, facilitating ligand movement in the search space. The exhaustiveness value was set to 8 to optimize the binding conformational analysis. The evaluation of the docked compound relied on the lowest binding energy (Kcal/mol). Finally, Discovery Studio 2021 was employed for 2D depictions of the docked complexes[17].

Results and Discussion

The essential oil components identified with their percentages are listed in Table 1 in order of their elution from the HP-5 column. The oil yield (w/w) was 0.5% based on the fresh weight basis. The GC-FID and GC-MS analysis of the essential oil revealed the presence of 34 chemical components constituting around 96.7%. Phenylpropanoids were the most dominant components found in the essential oil, which constituted seven components, accounting for 54.3% of the total composition and was characterized by its richness in elemicin (44.0%) and safrole (4.1%). In addition, sesquiterpene hydrocarbons were present in extraordinary amounts which comprised twenty components, accounting for 39.0% of the total composition, which was constituted predominantly by α-gurjunene (9.3%), β-caryophyllene (4.5%), germacrene D (3.6%), bicyclogermacrene (3.4%), and α-elemene (3.3%). The composition of the essential oil from *B. albosanguinea* in this study exhibits both similarities and differences compared to previous reports in the literature. Consistent with prior studies, compounds such as α-pinene, β-pinene, and limonene emerge as major constituents, reflecting the typical monoterpene hydrocarbons found in *Boesenbergia* species[18]. However, notable differences may exist in the relative abundance or presence of minor components. A review of the existing literature on essential oils of the genus *Boesenbergia* revealed that elemicin was found predominantly in *B. xiphostachya*[19] and from the hexane fraction of *B. pulcherrima*[20] roots. These subtle differences in the chemical components may be attributed to the differences in environmental and genetic factors, chemotype, and nutritional status of the plants, which may influence their oil composition[21].

The essential oil was also subjected to a preliminary test to determine anticholinesterase activity. Anticholinesterase activity was tested against AChE and BChE enzymes where it was compared with that of galantamine, as a standard drug in the treatment of Alzheimer's disease. The essential oil indicated moderate AChE (IC_{50} value 90.5 ± 0.2 µg/mL) and BChE (IC_{50} value 110.8 ± 0.2 µg/mL) inhibitory activity, compared to galantamine (AChE: IC_{50} value 2.5 ± 0.2 µg/mL, BChE: IC_{50} value 17.5 ± 0.2 µg/mL). The level of anticholinesterase activity of the essential oil was probably related to their richness in phenylpropanoid component, which is elemicin. The observed moderate AChE inhibitory activity of the essential oil from *B. albosanguinea* in our study warrants further discussion in relation to previous findings. While our study represents one of the first investigations into the anticholinesterase activity of *B. albosanguinea* essential oil, comparisons with similar studies on other *Boesenbergia* species can provide valuable insights. Previous research on Zingiberaceae essential oils has demonstrated varying degrees of AChE inhibitory activity across different species and geographical regions. Studies on *Hedychium gardnerianum* leaf essential oils have reported IC_{50} value 1 mg/mL for AChE inhibition[22]. These variations in activity levels may be attributed to differences in the chemical composition of the essential oils, particularly in terms of the relative abundance of key bioactive compounds such as phenylpropanoids. Elemicin, an alkenylbenzene constituent of natural oils of several plant species, is widely distributed in food, dietary supplements, and medicinal plants. Previous studies have shown that elemicin could react with DNA and induce genotoxicity in adult rat hepatocytes and mice. Moreover, exposure

Table 1. Chemical components identified in *B. albosanguinea* essential oil

No	Components	KI[a]	KI[b]	Percentage (%)[c]	Identifications[d]
1	Linalool	1095	1095	0.2	RI, MS
2	*trans*-Verbenol	1140	1140	0.4	RI, MS
3	Camphor	1142	1141	0.2	RI, MS
4	**Safrole**	1285	1285	**4.1**	RI, MS, Std
5	α-Cubebene	1342	1345	0.3	RI, MS
6	Cyclosativene	1370	1369	0.2	RI, MS
7	α-Copaene	1375	1374	1.8	RI, MS
8	Isoledene	1376	1376	0.3	RI, MS
9	β-Cubebene	1385	1387	0.2	RI, MS
10	β-Elemene	1390	1389	2.4	RI, MS
11	Methyl eugenol	1402	1403	1.2	RI, MS
12	**α-Gurjunene**	1410	1409	**9.3**	RI, MS, Std
13	Aristolene	1415	1416	0.4	RI, MS
14	**β-Caryophyllene**	1418	1417	**4.5**	RI, MS, Std
15	β-Copaene	1430	1430	1.5	RI, MS
16	γ-Elemene	1436	1434	2.6	RI, MS
17	Aromandendrene	1440	1439	1.2	RI, MS
18	α-Humulene	1450	1452	1.0	RI, MS
19	*epi*-Cubebol	1452	1453	1.6	RI, MS
20	Alloaromadendrene	1460	1458	0.4	RI, MS
21	α-Elemene	1476	1477	3.3	RI, MS
22	γ-Muurolene	1478	1478	0.7	RI, MS
23	Germacrene D	1484	1484	3.6	RI, MS
24	Bicyclogermacrane	1502	1500	3.4	RI, MS
25	α-Bourbonene	1505	1509	0.2	RI, MS
26	Myristicin	1515	1517	2.9	RI, MS
27	δ-Cadinene	1520	1522	1.7	RI, MS
28	**Elemicin**	1552	1555	**44.0**	RI, MS, Std
29	γ-Asarone	1570	1572	0.7	RI, MS
30	Spathulenol	1580	1578	0.3	RI, MS
31	Guaiol	1600	1602	0.3	RI, MS
32	(*Z*)-Asarone	1615	1616	1.2	RI, MS
33	τ-Muurolol	1645	1644	0.4	RI, MS
34	(*E*)-Asarone	1675	1675	0.2	RI, MS
	Group components				
	Phenylpropanoids			54.3	
	Oxygenated monoterpenes			0.8	
	Sesquiterpene hydrocarbons			39.0	
	Oxygenated sesquiterpenes			2.6	
	Total identified			96.7	

[a]Linear retention index experimentally determined using homologous series of C6-C30 alkanes; [b]Linear retention index taken from Adams, Wiley or NIST08 and literature; [c]Quantification was done by the external standard method using calibration curves generated by running GC analysis of representative authentic compounds; [d]RI, based on comparison of calculated RI with those reported in Adams; MS, based on comparison with Wiley; Std, based on comparison with standard compounds

to elemicin could result in hepatomegaly, accompanied by lower ratios of unsaturated- and saturated lysophosphatidylcholine in the plasma of mice. In addition, elemicin was reported to possess strong antibacterial and antifungal effects[23,24]. The presence of a conjugated double bond and a methoxyl group seems to be important for the acetylcholinestrase activity since elemicin containing these structural features has shown activity. Besides, the AChE/BChE activity of this compound can be explained by the hydrophobic interactions between hydrophobic active site of AChE/BChE and hydrocarbon skeleton of the phenylpropanoids[25]. In previous reports, AChE/BChE inhibition could be explained by the high content of monoterpenes. It has also been mentioned that 1,8-cineole, camphor, α-pinene, β-pinene, borneol, linalool have anticholinesterase activity[26]. This study showed that the essential oils had low amount of monoterpenoids, which contributed to moderate AChE/BChE inhibition.

The molecular docking analysis revealed important insights into *B. albosanguinea* essential oil major component interaction with the acetylcholinesterase target (PDB ID: 4EY7), elucidating its binding affinity and potential inhibitory effects against the cholinesterase enzyme (Fig. 1). Elemicin exhibited a notable docking score of -7.0 kcal/mol, revealing robust interactions within the receptor's binding pocket, engaging in three hydrogen bonding with Tyr124 and Asp74 by two of the methoxy oxygens and the hydrogen of the methoxy respectively, moreover, two π-π T-shaped interactions between elemicin aromatic ring and the Tyr337 and Tyr86 residues. One π-σ interaction was also observed with TYR341. Additionally, seven π-alkyl interactions with Tyr337, Tyr86, Phe338, Phe297, and His447 were observed, underscoring its potent inhibitory potential (Fig. 1). These findings delineate the binding mode and interaction of elemicin, providing valuable insights into its potential as a cholinesterase inhibitor for therapeutic applications.

Furthermore, the ADME (absorption, Distribution, Metabolism, and Excreation) properties of elemicin was assessed using swissadme webtool, this is essential for evaluating its potential in medicinal applications (Fig. 2). Notably, its lipophilicity is reflected in various logP values such as iLOGP (2.89), XLOGP3 (2.53), and WLOGP (2.44), contributing to its potential permeability. The solubility prediction Log S (Esol) (-2.69) indicated a soluble nature which was further supported by Ali solubility values (-2.76). The compound also displays high gastrointestinal absorption and permeability through the blood-brain barrier. The absence of violations in Lipinski's rule of five, Ghose's rule, Veber's rule and Muegge's rule demonstrate its favourable drug-like properties. Moreover, it

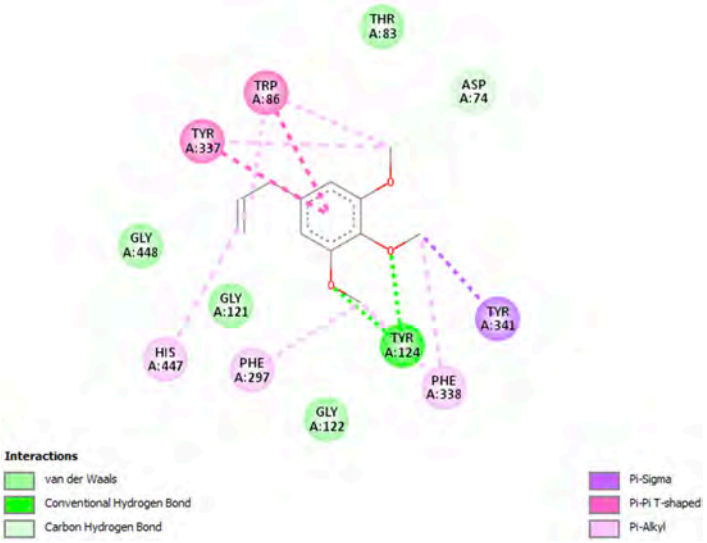

Figure 1. 2D interactions view of elemicin inside Acetylcholinesterase receptor

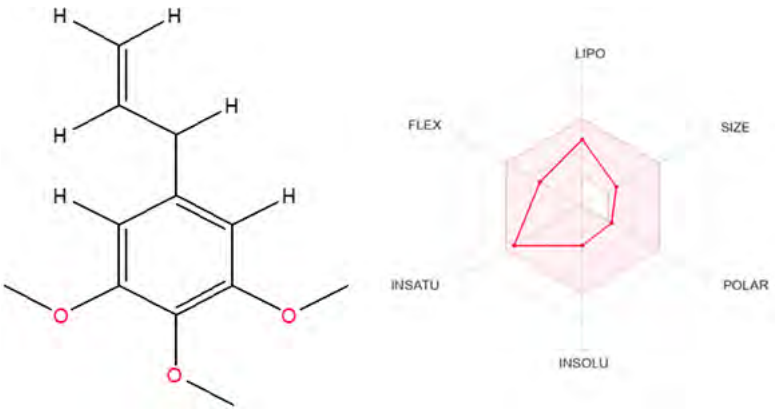

Figure 2. Oral bioavailability radar for elemicin showing how suitably fall within the physicochemical space

shows a bioavailability score of 0.55 emphasizing its potential for effective drug delivery.

Conclusion

This study represents a pioneering investigation into both the composition and anticholinesterase activities of the essential oil derived from *B. albosanguinea*. The findings from this study shed light on the chemical constituents of the essential oil and its potential pharmacological activities, particularly its ability to inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes. These results highlight the therapeutic potential of *B. albosanguinea* essential oil in the management of neurodegenerative disorders such as Alzheimer's disease (AD), where AChE inhibition is a key therapeutic target. Our findings suggest that essential oils, including those from *B. albosanguinea,* could serve as promising natural agents for food preservation, offering both antimicrobial properties and potential health benefits. This study also contributes to the growing body of research supporting the use of natural products as alternatives to synthetic additives in both food and pharmaceutical industries. Future research may focus on formulating *B. albosanguinea* essential oil for functional foods or therapies targeting oxidative stress-related and neurodegenerative conditions.

Acknowledgments

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Competing interests

The authors declare that no competing interest exists.

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LIPOXYGENASE INHIBITORY ACTIVITY OF PHYTOCONSTITUENTS ISOLATED FROM THE RHIZOMES OF *Boesenbergia albosanguinea* (Ridl.) Loes.

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ABSTRACT. This study aimed to investigate the compounds isolated from the rhizome extract of *Boesenbergia albosanguinea*. The extraction was performed using Soxhlet extraction of the dried powdered rhizome, following a polarity gradient of *n*-hexane, dichloromethane, and methanol. The phytochemicals were purified using chromatography techniques, and their structures were confirmed through spectroscopic analyses (IR, NMR, and MS) and comparison with existing literature. Additionally, the isolated compounds were evaluated for their lipoxygenase inhibitory activity. The isolation process successfully yielded eight phytochemicals belonging to various classes, including phenylpropanoid, chalcones, kavalactone, flavanones, flavonol, and flavone. These compounds were identified as elemicin (**1**), panduratin A (**2**), isopanduratin A (**3**), 5,6-dehydrokawain (**4**), pinostrobin (**5**), pinocembrin (**6**), kaempferol (**7**), and luteolin (**8**). Among these, luteolin (**8**) and kaempferol (**7**) demonstrated the most potent lipoxygenase inhibitory activity, with percentage inhibitions of 88.5% and 86.2%, respectively. These findings highlight the potential of *B. albosanguinea* as a natural source of lipoxygenase inhibitors, particularly in comparison to previously studied plant-based inhibitors. The strong inhibitory effects of luteolin (**8**) and kaempferol (**7**) underscore their relevance in the development of anti-inflammatory and nutraceutical formulations. This study contributes to the growing body of research on *Boesenbergia* species, offering a foundation for further pharmacological and industrial applications.

KEY WORDS: Zingiberaceae, *Boesenbergia albosanguinea*, Constituent, Flavonoid, Lipoxygenase

INTRODUCTION

Lipoxygenases (LOXs) are a group of monomeric enzymes responsible for catalyzing the oxidation of polyunsaturated fatty acids (PUFAs), such as linoleic, linolenic, and arachidonic acids, leading to the formation of hydroperoxides. These enzymes are broadly distributed across animals, plants, fungi, and cyanobacteria [1]. Moreover, 5-LOX is ubiquitous in mammalians and oxygenates carbon-5 of arachidonic acid, while 9-LOX and 13-LOX are plant LOXs that catalyze the oxygenation of linoleic and linolenic acids [2]. Lipoxygenases, inherent in the human body, play a vital role in stimulating inflammatory reactions. Overexposure to reactive oxygen species can result in inflammation, which then stimulates the synthesis of cytokines and the activation of

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LOXs. Numerous illnesses, including cancer, stroke, cardiovascular, and neurological conditions, are associated with inflammation [3, 4]. Leukotriene and prostaglandin production is aided by LOXs. They are linked to the onset of the disease, and blocking them is considered an essential first step in the prevention of the disease [5]. Enzyme activity inhibitors may be better candidates for chemopreventive intervention because these enzymes' inhibition directly reduces fatty acid metabolite production, with simultaneous reduction of the related inflammatory, proliferative, and metastatic processes that lead to the development of cancer [6, 7]. Thus, the discovery of new lipoxygenase inhibitors with potent inhibitory activity is needed. In fact, in a few studies, LOX inhibitors have prevented carcinogen-induced lung adenomas and rat mammary gland cancer.

Zingiberaceae, commonly known as a ginger family, belongs to the order Zingiberales. It is a family of flowering plants made up of about 50 genera with a total of about 1600 known species, distributed throughout tropical Africa, Asia, and the Americas. Some parts of the plants from the Zingiberaceae family are usually aromatic, well-known for their ornamental and unique flavours. It is also a common ingredient in food, spices, medicines, dyes, and perfumes [8, 9]. For instance, rhizome extracts of *Zingiber zerumbet* have been used in Malay traditional medicine to treat inflammation and pain-mediated diseases, worm infestation, and diarrhea [10]. Besides, *Alpinia galanga* is useful against lumbago, rheumatic pain, sore throat, diabetes, tubercular glands, bronchitis, and catarrhal affection [11]. Furthermore, the leaves of *Curcuma aromatica* and *C. longa* are utilized for treating bruises, sprains, snake bites, skin ailments, and a range of inflammatory conditions [12].

Boesenbergia is a genus of the Zingiberaceae family, characterised by its small-sized species. The genus is classified within the subtribe *Zingiberae* of the family Zingiberoideae. The *Boesenbergia* genus currently includes about 99 species, with Thailand and Borneo being the main centres of diversity. Many researchers have proven that the rhizome part of *Boesenbergia* species displayed health-benefit properties. Besides, *Boesenbergia* serves a range of purposes, including food, medicinal applications, and ornamental use [13]. The extracts of this genus are rich in flavonoids, diterpenoids, and polyphenols, all of which possess a wide range of pharmacological activities such as anticancer, anti-inflammatory, antimicrobial, antiviral, antidiabetic, antiallergic, antiobesity, hepatoprotective, vasorelaxant, and aphrodisiac activities [14].

Boesenbergia albosanguinea is mainly distributed in Malaysia (Langkawi, Pulau Langgun) and Thailand (Satun Province). It can be found on limestone outcrops in shaded habitats in close proximity to the sea. In the Langkawi populations, the plants tend to be generally less robust, shorter in height with narrower, shorter leaves. The leaf sheaths are red but the color does not extend onto the rachis. The inflorescences are more cylindrical, less flattened with slightly longer, narrower bracts which sometimes deflex slightly away from the rachis on some plants. Flower shape and color are nearly identical to the Thai populations, albeit slightly smaller [15]. Recently, investigation of the essential oil revealed the presence of 34 components, accounting for 96.7% of the total oil. The most abundant components were elemicin (44.0%), α-gurjunene (9.3%), β-caryophyllene (4.5%), and safrole (4.1%). Besides, the rhizome oil showed a moderate inhibitory effect was observed for acetylcholinesterase and butyrylcholinesterase enzymes with IC_{50} values of 90.5 and 110.8 μg/mL, respectively [16].

In our attempt to isolate natural products for drug discovery and development using a rational approach as a part of a laboratory project to re-investigating medicinal plants, we initiated a study to isolate constituents from the rhizome of *Boesenbergia albosanguinea*. To the best of our knowledge, no report exists on their chemical constituents and its lipoxygenase inhibitory activity.

RESULTS AND DISCUSSION

Eight compounds were successfully identified from the rhizome of *B. albosanguinea*, which were characterized as elemicin (**1**), panduratin A (**2**), isopanduratin A (**3**), 5,6-dehydrokawain (**4**),

pinostrobin (**5**), pinocembrin (**6**), kaempferol (**7**), and luteolin (**8**). They were all identified by analyzing their spectroscopic data and comparing them with the reported literature. Their chemical structures are shown in Figure 1.

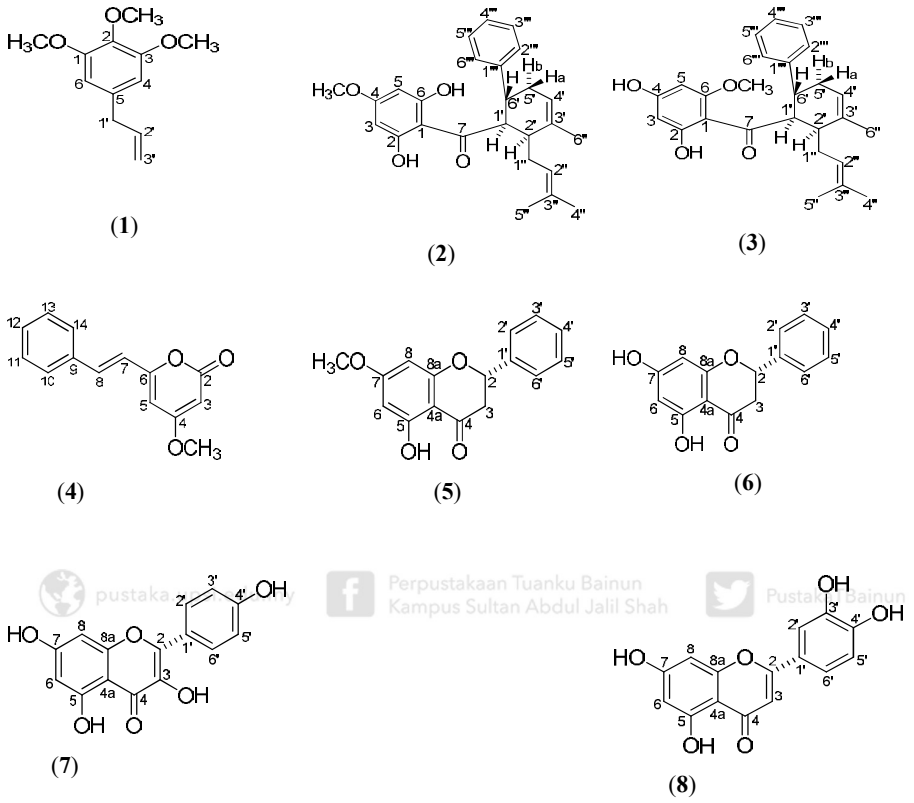

Figure 1. Chemical structures of isolated constituents from *B. albosanguinea.*

Compound (**1**) was identified as elemicin, it exhibited distinctive signals in its NMR spectrum, confirming its identity. The ^{1}H NMR spectrum reveals a three singlet signal, δ 3.87 and 3.85 corresponding to the methoxyl group at C-1, C-2, and C-3. Another singlet signal resonance at δ 6.43 indicates the presence of aromatic protons of H-4 and H-6. A doublet signal was appeared at δ 3.36 with a coupling constant of 6.8 Hz, characteristic of the methylene proton of H-1′. Besides, a doublet of doublet signal was displayed at δ 5.11 which attributed to protons H-3′a and H-3′b. A multiplet signal at δ 5.98 integrated for was assigned to the methine proton, H-2′. The ^{13}C NMR spectrum showed the presence of twelve carbons which attributed to four quaternary, three methine, two methylene, and three methoxyl carbons. The DEPT spectra revealed the existence of quaternary carbons resonated at δ 153.2 (C-1/C-3) and 136.4 (C-2). The methine carbons were observed at δ 105.5 (C-4/C-6) and 137.2 (C-2′). The methylene carbons resonate at δ 40.5 (C-1′) and 115.9 (C-3′). The methoxyl carbons were presented at δ 56.0 (1/3-OCH_3) and 60.8 (2-OCH_3).

Compound (**2**) was identified as panduratin A. The ^{1}H NMR spectrum revealed singlet signals assignable to three methyls at δ 1.51 (H-4″ and H-5″) and 1.77 (H-6″). Two set doublets of doublet

of doublet were appeared at δ 2.05/2.38 (H-5′a/H-5′b) and δ 2.16/2.27 (H-1″a/H-1‴b) which attributed to methylene protons. Besides, three methine protons were displayed at at δ 2.62 (ddd), 3.42 (ddd), and 4.67 (dd), which were corresponded to H-2′, H-6′, and H-1′, respectively. Two tri-substituted olefins were resonated at δ 4.85 (H-2″) as triplet (J = 6.9 Hz) and 5.42 (H-4′) as doublet of doublet (J = 3.0 and 5.0 Hz). In addition, seven aromatic protons were discovered at δ 5.87 (H-3/H-5), 7.10 (H-4‴), 7.21 (H-2‴/H-6‴/H-3‴/H-5‴), together with a methoxyl group at δ 3.74 (4-OCH_3). The ^{13}C NMR spectrum exhibited 26 signals of carbon present as one methoxyl carbon at δ 55.0 (4-OCH_3), one carbonyl at δ 207.0 (C-7), two methylene carbons 29.6 (C-1″), 35.8 (C-5′), three methyl carbons at δ 17.3 (C-5″), 22.9 (C-6″), 25.5 (C-4″), seven quaternary carbons δ 41.8 (C-2′), 105.6 (C-1), 137.3 (C-3′), 145.7 (C-1‴), 163.0 (C-2/C-6), 167.6 (C-4), and twelve methine carbons at δ 37.1 (C-6′), 41.8 (C-2′), 54.0 (C-1′), 96.6 (C-3/C-5), 122.6 (C-4′), 124.1 (C-2″), 125.6 (C-4‴), 127.2 (C-2‴/C-6‴), and 127.9 (C-3‴/C-5‴). The relative stereochemistry of C-l′, C-2′, and C-6′ was established by comparing the coupling constants with those of saggenon C and D [18]. These data lead to the conclusion that H-1′ and H-2′ are *cis*-oriented (J = 4.7 Hz) and H-1′/H-6′ have a *trans* relationship (J = 11.4 Hz).

Compound (**3**) was identified as isopanduratin A. The ^{1}H NMR spectrum was closely resembled to the ^{1}H NMR spectrum of panduratin A (**1**) except the position of the methoxyl group. Compound (**2**) features a methoxy group at C-6 position, while compound (**1**) features a methoxy group at C-4 position. The HMBC spectrum confirmed this position by showing the correlation of 6-OCH_3 (δ 55.7) with C-6 (δ 162.4). The ^{13}C NMR spectrum and DEPT spectra showed the presence of 26 signals attributed to 26 carbons, which were found similar to those of compound (**1**).

Compound (**4**) was identified as 5,6-dehydrokawain, displaying characteristic signals in its ^{1}H NMR spectrum, revealing the presence of a methoxyl group at 4-OCH_3 were detected at δ 3.85. Two doublets observed at δ 6.61 (J = 16.0 Hz) and 7.54 (J = 16.0 Hz) were assigned to olefinic protons H-7 and H-8, respectively. The large coupling constant, J = 16.0 Hz suggests that these protons are in a *trans*-orientation. Another two sets of doublets appeared at δ 5.52 and 5.97 (J = 2.2 Hz) were attributed to olefinic protons H-3 and H-5, of pyran-2-one respectively. The aromatic protons (H-10 to H-14) were observed as multiplet signals at δ 7.36-7.42. The ^{13}C NMR and DEPT spectra revealed the presence of fourteen carbons; one methoxy carbon at δ 55.9 (4-OCH_3), nine methine carbons at δ 88.8 (C-3), 101.3 (C-5), 118.6 (C-7), 127.4 (C-10/C-14), 128.9 (C-11/C-13), 129.4 (C-12), and 135.2 (C-8), three quaternary carbons at δ 135.8 (C-9), 163.9 (C-6), and 158.6 (C-4), and a carbonyl carbon at δ 171.0 (C-2).

Compound (**5**) was identified as pinostrobin, which exhibited characteristic signals in its ^{1}H NMR spectrum, confirming the presence of a hydroxyl group which was represented by a singlet signal at δ 12.05. Another singlet peak was observed at δ 3.84, assigned to a methoxy group (7-OCH_3). The *meta*-coupled signals appeared at δ 6.19 and 6.11 (J = 2.2 Hz), which were attributed to aromatic protons H-6 and H-8, respectively. In addition, three set doublets of doublet signal, each integrated for one proton were observed at δ 2.85 (J = 3.0 Hz and 17.1 Hz), 3.12 (J = 13.0 Hz and 17.1 Hz) and 5.45 (J = 3.0 Hz and 13.1 Hz,) were assigned for H-3a, H-3b and H-2, respectively. In addition, a multiplet signal resonated at δ 7.42-7.47 integrating for five protons of aromatic protons, H-2′–H-6′ of ring B. Besides, based on the chemical shift pattern comparison of H-2, H-3a, and H-3b, the configuration of H-3 was deduced as R configuration [19]. The ^{13}C NMR and DEPT spectra indicated the presence of sixteen carbons including one methyl, one methylene, one carbonyl carbon, eight methine, and five quaternary carbon atoms. The carbon signal of methoxy group (7-OCH_3) was clearly assigned at δ 55.7, while the carbonyl carbon (C-4) was also observed at δ 195.8. The single signal position of methylene carbon (C-3a/C-3b) was observed at δ 43.4. Eight methine carbons were detected at δ 79.3 (C-2), 94.3 (C-8), 95.2 (C-6), 126.2 (C-2′/C-6′) and 128.9 (C-3′/C-4′/C-5′) as well as five quaternary carbons at δ 103.2 (C-4a), 138.4 (C-1′), 162.8 (C-8a), 164.2 (C-5) and 168.0 (C-7).

Compound (**6**) was identified as pinocembrin. The ^{1}H NMR spectrum was almost identical to compound (**5**). The similarities of those spectra were observed as the presence of meta-coupled signals, the multiplet peak for aromatic protons, and the singlet peak at the downfield region (5-OH). The only difference between both spectra was the absence of a methoxy signal at C-7 with additional proton OH in the ^{1}H NMR spectrum of compound (**6**). Besides, based on the chemical shift pattern comparison of H-2, H-3a, and H-3b, the stereochemistry of H-3 was deduced as R configuration [20]. The ^{13}C NMR and DEPT spectra exhibited the presence of fifteen carbons, embracing of one methylene carbon, eight methine carbons, five quaternary carbons, and a carbonyl carbon.

Compound (**7**) was identified as kaempferol. The ^{1}H NMR spectrum showed one proton singlet at δ 6.15 which was assigned for the aromatic proton at H-6 and one proton singlet δ 6.40 for the aromatic proton at H-8. The peak with two protons at δ 8.00 appeared as the doublet of doublet was assigned to H-2' and H-6'. Another doublet of doublet peak with two protons at δ 6.89 was assigned for H-5' and H-3'. The ^{13}C NMR spectrum established the resonances of fifteen carbon atoms. Characterization of these signals as six methines appeared at δ 98.7 (C-6), 94.0 (C-8), 115.9 (C-3'/C-5'), 130.0 (C-2'/C-6') and nine quaternary carbons resonated at δ 103.5 (C-4a), 122.1 (C-1'), 136.2 (C-3), 147.3 (C-2), 159.7 (C-4'), 156.7 (C-8a), 161.2 (C-5), 161.2 (C-7), and 176.4 (C-4).

Compound (**8**) was identified as luteolin, displaying characteristic signals in its NMR spectrum, similar to kaempferol (**7**) except for an additional singlet signal at δ 6.47 which was attributed to H-3. In addition, the ABX aromatic spin system signals which were observed at δ 7.31 (1H, d, J = 2.3), 7.28 (1H, d, J = 1.7 Hz), and 6.74 (1H, d, J = 8.1 Hz), and were assigned to methine protons of H-2′, H-6′, and H-5′ in B ring, respectively. The fifteen carbons established from the ^{13}C NMR spectrum were categorized into six methines, nine quaternary carbons, and one carbonyl.

Compounds (**1–8**) have been previously reported from several *Boesenbergia* species. Compound (**1**) has been reported as a major component in the roots of *B. pulcherrima* [21] and *n*-hexane extract of *B. xiphostachya* [22]. Compounds (**2-7**) were reported previously from the rhizomes of *B. rotunda* [23], whereas compound (**8**) was isolated from *B. armeniaca* [24].

As a summary, the chemical constituents of *B. albosanguinea* from Malaysia were found to be rich in flavonoids. Flavonoids represent one of the largest classes of plant secondary metabolites and are involved in a multitude of physiological functions, including UV protection, insect attraction, pathogen defense, symbiosis, and variation of flower color [25]. Flavonoids have been associated with many favorable agronomic traits and health benefits to humans, and their metabolic engineering is therefore an important target for plant biotechnology [26]. To the best of our knowledge, all compounds were isolated from *B. albosanguinea* for the first time.

Recently, attention has been paid to the preventative and medicinal value of dietary components, especially secondary metabolites with known inhibitory properties against LOXs. There is evidence that at least several hydrogen-donating organic molecules are co-oxidized by plant and mammalian LOXs. These observations lead to the conclusion that LOX is a versatile biocatalyst for biotransformation of endobiotics and xenobiotics [27]. Based on the relevant potential of plants as a source of anti-inflammatory compounds, in the current study, the isolated constituents from *B. albosanguinea* were subjected to lipoxygenase inhibitory activity, and the results are shown in Table 1.

All isolated constituents showed strong lipoxygenase inhibitory activity, which gave inhibition values in the range of 73.0–88.5 µg/mL at a concentration of 1 mg/mL. Among them, luteolin (**8**) and kaempferol (**7**) have shown the highest an inhibition, which gave inhibition of 88.5% and 86.2%, respectively. The result was in line with previous data which reported that luteolin as the most potent inhibitor of the mammalian enzyme with an IC_{50} value of 0.6 µM [28]. Comparatively, synthetic inhibitors such as zileuton, a well-established 5-lipoxygenase inhibitor used in asthma management, exhibit IC_{50} values in the micromolar range (~0.5–2 µM). While

direct IC_{50} comparisons are necessary for a definitive conclusion, flavonoids like luteolin have previously shown IC_{50} values around 2–5 μM in LOX inhibition studies, suggesting competitive potency with synthetic alternatives. Remarkably, even flavone was found to be inhibitory, showing that the general presence of phenolic hydroxyl groups is not a pre-requisite for the inhibition. In particular, the 3-OH proved to be not essential but rather interfering, as indicated by the higher potency of the flavone luteolin than that of the flavonol quercetin. Presence of catechol in ring A or B enhanced the inhibitory potency but was not essential for it [29]. Meanwhile, recent studies demonstrated that several prenylated chalcones and hydroxychalcones were potent free radical scavengers and also inhibited the expression of adhesion molecules and iNOS by blocking the activation of NF-kB [30]. In a previous study, panduratin A (**2**) showed significant anti-inflammatory activity in a skin-inflammatory animal model [31]. Inflammation is still a condition that is harmful to public health, which brings great pain to patients. LOX not only refers to the oxidation of lipids, but also the involvement in producing leukotrien, which mediates the occurrence of inflammation. Inhibiting LOX activity is a prospective method to treat inflammation, for the reason that many specific compounds were designed and synthesized as LOX inhibitors.

Table 1. Lipoxygenase inhibitory activity of the isolated constituents.

Compounds	% inhibition at 1 mg/mL
Elemicin (**1**)	73.2
Panduratin A (**2**)	76.2
Isopanduratin A (**3**)	84.9
5,6-Dehydrokawain (4)	73.0
Pinostrobin (**5**)	73.2
Pinocembrin (**6**)	76.0
Kaempferol (**7**)	86.2
Luteolin (**8**)	88.5
Standard - Quercetin	81.9

EXPERIMENTAL

Plant material

The rhizomes of *B. albosanguinea* were collected from Langgun Island, Langkawi (August 2023) and identified by Dr Shamsul Khamis from Universiti Kebangsaan Malaysia (UKM). The voucher specimen (PL-13/2) was deposited at UKM Herbarium, UKM.

General experimental procedures

A Soxhlet extraction technique was applied to extract the phytochemicals from the dried rhizome using different polarity solvents (*n*-hexane, dichloromethane (DCM) and methanol). Column chromatography (CC) was performed using Merck silica gel 60 (70-230 mesh) as the stationary phase. Thin layer chromatography (TLC) analysis was performed on Merck precoated silica (SiO_2) gel F_{254} plates (0.22 mm thickness) to detect and monitor the presence of compound samples. The spots were visualized under UV light (254 and 365 nm) and included with spraying reagent vanillin sulphuric acid in MeOH followed by heating. Melting points were measured by comparing them with other literature. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a Bruker Avance 500 Spectrometer. Chemical shifts were reported in ppm and $CDCl_3$ as solvent. The residual solvent was used as an internal standard. The IR spectra were recorded on Perkin Elmer ETR and 1600 spectrophotometer series as KBr discs or thin film of NaCl discs. Mass spectral data were obtained from Orbitrap Exploris 240 Mass Spectrometer.

Extraction and isolation

The dried rhizome of *B. albosanguinea* (500 g) was grinded into powder and extracted with *n*-hexane, DCM, and methanol sequentially by soxhlet extraction. The extract was concentrated using rotary evaporation to afford the crude extracts. The *n*-hexane extract (BARH, 8 g) was fractionated by VLC and eluted with *n*-hexane:DCM: EtOAc to afford 10 major fractions (F1-F10). Fraction F2, F4, and F6 was purified by CC and eluted with *n*-hexane:DCM to afford compounds (**1**) (20 mg), (**2**) (40 mg) and (**3**) (30 mg), respectively. Purification of the DCM extract (BARE, 12 g) by CC eluted with *n*-hexane:EtOAc:MeOH afforded 8 fractions (F1-F8). Fraction F3 was crystallized and successfully yielded compound (**4**) (18 mg), whereas fraction F6 yielded compound (**5**) (15 mg). Fraction F7 was subjected to CC using *n*-hexane:EtOAc as the eluent, yielding compound (**6**) (14 mg). Purification of the methanol extract (BARM, 15 g) by CC eluted with *n*-hexane:EtOAc:MeOH afforded 9 fractions (F1-F9). The fractions F4 and F5 were further purified by PTLC and successfully afforded compounds (**7**) (8 mg) and (**8**) (8 mg), respectively.

Elemicin (**1**): colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.36 (2H, d, J = 6.8 Hz, H-1'), 3.85 (3H, s, 2-OCH_3), 3.87 (6H, s, 1/3-OCH_3), 5.11 (1H, dd, J = 10.1 and 1.8 Hz, H-3'a), 5.13 (1H, dd, J = 17.0 and 1.8 Hz, H-3'b), 5.98 (1H, m, H-2'), 6.43 (2H, s, H-4/H-6); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 40.5 (C-1'), 56.0 (1-OCH3), 56.0 (3-OCH_3), 60.8 (2-OCH_3), 105.5 (C-4/C-6), 115.9 (C-3'a/C-3'b), 136.4 (C-2), 137.2 (C-2'), 153.2 (C-1/C-3), 135.7 (C-5); EIMS: *m/z* 208 [M^+, $C_{12}H_{16}O_3$].

Panduratin A (**2**): white crystalline needle. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.51 (6H, s, H-4"/H-5"), 1.77 (3H, s, H-6"), 2.05 (1H, ddd, J = 3.0, 11.8, 18.0 Hz, H-5'b), 2.16 (1H, ddd, J = 7.0, 7.5, 15.0 Hz, H-1″a), 2.27 (1H, ddd, J = 7.3, 7.3, 14.9 Hz, H-1″b), 2.38 (1H, ddd, J = 4.6, 7.0, 17.7 Hz, H-5'a), 2.62 (1H, ddd, J = 4.7, 7.4, 7.8 Hz, H-2'), 3.42 (1H, ddd, J = 6.2, 10.8, 10.9 Hz, H-6'), 3.74 (3H, s, 4-OCH_3), 4.67 (1H, dd, J = 4.7 Hz, H-1'), 4.85 (1H, t, J = 6.9 Hz, H-2"), 5.42 (1H, dd, J = 3.0, 5.0 Hz, H-4'), 5.87 (2H, s, H-3/H-5), 7.10 (1H, m, H-4'"), 7.21 (4H, m, H-2'"/H-3'"/H-5'"/H-6'"); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 17.3 (C-5"), 22.9 (C-6"), 25.5 (C-4"), 29.6 (C-1"a/C-1"b), 35.8 (C-5'a/C-5'b), 37.1 (C-6'), 41.8 (C-2'), 54.0 (C-1'), 55.0 (4-OCH_3), 96.6 (C-3/C-5), 105.6 (C-1), 122.6 (C-4'), 124.1 (C-2"), 125.6 (C-4'"), 127.2 (C-2'"/C-6'"), 127.9 (C-3'"/C-5'"), 132.4 (C-3"), 137.3 (C-3'), 145.7 (C-1'"), 167.6 (C-4), 167.6 (C-2), 207.0 (C-7); EIMS: *m/z* 407 [M^+, $C_{26}H_{30}O_4$].

Isopanduratin A (**3**): yellow oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.52 (6H, s, H-4"/H-5"), 1.81 (3H, s, H-6"), 2.05 (1H, m, H-5'b), 2.10 (1H, m, H-1'a), 2.26 (1H, m, H-1'b), 2.43 (1H, m, H-5'a), 2.52 (1H, m, H-2'), 3.44 (1H, m, H-6'), 3.92 (3H, s, 6-OCH_3), 4.52 (1H, dd, J = 4.7 and 11.3 Hz, H-1'), 4.88 (1H, t, J = 7.0 Hz, H-2"), 5.45 (1H, br.s, H-4'), 5.94 (1H, s, H-3), 5.96 (1H, s, H-5), 7.11 (1H, m, H-4'"), 7.21 (2H, m, H-2'"/H-6'"), 7.23 (2H, m, H-3'"/H-5'"); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 17.8 (C-5"), 22.9 (C-6"), 25.6 (C-4"), 28.9 (C-1"a/b), 35.8 (C-5'a/b), 37.1 (C-6'), 42.6 (C-2'), 54.1 (C-1'), 55.7 (6-OCH_3), 90.9 (C-5), 96.8 (C-3), 106.6 (C-1), 120.9 (C-4'), 124.1 (C-2"), 125.5 (C-4'"), 127.0 (C-2'"/6'"), 128.3 (C-3'"/5'"), 131.8 (C-3"), 137.2 (C-3'), 147.1 (C-1'"), 162.7 (C-4/6), 167.5 (C-2), 206.3 (C-7); EIMS: *m/z* 405 [M+H^+, $C_{26}H_{30}O_4$].

5,6-Dehydrokawain (**4**): white solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.85 (3H, s, 4-OCH_3), 5.52 (1H, d, J = 2.2 Hz, H-3), 5.97 (1H, d, J = 2.1 Hz, H-5), 6.61 (1H, d, J = 16.0 Hz, H-7), 7.36-7.42 (1H, m, H-10/H-11/H-12/H-13/H-14), 7.54 (1H, m, H-8); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 55.9 (4-OCH_3), 88.8 (C-3), 101.3 (C-5), 118.6 (C-7), 127.4 (C-10/C-14), 128.9 (C-11/C-13), 129.4 (C-12), 135.2 (C-8), 135.8 (C-9), 158.6 (C-4), 163.9 (C-6), 171.0 (C-2); EIMS: *m/z* 229 [M^+, $C_{14}H_{12}O_3$].

Pinostrobin (**5**): white solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.85 (1H, dd, J = 3.0, 17.1 Hz, H-3b), 3.12 (1H, dd, J = 13.0, 17.1 Hz, H-3a), 3.84 (3H, s, 7-OCH_3), 5.45 (1H, dd, J = 3.0, 13.1 Hz, H-2), 6.09 (1H, d, J = 2.3 Hz, H-8), 6.11 (1H, d, J = 2.2 Hz, H-6), 7.42 (1H, m, H-3'/H-4'/H-5'), 7.47 (1H, m, H-2'/H-6'), 12.05 (1H, s, 7-OH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 43.4 (C-3a/b), 55.7

(7-OCH_3), 79.3 (C-2), 94.3 (C-8), 95.2 (C-6), 103.2 (C-4a), 126.2 (C-2'/C-6'), 128.9 (C-3'/C-4'/C-5'), 138.4 (C-1'), 162.8 (C-8a), 164.2 (C-5), 168.0 (C-7), 195.8 (C-4); EIMS: *m/z* 271 [M^+, $C_{16}H_{14}O_4$].

Pinocembrin (**6**): yellow solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.86 (1H, dd, J = 3.0, 17.1 Hz, H-3b), 3.12 (1H, dd, J = 3.0, 17.1 Hz, H-3a), 5.45 (1H, dd, J = 3.0, 13.0 Hz, H-2), 6.00 (2H, s, H-8), 6.03 (2H, s, H-6), 7.41-7.49 (1H, m, H-2'/H-3'/H-4'/H-5'/H-6'), 12.07 (1H, s, 5-OH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 43.4 (C-3a/b), 79.3 (C-2), 95.5 (C-8), δ 96.8 (C-6), 103.3 (C-4a), 126.2 (C-2'/C-6'), 128.9 (C-3'/C-4'/C-5'), 138.3 (C-1'), 163.1 (C-8a), 164.4 (C-5), 164.5 (C-7), 195.8 (C-4); EIMS: *m/z* 257 [M^+, $C_{15}H_{12}O_4$].

Kaempferol (**7**): yellow crystalline solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.15 (1H, d, J = 2.0 Hz, H-6), 6.40 (1H, d, J = 2.0 Hz, H-8), 6.89 (1H, d, J = 8.8 Hz, H-3'/H-5'), 8.00 (1H, d, J = 8.8 Hz, H-2'/H-6'), 12.44 (1H, s, 5-OH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 94.0 (C-8), 98.7 (C-6), 103.5 (C-4a), 115.9 (C-3'/C-5'), 122.2 (C-1'), 130.0 (C-2'/C-6'), 136.2 (C-3), 147.3 (C-2), 156.7 (C-8a), 159.7 (C-4'), 161.2 (C-5), 164.4 (C-7), 176.4 (C-4); EIMS: *m/z* 287 [M^+, $C_{15}H_{10}O_6$].

Luteolin (**8**): yellow crystalline solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.00 (1H, d, J = 2.0 Hz, H-6), 6.25 (1H, d, J = 2.0 Hz, H-8), 6.47 (1H, s, H-3), 6.74 (1H, d, J = 8.1 Hz, H-5'), 7.28 (1H, d, J = 1.7 Hz, H-6'), 7.31 (1H, d, J = 2.3 Hz, H-2'), 12.89 (1H, s, 5-OH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 94.8 (C-8), 100.1 (C-6), 102.3 (C-3), 102.8 (C-4a), 112.8 (C-2'), 116.3 (C-5'), 119.4 (C-6'), 120.4 (C-1'), 146.9 (C-3'), 152.4 (C-4'), 158 (C-8a), 161.8 (C-5/C-7), 164.1 (C-2); EIMS: *m/z* 285 [M^+, $C_{15}H_{14}O_6$].

Lipoxygenase inhibitory activity

Evaluation of lipoxygenase (LOX) assay of isolated constituents was prepared according to the standard protocol (Lipoxygenase inhibitor screening assay kit, Item No. 760700, Cayman Chemicals Co) [17]. Stock solution of the samples was prepared to obtain a concentration of 1 mg/mL. The prepared solutions were then introduced onto 96-well plates where the cells were distributed as blanks 1A-2A-1D (triplicate), positive control 1B-2B (duplicate), and 100% initial activity wells 1C-2C-2D (triplicate). The remaining wells were designated for inhibitor (tested samples) solutions in duplicate. The addition of the reagents was done according to the standard protocol, according to which 100 µL of assay buffer was added to the blank wells and 90 µL of lipoxygenase (15-LOX) enzyme and 10 µL of assay buffer were added to positive control wells. For the 100% initial activity wells, 90 µL of lipoxygenase enzyme and 10 µL of solvent (DMSO) were added. The inhibitor (tested samples) wells were charged with 90 µL of lipoxygenase enzyme and 10 µL of respective stock (tested samples) solution. The reaction was initiated by adding 10 µL of the substrate (AA) to all wells. The plate was then shaken for 5 min on an orbital shaker. Ultimately, 100 µL of chromogen solution (prepared according to standard protocol) was added to each well to stop the enzyme catalysis. The plate was incubated for 30 min and was read at 500 nm. The percentage inhibitions (I%) of the tested samples were calculated using the following equation: $I\% = [A_{\text{initial activity}} - A_{\text{inhibitor}} / A_{\text{initial activity}}] \times 100$; where $A_{\text{initial activity}}$ is the absorbance of 100% initial activity wells without sample and $A_{\text{inhibitor}}$ is the absorbance of sample/reference.

CONCLUSION

In the current study, the phytochemical investigation of the rhizome of *B. albosanguinea* has afforded eight compounds, which comprised of diarylheptanoids (panduratin A, isopanduratin A), kavalactone (5,6-dehydrokawain), chalcones (pinostrobin, pinocembrin), flavonol (kaempferol), flavone (luteolin), and phenylpropanoid (elemicin). Likewise, the process of isolating and identifying phytochemicals can enhance our understanding of the compositional variations of phytochemicals in this species, which could potentially serve as an anti-inflammatory agent. There is a growing body of evidence to suggest that 5-LOX derived ROS are involved in a variety

of pathological and inflammatory responses. However, the detailed signaling mechanisms through which LOX metabolites mediate the specific signaling pathways that are involved in ROS generation, have yet to be clearly demonstrated. Taken together, our results shed light on the anti-inflammatory activity of isolated constituents and provide further evidence for the potential use of *Boesenbergia* species as a functional food. However, the stability and bioavailability of the active principles must be thoroughly investigated before any application can be realized.

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SECONDARY METABOLITES ISOLATED FROM THE RHIZOMES OF *Boesenbergia albosanguinea* AND ITS ACETYLCHOLINESTERASE INHIBITORY ACTIVITY

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Boesenbergia is a genus of the family Zingiberaceae, which comprises about 80 species growing in regions ranging from India to China and Southeast Asia. Its rhizome has been traditionally used in folk medicine for the treatment of several diseases. The *Boesenbergia* species contains various secondary metabolites which can be classified into two major groups, namely, flavonoids and chalcone derivatives, which indicate a great benefit for drug discovery [1]. Since this plant is used in a wide range of traditional medicine applications, many thorough studies have been carried out to assess its secondary metabolites and pharmacology activities.

Boesenbergia albosanguinea (Ridl.) Loes. is a member of the genus *Boesenbergia,* which traditionally serves a range of purposes, including food, medicinal applications, ornamental use, and ritualistic practices. It is found growing in wet, shady forest areas on sandstone or quartz-derived soils, and is mainly distributed in Malaysia (Langkawi, Pulau Langgun) and Thailand (Satun Province) [1, 2]. The essential oil composition of *B. albosanguinea* has already been reported by us [3]. Tracing the current literature, nothing was found concerning the phytochemical study of *B. albosanguinea* growing in Malaysia. As part of our continuing search to explore secondary metabolites from *Boesenbergia* species, we have investigated the chemical constituents of the rhizomes of *B. albosanguinea.*

The rhizome of *B. albosanguinea* was collected from Langgun Island, Langkawi (6.43449° or 6°26′4″ N, 99.89486° or 99°53′42″ E) in August 2023, and identified by a botanist, Dr. Shamsul Khamis, from the Universiti Kebangsaan Malaysia. A voucher specimen (PL-13/23) was deposited at UPSI Herbarium. The dried rhizome of *B. albosanguinea* (500 g) was ground into powder and extracted with *n*-hexane, ethyl acetate, and methanol sequentially by cold extraction. The extract was concentrated using rotary evaporation to afford the crude extracts.

The *n*-hexane extract (BARH, 8 g) was fractionated by VLC and eluted with *n*-hexane–DCM–EtOAc to afford 10 major fractions (F1–F10). Fraction F2 was purified by column chromatography using *n*-hexane–DCM, yielding compound **1** (20 mg). Fraction F4 was similarly purified to afford compound **2** (40 mg). Fraction F6 underwent the same process, resulting in the isolation of compound **3** (30 mg). Purification of the ethyl acetate extract (BARE, 12 g) was done by CC eluted with *n*-hexane–EtOAc–MeOH affording 8 fractions (F1–F8). Fraction F3 was crystallized and successfully yielded compound **4** (18 mg), whereas fraction F6 yielded compound **5** (15 mg).

Fraction F7 was submitted for CC eluted with *n*-hexane–EtOAc and yielded compound **6** (14 mg). Purification of the methanol extract (BARM, 15 g) by CC eluted with *n*-hexane–EtOAc–MeOH afforded 9 fractions (F1–F9). Fractions F4 and F5 were further purified by PTLC and successfully afforded compounds **7** (8 mg) and **8** (8 mg), respectively. Fractions F7 and F8 were further crystallized to afford compounds **9** (10 mg) and **10** (11 mg), respectively.

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TABLE 1. Acetylcholinesterase Inhibitory Activity of Isolated Compounds from *B. albosanguinea*

Compound	IC_{50}, μM	Compound	IC_{50}, μM	Compound	IC_{50}, μM
1	> 100	**5**	65.2	**9**	> 100
2	0.9	**6**	>100	**10**	> 100
3	1.9	**7**	10.5	Galantamine	0.45
4	22.5	**8**	5.2		

Fractionation and purification of the extracts afforded ten compounds identified as elemicin (**1**), panduratin A (**2**), isopanduratin A (**3**), pinostrobin (**4**), pinocembrin (**5**), 5,6-dehydrokawain (**6**), kaempferol (**7**), luteolin (**8**), caffeic acid (**9**), and ferulic acid (**10**). Their structures were established on the basis of spectroscopic analysis and chemical evidence.

Elemicin (1), yellow oil. MS *m/z* 208 $[M]^+$, $C_{12}H_{16}O_3$. The NMR spectral data are reported in [4].

(–)-Panduratin A (2), white crystalline solid. MS *m/z* 407 $[M + H]^+$, $C_{26}H_{30}O_4$. The NMR spectral data are reported in [5].

(–)-Isopanduratin A (3), white crystalline solid. MS *m/z* 407 $[M + H]^+$, $C_{26}H_{30}O_4$. The NMR spectral data are reported in [6].

(–)-Pinostrobin (4), white crystalline solid. MS *m/z* 271 $[M + H]^+$, $C_{16}H_{14}O_4$. The NMR spectral data are reported in [7].

(–)-Pinocembrin (5), white crystalline solid. MS *m/z* 257 $[M + H]^+$, $C_{15}H_{12}O_4$. The NMR spectral data are reported in [8].

5,6-Dehydrokawain (6), white solid. MS *m/z* 229 $[M + H]^+$, $C_{14}H_{12}O_3$. The NMR spectral data are reported in [7].

Kaempferol (7), yellow crystalline solid. MS *m/z* 287 $[M + H]^+$, $C_{15}H_{10}O_6$. The NMR spectral data are reported in [9].

Luteolin (8), yellow crystalline solid. MS *m/z* 287 $[M + H]^+$, $C_{15}H_{10}O_6$. The NMR spectral data are reported in [9].

Caffeic acid (9), white solid, $C_9H_8O_4$. Based on the NMR spectral data and comparison with those reported in the literature [9].

Ferulic acid (10), white solid, $C_{10}H_{10}O_4$. Based on the NMR spectral data and comparison with those reported in the literature [9].

Compounds **1–10** have been previously reported from several *Boesenbergia* species. Compound **1** has been reported as a major component in the roots of *B. pulcherrima* [10] and the hexane extract of *B. xiphostachya* [11]. Compounds **2–7** were reported previously from the rhizomes of *B. rotunda* [12], whereas compound **8** was isolated from *B. armeniaca* [9]. Compounds **9** and **10** were reported previously from the leaves and stems of *B. pulchella* var. *attenuata* [9].

In this study, the acetylcholinesterase inhibitory activity of the isolated compounds was evaluated using the Ellman method, with the results presented in Table 1 [13]. Panduratin A (**2**) and isopanduratin A (**3**) exhibited the strongest activity compared to the other compounds, with IC_{50} values of 0.9 and 1.9 μM, respectively. Their structures include a conjugated aromatic ring and hydroxyl groups, which enable hydrogen bonding and π–π stacking with the AChE active site [14]. Additionally, the bulky hydrophobic substituents likely enhance interactions within the enzyme's hydrophobic gorge, increasing their potency. Meanwhile, luteolin (**8**) and kaempferol (**7**), with their polyhydroxyflavonoid structures, allow for moderate hydrogen bonding and better π–π stacking within the AChE active site [15].

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