BIOASSAY-GUIDED ISOLATION, CHARACTERISATION AND MOLECULAR DOCKING STUDY OF ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE INHIBITORS FROM *Horsfieldia polyspherula* AND *Horsfieldia tomentosa*

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by

IDRISS MOHAMMED

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF SCHEMES

Page

LIST OF SYMBOLS

Å	Angstrom
%	Percent
°C	Degree Celsius
α	Alpha
β	Beta
δ	Delta
λ	Lambda
π	Pi
μg mL^{-1}	Micrograms per milliliter
μM	Micromolar
^{13}C	Carbon NMR
^{1}H	Proton NMR
C	Carbon
cm	Centimeter
cm^{-1}	Per centimeter
g	Gram
H	Hydrogen
Hz	Hertz
J	Coupling constant
kcal mol^{-1}	Kilocalorie per mol
Kg	Kilogram
m/z	Mass to charge ratio

mg	Milligram
mg mL^{-1}	Milligram per milliliter
MHz	Megahertz
min	Minute
mL	Milliliter
mm	Millimeter
nm	Nanometer
$P < 0.05$	95% confidence

LIST OF ABBREVIATIONS

δ_C	Carbon chemical shift
δ_H	Proton chemical shift
s	Singlet
d	Doublet
dd	Doublet of doublet
dt	Doublet of triplet
m	Multiplet
t	Triplet
br s	Broad singlet
1D	One dimensional
2D	Two dimensional
3D	Three dimensional
^{1}H-NMR	Proton nuclear magnetic resonance
^{13}C-NMR	Carbon 13 nuclear magnetic resonance
IC_{50}	Inhibition concentration at 50 % activity
A-site	Acylation site
A	Aril
ABSTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
ACh	Acetylcholine
AChE	Acetylcholinesterase
ATChI	Acetylthiocholine iodide
AR	Analytical reagents
ACD	Available chemical directory

AD	Alzheimer's disease
ATP	Adenosine triphosphate
$AlCl_3$	Aluminium chloride
Aβ	Beta amylase
B	Biologically
BuChE	Butyrylcholinesterase
BuTChI	Butyrylthiocholine iodide
B	Bark
Br	Branch
BFE/FEB	Binding free energy
C	Concentration
COSY	^{1}H-^{1}H correlation spectroscopy
CC	Column chromatography
Conc.	Concentrated
CD_3OD	Deuterated methanol
$CDCl_3$	Deuterated chloroform
CH_3Cl	Chloroform
CH_3OH/MeOH	Methanol
CSD	Cambridge structural database
ChE	Cholinesterase
DCM	Dichloromethane
DEPT-135	Distortionless enhancement by polarization transfer at 135 degrees
DPPH	1,1-diphenyl-2-picrylhydrazyl
DDC	Dopa decarboxylase

DTNB	5,5'dithiobis (2-nitrobenzoic acid)
EtOAc	Ethyl acetate
FT-IR	Fourier transform infrared spectroscopy
F	Fruit
Fr	Fraction
FDA	Federal department agency
GAE	Gallic acid equivalent
H	*Horsfieldia*
HMBC	Heteronuclear multiple bond correlation
HSQC	Heteronuclear single quantum coherence
HRESIMS	High resolution electron spray ionization mass spectrometry
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
$HO^{\cdot}$	Hydroxide radical
HP	Horsepower
L	Leave
M	Mass
MDDR	MDL drug data report
MM2	Molecular mechanics 2
MYBIS	Malaysia biodiversity information system
NOESY	Nuclear Overhauser effect spectroscopy
NMR	Nuclear Magnetic Resonance
NCI	National cancer institute database
NaOH	Sodium hydroxide
Na_2CO_3	Sodium carbonate

NaH_2CO_3	Sodium bihydrogencarbonate
Na_2HCO_3	Bisodium hydrogencarbonate
NMDA	*N*-methyl-D-aspartate
NMR	Nuclear magnetic resonance
NP/N	Natural product
ND	Natural product derivative
NM	Natural product mimic
NO	Nitric oxide
$NaNO_3$	Sodium nitrate
n-Hex	Normal hexane
$^1O_2$	Singlet oxygen
O2	Diatomic oxygen
P	Pericarp
PBS	Phosphate buffer solution
PDB ID	Protein data bank identification data
pH	Potential of hydrogen
p-site	Peripheral site
QE	Quercetin equivalent
ROS	Reactive oxygen specie
SD	Standard deviation
S	Synthetic
S	Seed
Sc	Seed coat
Sb	Stem bark
sp	Specie

T	*Tau*
T	Trunk
Tm	Timber
Tw	Teig
Tr	Trunk
TLC	Thin layer chromatography
TMS	Tetramethylsilane
U	Unit
UV	Ultraviolet
V	Volume
v/v	Volume by volume
W	Wood
WFO	World forest organisation
WPR	World population review

LIST OF APPENDICES

PEMENCILAN BERPANDUKAN BIOASSAI, PENCIRIAN DAN KAJIAN PENGEDOKAN MOLEKUL BAGI PERENCAT ASETILKOLINESTERASE DAN BUTIRILKOLINESTERASE DARIPADA *Horsfieldia polysperula* DAN *Horsfieldia tomentosa*

ABSTRAK

Penyakit Alzheimer adalah suatu penyakit neurodegeneratif yang berkaitan dengan penuaan. Ciri-cirinya dapat ditentukan melalui pengumpulan beta-amiloid, penggumpalan simpulan neurofibril, tekanan oksidatif dan penyusutan paras asetilkolina. Spesies *Horsfieldia*, yang terkenal dengan khasiatnya, telah menghasilkan metabolit sekunder dengan pelbagai manfaat kesihatan. Kajian ini menekankan penemuan pelbagai metabolit sekunder beserta komposisi fenolik dan flavonoid dengan jumlah yang besar dalam ekstrak *H. polyspherula* dan *H. tomentosa*. Sebanyak tujuh belas sebatian unik telah dipencilkan, sepuluh antaranya daripada ekstrak etil asetat (EtOAc) kulit *H. Polyspherula*, manakala tujuh lagi daripada ekstrak EtOAc buah *H. tomentosa*. Oleh itu, sebatian yang telah dipencilkan daripada *H. polyspherula* ialah; 3,4-dihidroksibenzoik asid (**85**), 16-fenilheksadekanoik asid (**86**), undekilbenzena (**87**), asid laurik (**88**), asid miristik (**89**), asid pentadekanoik (**90**), 1-tridekena (**91**), trimiristin (**92**), *δ*-tokotrienol (**93**) dan stigmasterol (**94**). Sementara itu, untuk *H. tomentosa*, sebatian yang telah dipencilkan termasuklah trimiristin (**92**), (-)-sesamin (**95**), 1-(2,4,6-trihidroksifenil)dekan-1-on (**96**), 1-(2,4,6-trihidroksifenil)-12-fenil-dodekanon (**97**), 1-(2,6-dihidroksi-4-(oktiloksi)fenil)-11-undekan-1-on (**98**) dan 9-oktilsesamin (**99**) dan metil miristat (100), tiga antaranya iaitu 97-99 ialah sebatian baharu. Ekstrak metanol *H. polyspherula* dan pecahan pembahagian tak terlarut *n*-heksana *H. tomentosa* mempamerkan jumlah kandungan fenolik tertinggi

dengan nilai masing-masing iaitu 619.00 ± 1.74 dan 1788.43 ± 2.01 mg GAE/g. Sementara itu, ekstrak metanol *H. polyspherula* dan ekstrak etil asetat *H. tomentosa* menunjukkan jumlah kandungan flavonoid tertinggi dengan nilai masing-masing ialah 137.25 ± 24.06 dan 361.11 ± 26.24 mg/QE/g ($P < 0.05$). Ketaranya, aktiviti antioksidan yang tinggi telah dikenalpasti dalam ekstrak metanol *H. polyspherula* (7.27 ± 0.25 μg/mL) dan sebatian **93** (1.44 ± 0.19 μg/mL), manakala pecahan pembahagian tak terlarut *n*-heksana *H. tomentosa* dan sebatian **96** mempunyai nilai masing-masing iaitu 0.79 ± 0.07 and 6.02 ± 0.03 μg/mL, berbanding asid askorbik (kawalan) pada $P < 0.05$. Tambahan pula, ekstrak mentah etil asetat menunjukkan kesan rencatan kuat ke atas asetilkolinesterase (AChE) dan butirilkolinesterase (BuChE), dengan sebatian yang dipencilkan mempamerkan kesan rencatan IC_{50} yang tinggi hingga sederhana pada $P < 0.05$ apabila dibandingkan dengan ubat standard (eserine), khasnya *δ*-tokotrienol (**93**) (AChE: 27.68 μM; BuChE 37.13 μM) daripada *H. polyspherula* dan 1-(2,6-dihidroksi-4-(oktiloksi)-fenil)-11-undekan-1-on (**98**) (AChE: 3.85 μM; BuChE: 9.43 μM) daripada *H. tomentosa*. Kajian pengedokan molekul menunjukkan tenaga pengikatan yang memuaskan antara - 6.7 dan - 10.7 kcal mol^{-1} untuk sebatian-sebatian ini serta interaksinya telah dibandingkan dengan ubat standard (eserine). Oleh itu, antara sebatian yang dipencilkan, sebatian **93** daripada kulit *H. polyspherula* dan sebatian **98** daripada buah *H. tomentosa* telah terbukti menjadi sebatian utama yang sesuai untuk pengoptimuman lanjut sebagai agen yang berpotensi melawan penyakit Alzheimer.

BIOASSAY-GUIDED ISOLATION, CHARACTERISATION AND MOLECULAR DOCKING STUDY OF ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE INHIBITORS FROM *Horsfieldia polyspherula* AND *Horsfieldia tomentosa*

ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disorder associated with ageing. It is characterised by beta-amyloid deposition, aggregation of neurofibrillary tangles, oxidative stress and decreased acetylcholine levels. *Horsfieldia* species, known for their medicinal properties, have yielded secondary metabolites with various health benefits. This research highlighted the emergence of various secondary metabolites coupled with a considerable amount of phenolic and flavonoid constituents in the extracts of *H. polyspherula* and *H. tomentosa*. A total of seventeen unique compounds were isolated, ten of which were from the ethyl acetate (EtOAc) extract of the bark of *H. polyspherula*, while another seven from the EtOAc extract of *H. tomentosa* fruit. Hence, the isolated compounds from *H. polyspherula* are; 3,4-dihydroxybenzoic acid (**85**), 16-phenylhexadecanoic acid (**86**), undecylbenzene (**87**), lauric acid (**88**), myristic acid (**89**), pentadecanoic acid (**90**), 1-tridecene (**91**), trimyristin (**92**), *δ*-tocotrienol (**93**) and stigmasterol (**94**). Meanwhile, for *H. tomentosa*, the isolated compounds include trimyristin (**92**), (-)-sesamin (**95**), 1-(2,4,6-trihydroxyphenyl)decan-1-one (**96**), 1-(2,4,6-trihydroxyphenyl)-12-phenyl-dodecan-one (**97**), 1-(2,6-dihydroxy-4-(octylo-xy)phenyl)-11-undecan-1-one (**98**), 9-octyl-sesamin (**99**) and methyl myristate (**100**), three of which **97-99** are new. The methanol extract of *H. polyspherula* and the *n*-hexane non-soluble partition fraction of *H. tomentosa* significantly exhibited the highest total phenolic content of 619.00 ±

1.74 and 1788.43 ± 2.01 mg GAE/g, respectively ($P < 0.05$). Meanwhile, the methanol extract of *H. polyspherula* and the ethyl acetate extract of *H. tomentosa* showed the highest total flavonoid content of 137.25 ± 24.06 and 361.11 ± 26.24 mg/QE/g respectively ($P < 0.05$). Significantly, strong antioxidant activity was observed in the methanol extract of *H. polyspherula* (7.27 ± 0.25 µg/mL) and compound **93** (1.44 ± 0.19 µg/mL), while *n*-hexane non-soluble partition fraction of *H. tomentosa* and compound **96** having 0.79 ± 0.07 and 6.02 ± 0.03 µg/mL, respectively, compared to ascorbic acid (control) at $P < 0.05$. Additionally, the ethyl acetate crude extracts exhibited potent inhibitory effects on acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), with the isolated compounds significantly showing strong to moderate IC_{50} inhibitory effects at $P < 0.05$ when compared to standard drug (eserine), in particular *δ*-tocotrienol (**93**) (AChE: 27.68 µM; BuChE 37.13 µM) from *H. polyspherula* and 1-(2,6-dihydroxy-4-(octyloxy)phenyl)-11-undecan-1-one (**98**) (AChE: 3.85 µM; BuChE: 9.43 µM) from *H. tomentosa*. Molecular docking studies revealed favourable binding energies for these compounds between - 6.7 and - 10.7 kcal mol^{-1} and their interactions were compared with the standard drug (eserine). Hence, among the isolated compounds, compound **93** from the bark of *H. polyspherula* and compound **98** from the fruit of *H. tomentosa* proved to be promising lead structures for further optimisation as potential multipotent agents against Alzheimer's disease.

CHAPTER 1

INTRODUCTION

1.1 Natural products

Humans have been given the precious gift of the plant kingdom, a source they have relied on for over a millennium for fundamental necessities such as the management and deterrence of array of diseases. Natural products, i.e. metabolites derived from nature (Zhu *et al*., 2023), play a crucial part in drug discovery and development. This is particularly evident in the increasing evaluation of compounds derived from natural products in clinical trials (Liu, *et al.*, 2023).

Previously, exclusive pharmaceutical interventions to treat diseases included crude and semi-pure extracts from plants, animals, microbes and minerals (Jiménez-González *et al.*, 2023). Subsequently, the concept of drug utilisation was postulated. The proposition that the impact of a medication on the human body is influenced by a precise interaction between the drug molecule and biological activity resulted in the inference that the efficacy of a drug lies in the individual chemical compounds of the extracts and does not depend on a mystical force of life (Martorana *et al*., 2022). Consequently, this era marked the beginning of a fundamentally new era in drug discovery, in which pure, isolated chemicals replaced extracts as the established standard for the treatment of disease (Bolouri *et al*., 2022). It is approximated that 14-28 % of higher plant species are utilised for medicinal purposes and 74 % of the pharmacologically active plant constituents were discovered after tracking the ethnobotanical use of the plant (Newman & Cragg, 2020). Some of these medicinal compounds isolated from plants are presented in Table 1.1 and Figure 1.1.

Table 1.1 List of chemical compounds isolated from other plants.

S/N	Compound	Treatment	Plant
1.	Digitoxin (**1**)	Heart failure and cardiac arrythmias	*Digitalis lanata*
2.	Morphine (**2**)	Pain and temperature	*Papaveraceae somniferum*
3.	Aspirin (**3**)	Pain and fever	*Salix alba*
4.	Vinblastine (**4**)	Cancer	*Caranthus roseus*
5.	Lysergic acid diethylamide (**5**)	Psychiatric illness	Fungus (*Claviceps purpurea*)
6.	Quinine (**6**)	Malaria	*Cinchona calisa*

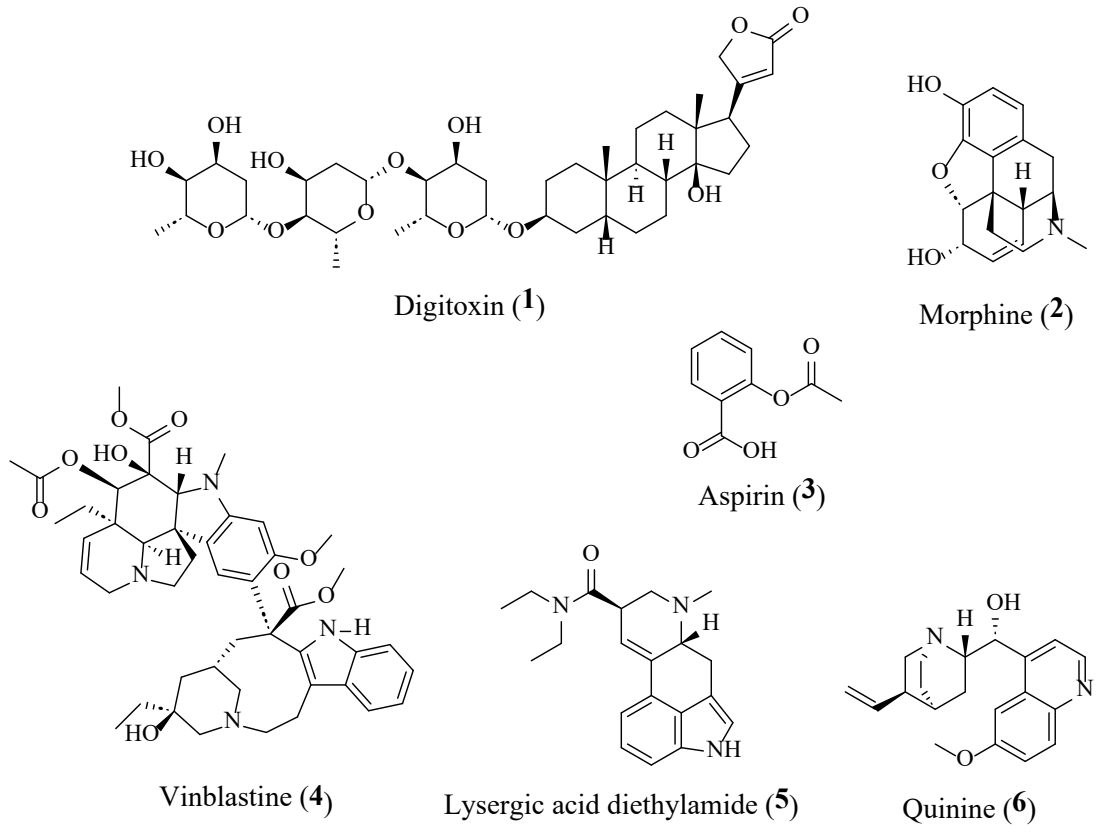


Figure 1.1 Structures of chemical compounds isolated from other plants.

Malaysia is the second most mega-diverse region in the world with a total of 13,739 plant species (Hamidah *et al*., 2022). The great botanical diversity in Malaysia is attributed to a spectrum of climatic, geological and geomorphological factors

(Khanalizadeh *et al*., 2023). Peninsular Malaysia and East Malaysia (Sabah and Sarawak) together harbour about 8,300 and 12,000 plant species respectively, of which 30 % and 42 % occur only in the respective geographical regions (Hamida *et al*., 2020). In Peninsular Malaysia, at least 28.64 % of the assessed taxa are categorised as threatened on the Malaysian Red List. In addition, a remarkable 98 % of the 96 sendemic taxa included in the same Red List are classified as threatened (Hamidah *et al*., 2022).

1.2 Alzheimer's disease

Alzheimer's disease (AD) was first described by Alois Alzheimer, a German scientist, in 1907. It is an age-related neurodegenerative process classified by a progressive loss of cognitive abilities and is closely linked to a defect in the central cholinergic system (Salerno *et al*., 2023). Although the aetiology of the disease is still poorly understood, several factors such as beta-amyloid deposition, protein aggregation, oxidative stress or low acetylcholine levels are believed to play an important role in the pathology of the disease (Anwal, 2021). Alzheimer's disease is reported to affect 55 million people and this value is expected to appreciate to 131 million by 2050 if no cure or treatment is found for the onset or progression of the disease (Lai *et al*., 2022; Tachibana *et al.*, 2024).

The pharmacological treatment of Alzheimer's disease is mostly based on the use of cholinesterase inhibitors. Cholinesterase (ChE) inhibitors are commonly prescribed to improve cholinergic signalling in the hope of restoring cognitive function (Zahedi *et al.*, 2023), which is partially impaired by the degeneration of cholinergic innervation in the hippocampus and cortex (Igarashi, 2023). Acetylcholinesterase facilitates the hydrolysis of acetylcholine in both the central and peripheral nervous

system, interrupting the transmission of impulses from neurons to postsynaptic membranes or muscles (Inoue *et al*., 2023). At the same time, butyrylcholinesterase (pseudocholinesterase) is thought to hydrolyse a variety of choline bases by enzymatically degrading harmful toxins, thus reducing the risk of potential damage or deactivation (Khan *et al*., 2023).

1.3 Problem statements

Alzheimer's disease (AD) presents a major public health challenge in the 21st century as it is emerging as the leading cause of dementia (Marasco, 2020). As the global population ages, the incidence of this weakening condition continues to rise consistently. Currently available treatments for AD face limitations, with approved drugs such as rivastigmine, galantamine and donepezil cause adverse effects on the digestive system, while N-methyl-D-aspartate (NMDA) receptor antagonists can lead to signalling dysfunction and gradual degeneration of neurons (Riederer, 2021). Although the recently approved drug lecanemab shows promise in targeting amyloid deposition, it is still in the third phase of clinical trials (Vejandla *et al*., 2024). These limitations in potent drugs, particularly cholinesterase inhibitors, have spurred a global research effort to find new inhibitors (Mezeiova *et al*., 2024). Preliminary bioassay studies conducted by our research group with various *Horsfieldia* species available in Malaysia showed significant acetylcholinesterase inhibition rates of 89 % and 98 % for *H. polyspherula* and *H. tomentosa* at 100 µg/mL, respectively. These promising results encourage further investigation of *H. polyspherula* and *H. tomentosa* for potential inhibitors. Therefore, in the present study, *H. polyspherula* and *H. tomentosa* will be evaluated for their antioxidant and anticholinesterase inhibitory properties via bioassay guided mode, followed by molecular interaction and orientation studies of

promising compounds with cholinesterase enzymes using *in silico* molecular docking techniques.

1.4 Significance of the study

In this research, the chemical compositions of *H. polyspherula* and *H. tomentosa* will be investigated using isolation, purification and characterisation techniques. Through this study, we aim to draw attention to these two indigenous Malaysian plants that have been categorised by the Malaysian Biodiversity Information System (Mybis) in the Red List as being of low concern, unassessed and endangered. The identification and promotion of these plants through this study can contribute to their conservation. In addition, the bioactive compounds discovered in the plants may be valuable for medicinal purposes, while the nature of the compounds can be explored. Therefore, this study focuses on isolating and investigating the chemical constituents of these two Malaysian plants, evaluating their potential as cholinesterase inhibitors, and assessing their antioxidant activity.

1.5 Objectives of the study

The general objectives of the study is to enhance knowledge and understanding of the secondary metabolites from *Horfieldia polyspherula* bark and fruits of *Horsfieldia tomentosa* in order to explore their potential as therapeutic agents for Alzheimer's Disease (AD) through the cholinesterase inhibition pathway. This research aims to foster the development of natural inhibitors from these plant sources, contributing to the advancement of natural products in addressing the challenges of age-related diseases such as AD.

The specific objectives are.

1. To isolate and purify the secondary metabolites of *H. polyspherula* bark and *H. tomentosa* fruits using different chromatographic techniques.

2. To elucidate and characterise the isolated compounds derived from *H. polyspherula* and *H. tomentosa* through the application of diverse spectroscopic techniques.

3. To evaluate the *in vitro* biological activities of the extracts, fractions and compounds from *H. polyspherula* and *H. tomentosa* as acetylcholinesterase and butyrylcholinesterase inhibitors.

4. To evaluate the *in silico* biological activities of the potent isolated compounds from *H. polyspherula* and *H. tomentosa* as acetylcholinesterase and butyrylcholinesterase inhibitors.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview

This chapter provides a comprehensive insight into the Myristicaceae family, with a special focus on the genus *Horsfieldia*. The family Myristicaceae represents a diverse botanical group, and within this taxonomic classification the genus *Horsfieldia* occupies a central position. In this chapter, the reported secondary metabolites are presented, with a particular focus on the substances isolated from the genus *Horsfieldia*. In addition, relevant information on Alzheimer's disease is discussed, with a focus on cholinesterase. Finally, this chapter explains the development of oxidative stress and highlights the imbalance between free radicals and the body's antioxidant defences.

2.2 Myristicaceae family

2.2.1 Distribution and habitat

The Myristicaceae is a pantropical family of trees spread in the Tropical Rain forests, chiefly at low and medium altitude though few are confined to the mountains. They are amid the five to ten widely widespread and significant tree families in most of the world's humid tropical lowland forests. This family is found in Africa, tropical Asia, Oceania and tropical America (Mao *et al.*, 2023). The family consists of 21 accepted and 10 unclassified genera and 531 and 71 unclassified species (WFO, 2024). The reported genera of Myristicaceae in Asia-Pacific region are *Myristica* (152 species), *Horsfieldia* (97 sp.), *Knema* (75 sp.), *Virola* (60 sp.), *Gymacranthera* (24 sp.), *Endocomia* (4 sp.) and *Paramyristica* (1 sp.) (WFO, 2024). In Sabah and

Sarawak, the family is represented by 5 genera with 110 species, of which several species are local endemics to Lambir Hills National Park, Sarawak, Pasoh Forest Reserve, Peninsula Malaysia, and Borneo (WFO, 2024).

The trees are classified based on the genus, the bark is sometimes smooth, dark brown with openings, while the inner bark is fibrous and reddish-brown (Armstrong & Wilson, 1978; Mao *et al.*, 2023). The twigs are olive green, alternately arranged while the leaves are long, the flowers are predominantly small, and has colours such as yellow, pink or red and occasionally exudes a sweet fragrance. The fruits are round to elongated, typically pointed and turn yellow or red when ripe (Figure 2.1). They have a thick fleshy wall, while the seeds are blackish and contrast with a red centre (Kitamura & Poonswad, 2013; Mao *et al.*, 2023).

Figure 2.1 Picture of Myristicaceae; (A) leaves, (B) fruits, (C) trunk/stem and (D) flower.

2.2.2 Classification of Myristicaceae

The Myristicaceae family is classified as (WFO, 2024):

Kingdom: Plantae
Subkingdom: Viridaeplantae
Class: Magnoliopsida
Subclass: Magnoliidae
Order: Myristicales
Family: Myristicaceae

Genus:

Bicuiba	*Gymnacranthera*	*Osteophloeum*
Brochoneura	*Haematodendron*	*Otoba*
Cephalosphaera	***Horsfieldia***	*Paramyristica*
Coelocaryon	*Iryanthera*	*Pycnanthus*
Compsoneura	*Knema*	*Scyphocephalium*
Doyleanthus	*Mauloutchia*	*Staudtia*
Endocomia	*Myristica*	*Virola*

2.2.3 Traditional importance of Myristicaceae

Plants belonging to the Myristicaceae family are known for their medicinal importance, which includes properties such as anti-inflammatory, antiviral, antitumour, antioxidant, cytotoxic and antileishmanial activities (Gupta & Rajpurohit, 2011; Salihu *et al.*, 2023). The latex obtained from the bark of certain species of this family is used in fussion with warm water to manage stomach infections, while the stem bark of some species is traditionally used to treat wounds and pimples (Salleh & Ahmad, 2017). In addition, a mixture of leaves, twigs, seeds and bark is also used to treat oral problems and fungal infections of the skin (Al-Rawi *et al*., 2024). Some species are also used to manage illnesses such as rheumatism, cholera, psychosis,

stomach cramps, nausea, diarrhoea and as an aphrodisiac (Al-Rawi *et al*., 2024; Leela, 2008).

2.3 The genus *Horsfieldia*

2.3.1 Distribution and botany

Horsfieldia belongs morphologically to the Myristicaceae because it corresponds to the biological motif of the Myristaceae (Armstrong & Wilson, 1978; Suthiwong *et al*., 2021). The genus *Horsfieldia* (Myristicaceae) comprises more than one hundred species distributed in Malaysia, Sri Lanka, the Caroline Islands, North-East India, and South-East China. Thirty-nine (39) of these species occur in Sabah and Sarawak, nine of which are endemic (i.e. they only occur in certain regions of Malaysia). The genus is best known for spices and is referred to as nutmeg in English, *pendarahan* or *darah-darah* in Malaysia, and *jela-bala* in Kenya (Al-Mekhlafi *et al.,* 2013; Soepadmo & Wong, 2007; Suthiwong *et al*., 2021). These dioecious trees have ribbed branches that usually branch several times, but not from the base, and alternate (distichous) rows of leaves along the branches. They have leaf blades up to 45 cm long, which are fragile when dry. The flowers are typically small, 0.5 to 4 mm in diameter, with the male bud being smaller than the female bud (Armstrong & Wilson, 1978; Soepadmo & Wong, 2007; Suthiwong *et al*., 2021; Zhang *et al*., 2023).

2.4 Previous chemical constituents isolated from *Horsfieldia* species

A literature search revealed that only fourteen (14) species of the genus *Horsfieldia* have been worked upon, of which one hundred and fifty-seven (157) isolated compounds were reported between 1982 and 2023. Reported secondary metabolites include alkaloids, terpenoids, lignins, essential oil and other compounds.

Table 2.1 and Figure 2.2 have listed compounds isolated from *Horsfieldia* species that showed activity as anti-cholinesterase and antioxidant properties.

Table 2.1 Chemical constituents isolated from *Horsfieldia* species that shows anti-cholinesterase and antioxidant properties.

Species	**Part**	**Compound**	**Reference**
H. kingii	Tw & L	1-(2'-hydroxy-4'-methoxyphenyl)-3-(4''-hydroxy-3''-methoxyphenyl)-propane (**7**)	Liu *et al*., 2019
		Horsfielenide A (**8**)	
		1-(2', 4'-dihydroxy-3'-methylphenyl)-3-(3''-methoxy-4''-hydroxyphenyl)-propane (**9**)	
		1-(2',4'-dihydroxyphenyl)-3-(3'',4''-methylenedioxyphenyl)-propane-2'-*O*-*β*-D-glucopyranose (**10**)	
		Bauhiniasin (**11**)	
		1,3-Di-(4-hydroxyphenyl)-propane (**12**)	
		1-(4-hydroxy-2-methoxyphenyl)-3-(4-hydroxy-3-methoxyphenyl)-propane (**13**)	
		Horsfielenide B (**14**)	
		Maculatin (**15**)	
		2,5'-Desmethoxyyatein (**16**)	
		Bursehernin (**17**)	
		Horsfielenide C (**18**)	
		Horsfielenide D (**19**)	
		Horsfielenide E (**20**)	
		Horisfieldone A (**21**)	Zhan *et al*., 2019
		Horisfieldone B (**22**)	
		1-(2'-hydroxy-4'-methoxyphenyl)-3-(4''-hydroxy-3''-methoxyphenyl)-propane (**7**)	Zhan *et al*., 2020
		(-)-Kobusin (**23**)	
		Virolane (**24**)	
		Virolanol B (**25**)	
		Virolanol C (**26**)	
		(+)-Catechin (**27**)	
		(+)-Eudesmin (**28**)	
		(+)-Phillygenin (**29**)	
		3'-Desmethylarctigenin (**30**)	
		(-)-Hinokinin (**31**)	
		Matairesinol (**32**)	

Table 2.1 (Continued)

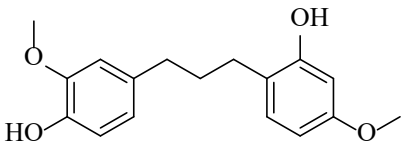
Species	Part	Compound	Reference
H. kingii	Tw & L	3',4'-de-*O*-methylenehinokinin (**33**) Horsfielenide C (**18**) Horsfielenide D (**19**) Horsfielenide E (**20**) Horsfielenidine A (**34**) 1-(2'-hydroxy-4'-methoxyphenyl)-3-(3",4"-methylene-dioxyphenyl)-propan-2-ol (**35**)	Zhan *et al*., 2020
		Matairesinol (**32**) Horsfielenigan A (**36**) Horsfielenigan B (**37**) Horsfielenigan C (**38**) 3-(3",4"-dihydroxy-benzyl)-2-(4'-hydroxy-3'-methoxy-benzyl)butyrolactone (**39**) Horsfielenidine B (**40**) Horsfielenidine C (**41**)	Zhang *et al*., 2023
	L	Horsfiequinone A (**42**) Horsfiequinone G (**43**) Horsfielenide F (**44**) Horspirotone A (**45**) Horspirotone B (**46**) Methyl spirobroussonin B (**47**)	Wang *et al*., 2023
H. macrobotrys	Sc	1-(2,4,6-trihydroxyphenyl)-9-phenylnonan-1-one (**48**) Malabaricone A (**49**)	Ramadhan & Phuwapraisirisan, 2015
H. motleyi	P	1-(2,4,6-trihydroxyphenyl)-9-phenylnonan-1-one (**48**) 2,4-Dihydroxychalcone (**50**) Horsfieldone A (**51**) Myristinin D (**52**) Myristinin E (**53**) Myristinin G (**54**)	Ramadhan *et al*., 2018
H. spicata	W, Tw & L	Dihydrocubebin (**55**) Horsfieldin (**56**) Myristicyclin A (**57**) Myristicyclin B (**58**) Cubebin (**59**) 1-(2,6-dihydroxyphenyl)-1-decanone (**60**)	Lu *et al*., 2014

Table 2.1 (Continued)

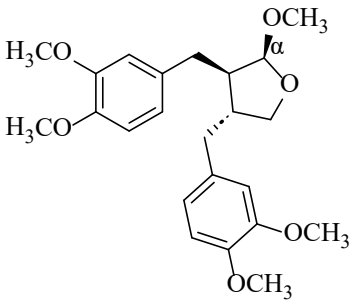
Species	Part	Compound	Reference
H. spicata	L	3',5-Dihydroxy-7,4dime-thoxyflavone (**61**) 5,7'-Dihydroxy-3(4'-hydroxy-benzyl)chromone (**62**) Kadsurenin I (**63**) Ent-16a, 17-hydroxy-19-kauranoic acid (**64**)	Megawati *et al*., 2023
H. superba		Horsfiline (**65**) 6-Methoxy-2-methyl-1,2,3,4- tetrahydron-*β*-carboline (**66**) 5-Methoxy-*N,N*-dimethyltryptamine (**67**)	Jossang *et al*., 1991
	Sb	(-)-5,6-Dihydro-6-undecyl-2*H*-pyran- 2-one (**68**) (-)-5,6-Dihydro-6-tridecyl-2*H*-pyran-2-one (**69**) (-)-3,4',7-Dihydroxy-3'-methoxy-fla-van (**70**) (-)-Viridiflorol (**71**) *β*-Sitosterol (**72**) Methyl 2,4-dihydroxy-6-methylbenz-oate (**73**) Methyl 2,4-dihydroxy-3,6-dimethyl-benzoate (**74**) (-)-4'-Hydroxy-7-methoxyflavanone (**75**) (-)-4',7-Dihydroxyflavan (**76**) (-)-4',7-Dihydroxy-3'-methoxyflavan (**77**) (+)-3,4',7-Trihydroxyflavan (**78**) (+)-Catechin (**27**) Epicatechin (**79**) (-)-7-Hydroxy-3',4'-methylenedioxyflavan (**80**) (+)-2',3,4-Trihydroxy-4'-methoxydihydrochalcone (**81**) 3',4',7-Trihydroxyflavone (**82**) Hexadecenoic acid (**83**) Hexacosanoic acid (**84**) 3,4-Dihdroxybenzoic acid (**85**)	Al-Mekhlafi *et al*., 2013

*Indicators for part of the plant used

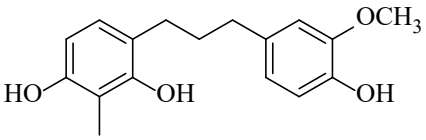
L = leave Tw = twig
Sc = seed coat P = pericarp
Sb = stem bark W = wood



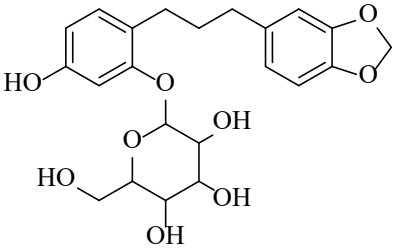
1-(2'-hydroxy-4'-methoxyphenyl)-3-(4''-hydroxy-3''-methoxyphenyl)-propane (**7**)



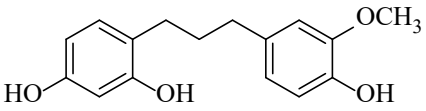
Horsfielenide A (**8**)



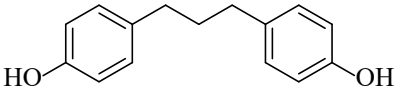
1-(2',4'-dihydroxy-3'-methylphenyl)-3-(3''-methoxy-4''-hydroxyphenyl)-propane (**9**)



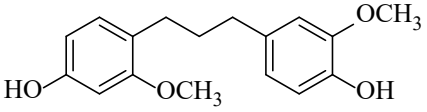
1-(2',4'-dihydroxyphenyl)-3-(3'',4''-methylenedioxyphenyl)-propane-2'-*O*-β-D-glucopyranose (**10**)



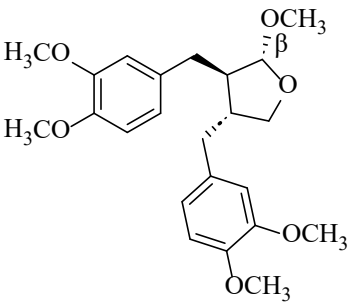
Bauhiniasin (**11**)



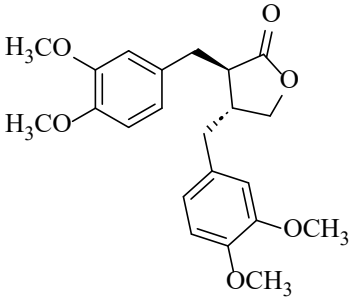
1,3-di-(4-hydroxyphenyl)-propane (**12**)



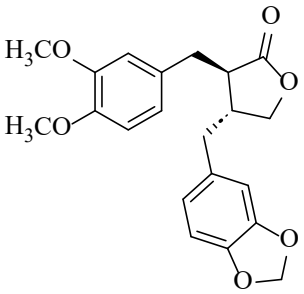
1-(4-hydroxy-2-methoxyphenyl)-3-(4-hydroxy-3-methoxyphenyl)-propane (**13**)



Horsfielenide B (**14**)

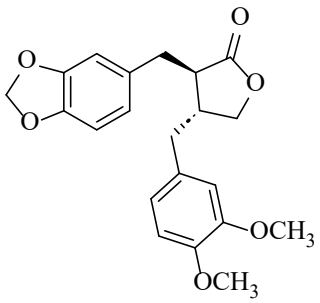


Maculatin (**15**)

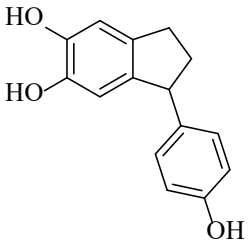


2,5'-desmethoxyyatein (**16**)

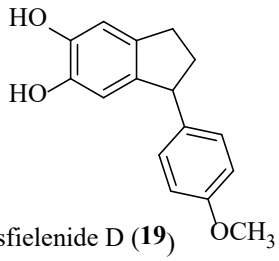
Figure 2.2 Chemical structures of isolated compounds from *Horsfieldia* species that shows anti-cholinesterase and antioxidant properties



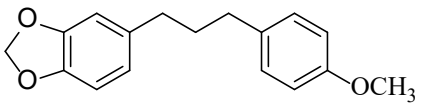
Bursehernin (**17**)



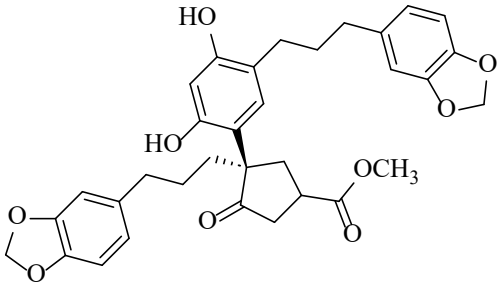
Horsfielenide C (**18**)



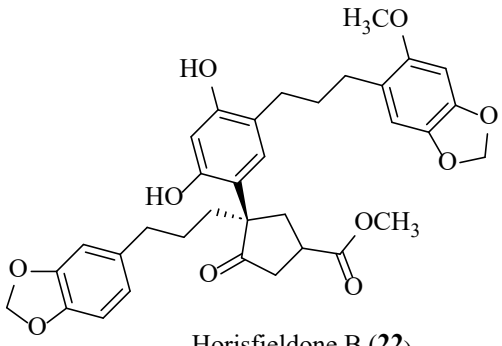
Horsfielenide D (**19**)



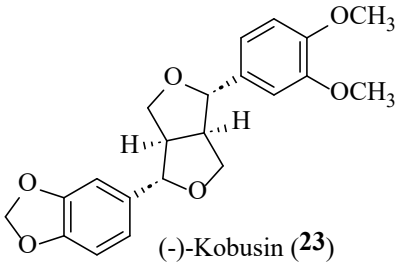
Horsfielenide E (**20**)



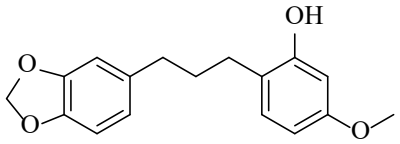
Horisfieldone A (**21**)



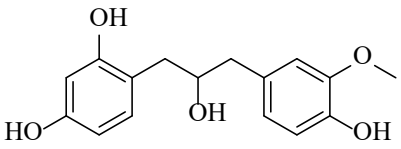
Horisfieldone B (**22**)



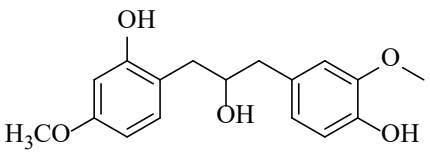
(-)-Kobusin (**23**)



Virolane (**24**)

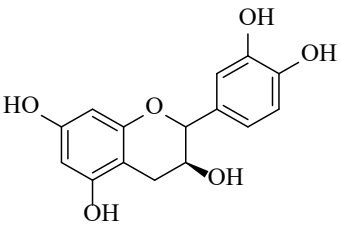


Virolanol B (**25**)

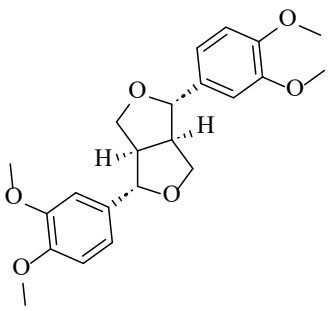


Virolanol C (**26**)

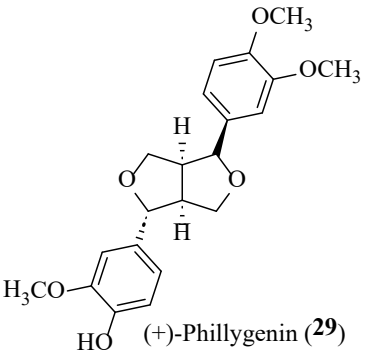
Figure 2.2 (Continued)



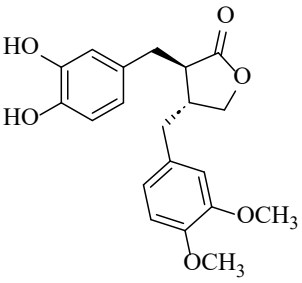
(+)-Catechin (**27**)



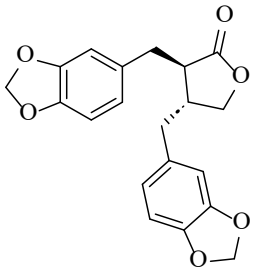
(+)-Eudesmin (**28**)



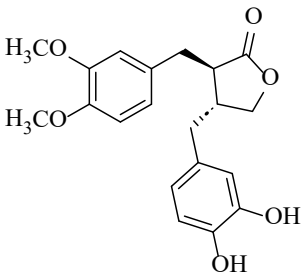
(+)-Phillygenin (**29**)



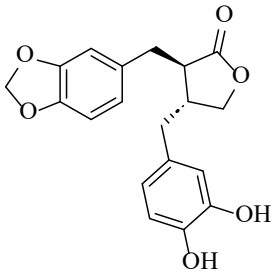
3'-desmethylarctigenin (**30**)



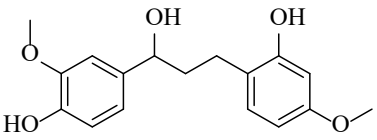
(-)-Hinokinin (**31**)



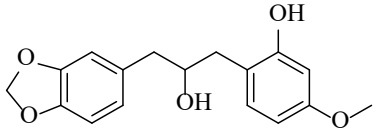
Matairesinol (**32**)



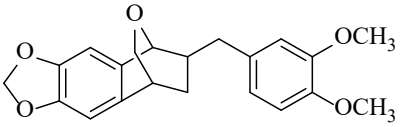
3',4'-de-*O*-methylenehinokinin (**33**)



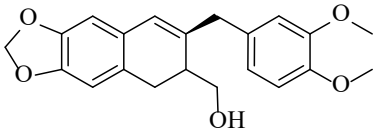
Horsfielenidine A (**34**)



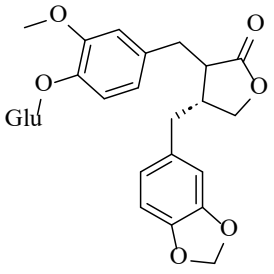
1-(2'-hydroxy-4'-methoxyphenyl)-3-(3",4"-methylene-dioxyphenyl)-propan-2-ol (**35**)



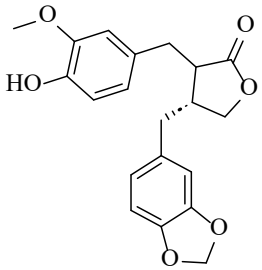
Horsfielenigan A (**36**)



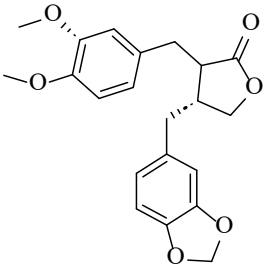
Horsfielenigan B (**37**)



Horsfielenigan C (**38**)

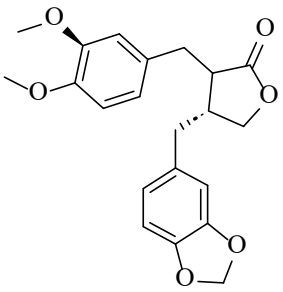


3-(3",4"-dihydroxy-benzyl)-2-(4'-hydroxy-3'-methoxybenzyl)butyr-olactone (**39**)

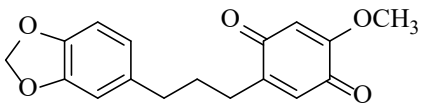


Horsfielenidine B (**40**)

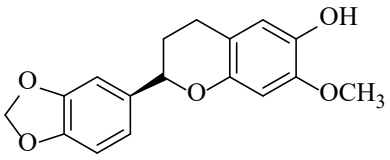
Figure 2.2 (Continued)



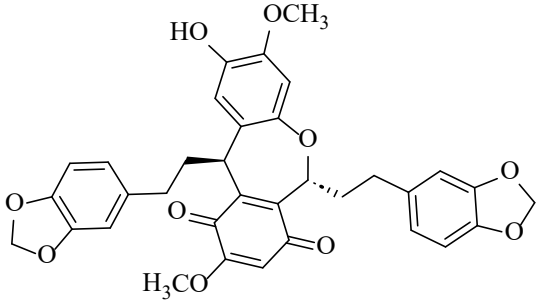
Horsfielenidine C (**41**)



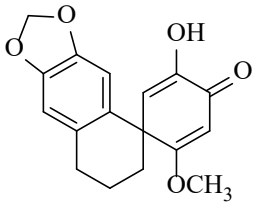
Horsfiequinone A (**42**)



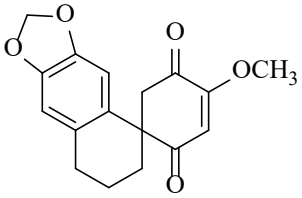
Horsfielenide F (**44**)



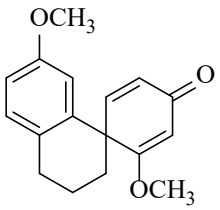
Horsfiequinone G (**43**)



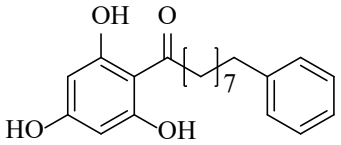
Horspirotone A (**45**)



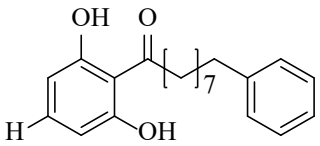
Horspirotone B (**46**)



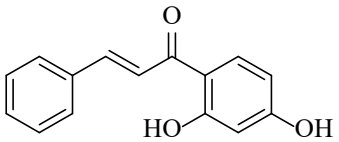
Methyl spirobroussonin B (**47**)



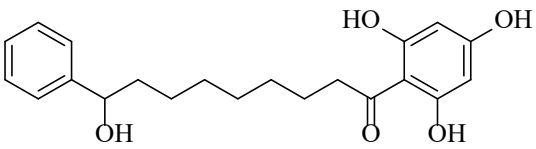
1-(2,4,6-trihydroxyphenyl)-9-phenylnonan-1-one (**48**)



Malabaricone A (**49**)

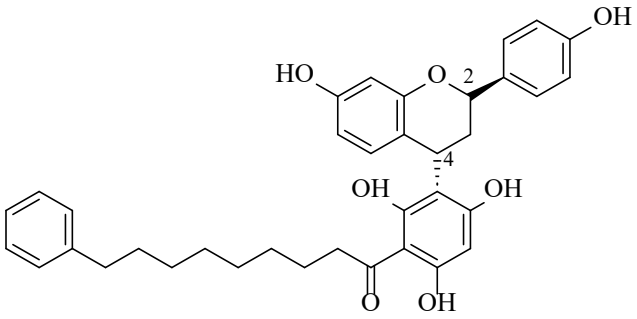


2,4-dihydroxychalcone (**50**)

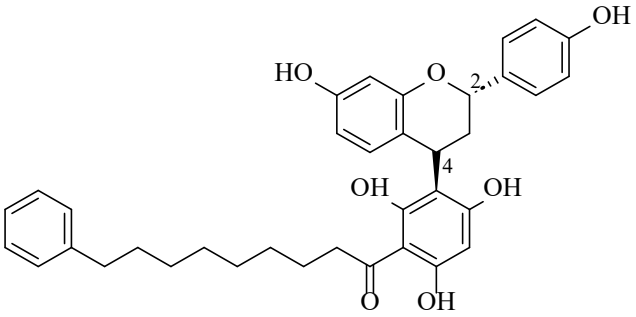


Horsfieldone A (**51**)

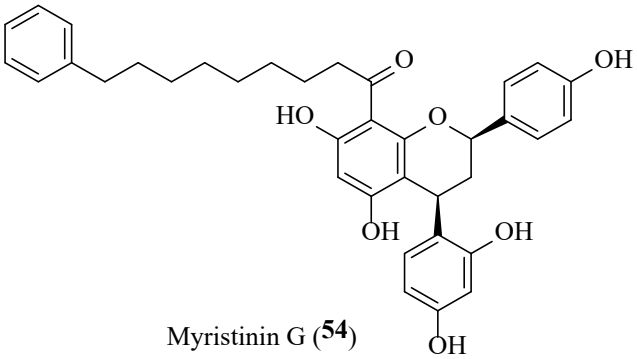
Figure 2.2 (Continued)



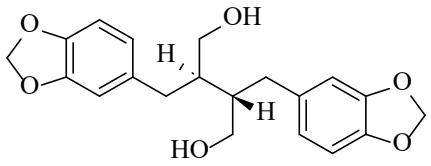
Myristinin D (**52**)



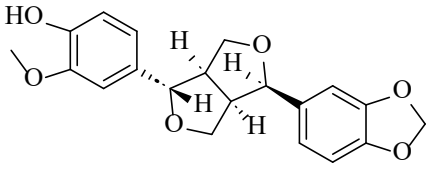
Myristinin E (**53**)



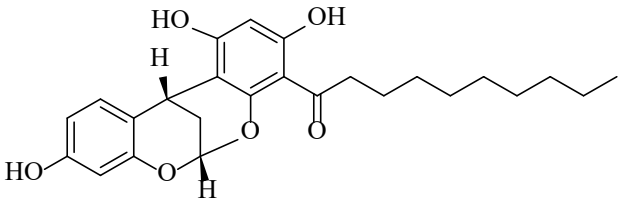
Myristinin G (**54**)



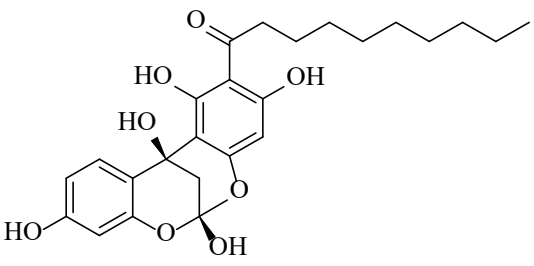
Dihydrocubebin (**55**)



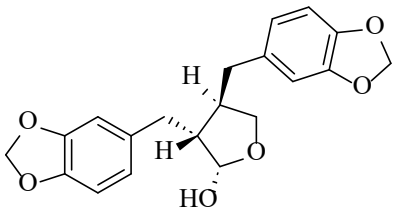
Horsfieldin (**56**)



Myristicyclin A (**57**)

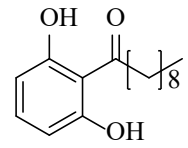


Myristicyclin B (**58**)

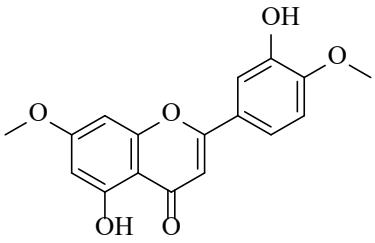


Cubebin **(59)**

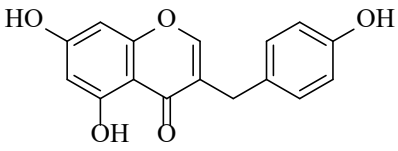
Figure 2.2 (Continued)



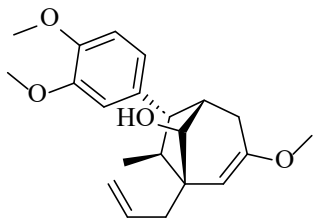
1-(2,6-dihydroxyphenyl)-1-decanone (**60**)



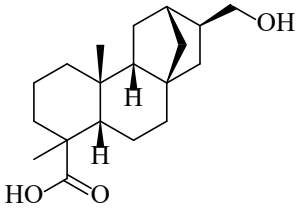
3',5-dihydroxy-7,4dimethoxyflavone (**61**)



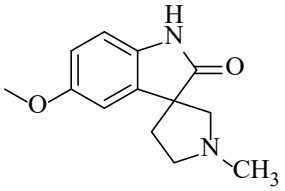
5,7'-dihydroxy-3(4'-hydroxybenzyl)chromone (**62**)



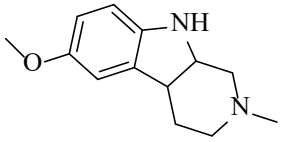
Kadsurenin I (**63**)



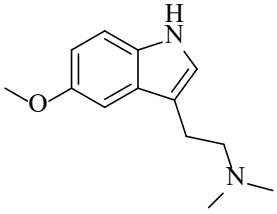
ent-16a, 17-hydroxy-19-kauranoic acid (**64**)



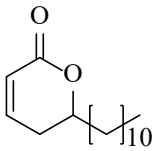
Horsfiline (**65**)



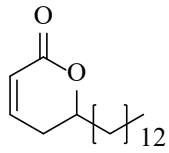
6-methoxy-2-methyl-1,2,3,4-tetrahydron-*β*-carboline (**66**)



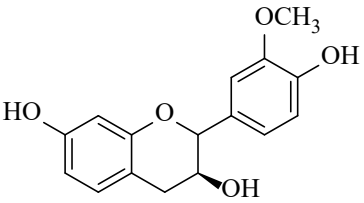
5-methoxy-*N,N*-dimethyltryptamine (**67**)



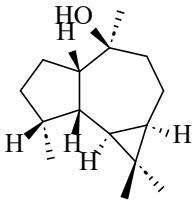
(-)-5,6-dihydro-6-undecyl-2H-pyran- 2-one (**68**)



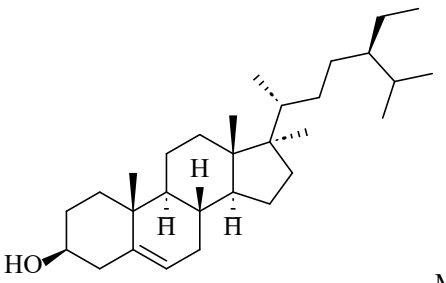
(-)-5,6-dihydro-6-tridecyl-2H-pyran-2-one (**69**)



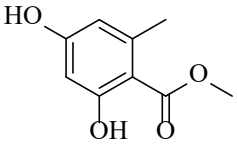
(-)-3,4',7-dihydroxy-3'-methoxyflavan (**70**)



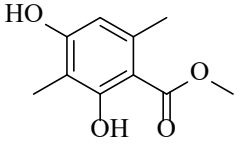
(-)-Viridiflorol (**71**)



β-Sitosterol (**72**)

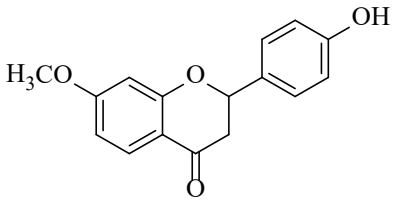


Methyl 2,4-dihydroxy-6-methylbenzoate (**73**)

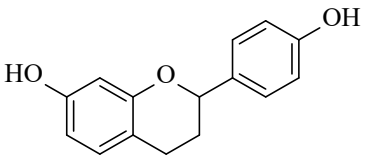


Methyl 2,4-dihydroxy-3,6-dimethylbenzoate (**74**)

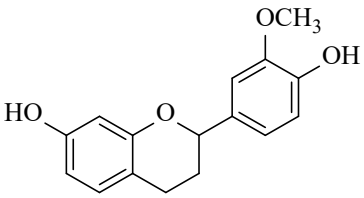
Figure 2.2 (Continued)



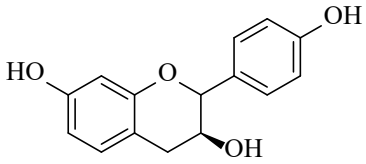
(-)-4'-hydroxy-7-methoxyflavanone (**75**)



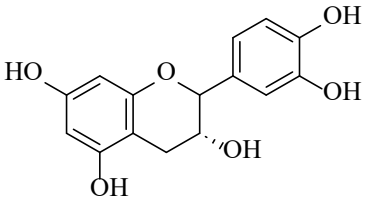
(-)-4'7-dihydroxyflavan (**76**)



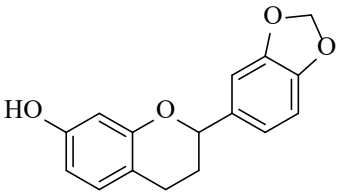
(-)-4'7-dihydroxy-3'-methoxyflavan (**77**)



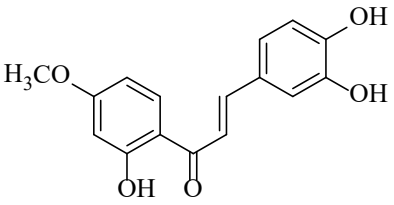
(+)-3,4',7-trihydroxyflavan (**78**)



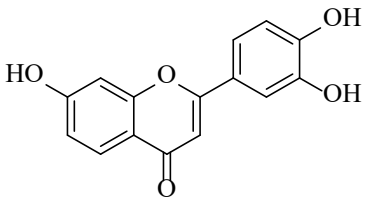
Epicatechin (**79**)



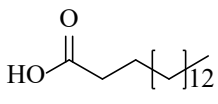
(-)-7-hydroxy-3',4'-methyl-enedioxyflavan (**80**)



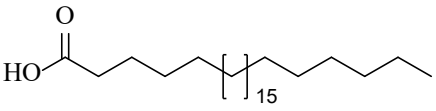
(+)-2',3,4-trihydroxy-4'-metho-xydihydrochalcone (**81**)



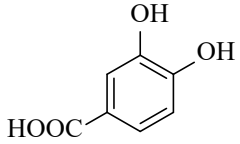
(+)-3,4',7-trihydroxyflavone (**82**)



Hexadecanoic acid (**83**)



Hexacosanoic acid (**84**)



3,4-dihdroxybenzoic acid (**85**)

Figure 2.2 (Continued)

2.5 Reported biological activities of *Horsfieldia* species

Horsfieldia genus was reported to have antifungal, antidiabetic, anti-inflamation, antimalarial, antimicrobial, immunosuppressant and cytotoxic activities (Du *et al*., 2017; Du *et al*., 2018; 2001; Liu *et al*., 2014; Liu *et al*., 2019; Ma *et al*., 2014; Ma *et al*., 2015; Miyake *et al*., 1992; Peng *et al*., 2016; Suthiwong *et al*., 2021; Wang *et al*. 2023). Table 2.2 provides a concise overview of the biological activities associated with certain *Horsfieldia* species as documented in the existing literature. It covers a broad spectrum of their biological properties agaist Alzheimer's disease, including potential anti-cholinesterase, antioxidant and human dopa decarboxylase. The objective of presenting this data is to deepen the awareness of the pharmacological capabilities attributed to *Horsfieldia* species.

Table 2.2 Previous biological activities from genus *Horsfieldia*

Biological activity	Species	Findings
Acetylcholinesterase	*H. superba*	The CH_3Cl and EtOAc extracts exhibited acetylcholinesterase inhibition, with respective IC_{50} values of 72 µg/mL and 60 µg/mL. Moreover, compounds 5,6-dihydro-6-undecyl-2H-pyran-2-one (**68**), 5,6-dihydro-6-tridecyl-2H-pyran-2-one (**69**), viridiflorol (**71**) and (-)-4'-hydroxy-7-methoxyflavan (**75**) displayed IC_{50} values exceeding 100 µM (Al-Mekhlafi *et al*., 2013).
Antidiabetic	*H. polyspherula*	The leaf and stem of *H. polyspherula* DCM extract showed excellent inhibition of > 90 % against both *α- amylase* and *α-glucosidase* at concentrations of 7.8 and 10.0 µg/mL, respectively. In addition, the IC_{50} for leaf and stem with respect to α-amylase is 2.4 ± 0.4 µg/mL and 1.6 ± 0.2 µg/mL, respectively, while *α-glucosidase* indicates 4.2 ± 0.9 and 2.4 ± 0.4 µg/mL for leaf and stem, respectively (Mohamad Jemain *et al*., 2011).

Table 2.2 (Continued)

Biological activity	Species	Findings
Antifungal	*H. tomentosa*	The 200 µg/mL EtOH extract showed activity against the growth of rice fungi using the mycelial growth test at $p< 0.05$. The results showed a percentage inhibition of 54.0 ± 2.0 %, 82.8 ± 2.6 %, 50.6 ± 0.7 %, 43.0 ± 0.8 % and 59.2 ± 0.4 % for *Bipolaris satariae*, *Curvularia lunata, Curvularia oryzae* and *Sclerotium rolfsii*, respectively (Plodpai *et al.*, 2013).
Antioxidant	*H. macrobotrys*	DPPH scavenging activity of 1-(2,4,6-trihydroxyphenyl)-9-phenylnonan-1-one (**48**) showed good antioxidant potency of IC_{50} 2.6 mM compared to standard (ascorbic acid) which has IC_{50} of 2.5 mM only, while malabaricone A (**49**) showed IC_{50} of 6.2 mM (Ramadhan & Phuwapraisirisan, 2015).
	H. motleyi	Myristinin D (**52**), myristinin E (**53**) and myristinin G (**54**) showed stronger DPPH and ABTS radical scavenging activity than the control ascorbic acid with IC_{50} of 47.1, 40.3 and 279.9 µM for DPPH, while 23.7, 20.3 and 54.3 µM for ABTS compared to ascorbic acid with 2450 µM and 751.2 µM for DPPH and ABTS radical scavenging, respectively (Ramadhan *et al*., 2018).
	H. spicata	Both the crude extract and the fractions have exhibited moderate activity against *α-glucosidase*, in comparison to Quercetin, the positive control. (Megawati *et al*., 2023).
Human Dopa decarboxylase	*H. kingii*	Horsfielenide C (**18**), horsfielenide D (**19**) and horsfielenide E (**20**) are active against the enzyme DDC (dopa decarboxylase, merging biomarker for Parkinson's disease (Liu e*t al.*, 2019). Horisfieldone A (**21**) and Horisfieldone B (**22**) exhibited very good inhibitory activity against human DDC with an IC_{50} value of 35.1 ± 3.9 and 67.1 ± 3.7, respectively (Zhan *et al*., 2019).

2.6 The significance of medicinal chemistry in Alzheimer's disease

Medicinal chemistry is essential for advancing the development of drugs for Alzheimer's disease (AD) by isolating bioactive compounds from natural sources, targeting specific elements such as beta-amyloid, *tau* protein and acetylcholinesterase. These targets play a central role in the development of plaques and tangles in the brain and contribute to neuronal impairment and cell death (Mahdi *et al*., 2021). Compounds that show promise for modulating these molecular targets are optimised for their pharmacological properties and progress through the preclinical and clinical phases as potential drug candidates (Wang *et al*., 2024).

2.7 Alzheimer's disease

2.7.1 Statistics of Alzheimer's disease

Alzheimer's disease (AD) is an incremental neurodegenerative illness that poses a major challenge for healthcare and neuroscience. Despite the rapid increase in the number of individuals living with the disease, only a quarter are reportedly officially diagnosed. Globally, 55 million people are affected and this figure is expected to rise to 131 million by 2050 if no effective treatments are found for the onset or progression of the disease (Tachibana *et al*., 2024). According to the World Health Rankings, Alzheimer's accounts for 60 % of age-related diseases, followed by cerebrovascular disease, Lewy disease and frontotemporal dementia. It is also the third leading cause of death worldwide, with findings indicating that women are more commonly affected, as shown in Figure 2.3 and Table 2.3 (Rawat *et al*., 2024; WPR, 2024). This might be due to their lengthier life expectancy in contrast to men. The report also estimates that 19 % and 17 % of all deaths per 100,000 in Nigeria and Malaysia are due to Alzheimer's disease, with the proportion of women in Nigeria and

Malaysia being 21 % and 19 % respectively, and men 17 % and 15 % respectively (Rawat *et al*., 2024; WPR, 2024).

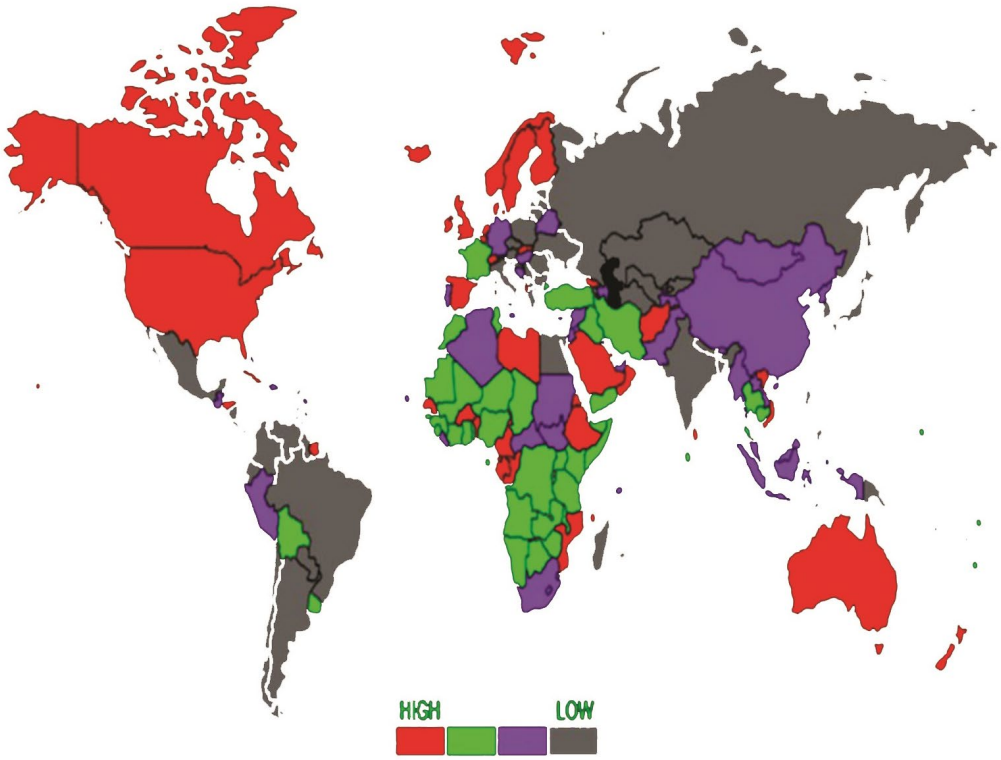


Figure 2.3 Map of areas with Alzheimer's disease (WPR, 2024).

Table 2.3 List of the ten countries with the highest Alzheimer's disease death rates in the world (WPR, 2024).

S/N	Country	(%) per 100,000		
		Total	Male	Female
1	Finland	54.65	52.10	55.32
2	United Kingdom	42.70	38.50	45.25
3	Slovakia	38.15	15.61	47.53
4	Albania	36.92	22.80	47.71
5	Iceland	35.59	31.16	38.94
6	Brunei	33.87	31.25	36.30
7	Netherland	33.78	30.48	35.54
8	United State	33.26	28.37	36.18
9	Ireland	32.23	27.11	35.64
10	Sweden	30.96	28.51	32.42