ISOLATION, CHARACTERIZATION, ANTI-HYPERGLYCEMIC ACTIVITY AND MOLECULAR DOCKING STUDIES OF CONSTITUENTS ISOLATED FROM *ENDIANDRA KINGIANA* AND *BEILSCHMIEDIA LUMUTENSIS* (LAURACEAE)

NUR AMIRAH BINTI SAAD

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by

NUR AMIRAH BINTI SAAD

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LIST OF SYMBOLS

%	Percent
°C	Degree Celsius
µg mL^{-1}	Micrograms per milliliter
µM	Micrometer
^{13}C	Carbon NMR
^{1}H	Proton NMR
Å	Angstrom
br	Broad
C	Carbon
cm	Centimeter
cm^{-1}	Per centimeter
δ_C	Chemical shift carbon
δ_H	Chemical shift hydrogen
E	Trans
g	Gram
H	Hydrogen
Hz	Hertz
J	Coupling constant
kcal mol^{-1}	Kilocalorie per mol
kg	Kilogram
m	Meter
m/z	Mass to charge ratio
mg	Milligram
mg mL^{-1}	Milligram per milliliter
MHz	Megahertz

min	Minute
mL	Milliliter
mL min^{-1}	Milliliter per minute
mm	Millimeter
Nm	Nanometer
O	Oxygen
ppm	Parts per million
R	Substituent group
Z	*Cis*
α	Alpha
β	Beta
λ	Lambda
π	Pi

LIST OF ABBREVIATIONS

1D	One dimensional
2D	Two dimensional
3D	Three dimensional
A549	Human lung epithelial cancer cell line
AChE	Acetylcholinesterase
AGIs	α-glucosidase inhibitors
AR	Analytical reagents
CC	Column chromatography
CD_3OD	Deuterated methanol
$CDCl_3$	Deuterated chloroform
CH_2	Methylene group
C-MAF	Cytotoxicity – Macrophage activating factors
CoA	Coenzyme A
COOH	Carboxylic acid
COSY	^1H-^1H correlated spectroscopy
d	Doublet
DCM	Dichloromethane
dd	Doublet of doublet
DEPT135	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethyl sulfoxide
DNS	Dinitrosalicyclic acid
dt	Doublet of triplet
EtOAc	Ethyl acetate
FTIR	Fourier-transform infrared spectroscopy
H_2SO_4	Sulphuric acid
HCT116 p53	Colon cancer cell
HMBC	Heteronuclear multiple bond correlation
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear single quantum coherence
HT-29	Human colon cancer cell line

IC_{50}	Inhibition concentration at 50%
IDF	International Diabetes Federation
iNOS	Inducible nitric oxide synthase
m	Multiplet
MBC	Minimum bactericidal concentration
MD	Molecular docking
MDA-MB-231	Human breast carcinoma cell
MeOH	Methanol
MGAM	Maltase
MIC	Minimum inhibitory concentration
NaOH	Sodium hydroxide
NCI-H460	Human non-small cell lung carcinoma
NFAT	Nuclear factor of activated T-cells
NHMS	National Health and Morbidity Surveys
NMR	Nuclear magnetic resonance
PDB ID	Protein data bank identification data
pH	Potential of hydrogen
pNPG	4-nitrophenyl α-D-glucopyranoside
RCDA	radical cation activated Diels-Alder
Rf	Retention factor
s	Singlet
t	Triplet
TLC	Thin layer chromatography
TMS	Tetramethylsilane
U87MG	Human glioblastoma cell
UHPLC-MS/MS	Ultra-high performance liquid chromatography-tandem mass spectrometer
UV	Ultraviolet
WHO	World Health Organization

LIST OF APPENDICES

PEMENCILAN, PENCIRIAN, AKTIVITI ANTI-HIPERGLISEMIK DAN KAJIAN PENGEDOKAN MOLEKUL SEBATIAN KIMIA DARIPADA *ENDIANDRA KINGIANA* DAN *BEILSCHMIEDIA LUMUTENSIS* (LAURACEAE)

ABSTRAK

Kajian awal ke atas kulit kayu *Endiandra kingiana* (*E. kingiana*) dan *Beilschmiedia lumutensis* (*B. lumutensis*) menunjukkan keberkesanan sebagai agen anti-hiperglisemik terhadap aktiviti perencatan enzim α-amilase dan α-glucosidase, yang mendorong penyiasatan kimianya. Asid endiandrik dan kingianin adalah dua kumpulan utama poliketida siklik yang diasingkan daripada kedua-dua spesies. Proses pengasingan dan penulenan ekstrak aktif *E. kingiana* dan *B. lumutensis* memencilkan tiga belas sebatian. *E. kingiana* memencilkan sepuluh sebatian yang diketahui; lima asid endiandrik; kingianic acid A (**141**), kingianic acid B (**143**), kingianic acid E (**145**), endiandric acid M (**141**), tsangibeilin B (**99**), dua kingianin; kingianin A (**30**), kingianin F (**33**), dua flavonoid; epicatechin (**164**), catechin (**164**) dan satu sesquiterpena; daibuoxide (**168**). Manakala bagi *B. lumutensis*, tiga asid endiandrik baru telah dipencilkan dan dinamakan sebagai lumutensic acid A (**169**), lumutensic acid B (**170**) and lumutensic acid C (**171**). Struktur telah ditentukan dengan pelbagai teknik spektroskopi seperti 1D dan 2D NMR, FTIR, HRMS dan perbandingan kajian literatur. Sebatian terpencil terpilih telah diuji selanjutnya untuk aktiviti perencatan anti-hiperglisemik terhadap enzim α-amilase dan α-glucosidase. Sebatian **143** menunjukkan perencatan tertinggi dengan nilai IC_{50} 0.02 ± 0.3 mg mL^{-1} bagi enzim α-amilase. Selain itu, untuk α-glucosidase, sebatian **99**, **145** dan **143** mendedahkan perencatan terbaik dengan nilai IC_{50} masing-masing 0.22 ± 0.02 mg mL^{-1}, 0.26 ± 0.03

mg mL^{-1} dan 0.28 ± 0.01 mg mL^{-1}. Kajian pendokkan molekul mendapati sebatian **143** dan **99** terikat ke dalam tapak aktif terminal C α-amilase pankreas manusia (ID PDB: 2QV4) dan Maltase-Glucoamylase (MGAM) manusia (ID PDB: 3TOP), seterusnya bertepatan dengan aktiviti perencatan terhadap enzim α-amilase dan α-glukosidase. Oleh itu, sebatian **143** yang bertindak sebagai perencat dwi mampu menjadi pemilihan utama dalam pembangunan baru bagi agen anti-hiperglisemik daripada sebatian semulajdi.

ISOLATION, CHARACTERIZATION, ANTI-HYPERGLYCEMIC ACTIVITY AND MOLECULAR DOCKING STUDIES OF CONSTITUENTS ISOLATED FROM *ENDIANDRA KINGIANA* AND *BEILSCHMIEDIA LUMUTENSIS* (LAURACEAE)

ABSTRACT

A preliminary study upon the bark of *Endiandra kingiana* (*E. kingiana*) and *Beilschmiedia lumutensis* (*B. lumutensis*) revealed promising anti-hyperglycemic agents against α-amylase and α-glucosidase inhibition activity, which prompted its chemical investigation. Endiandric acids and kingianin series were two main groups of cyclic polyketides isolated from both species. Isolation and purification process of the active extracts of *E. kingiana* and *B. lumutensis* afforded thirteen compounds. *E. kingiana* yielded ten compounds comprising of five endiandric acids; kingianic acid A (**141**), kingianic acid B (**143**), kingianic acid E (**145**), endiandric acid M (**141**), tsangibeilin B (**99**), two kingianins; kingianin A (**30**), kingianin F (**33**), two flavonoids; epicatechin (**164**), catechin (**164**) and one sesquiterpene; daibuoxide (**168**). Meanwhile for *B. lumutensis*, three new endiandric acids were isolated identified as lumutensic acid A (**169**), lumutensic acid B (**170**) and lumutensic acid C (**171**). Their structures were elucidated by multiple spectroscopic techniques, for instance 1D and 2D NMR, FTIR, HRMS and comparison with the literature data. The selected compounds were further tested for anti-hyperglycemic inhibition activity against α-amylase and α-glucosidase enzymes. Compound **143** showed the highest inhibition with IC_{50} value of 0.02 ± 0.3 mg mL^{-1} for α-amylase. For α-glucosidase, compounds **99, 145** and **143** revealed potent inhibition with IC_{50} values of 0.22 ± 0.02 mg mL^{-1}, 0.26 ± 0.03 mg mL^{-1} and 0.28 ± 0.01 mg mL^{-1}, respectively. The molecular docking study revealed

that compounds **143** and **99** bound into the active site of the C-terminal of human pancreatic α-amylase (PDB ID: 2QV4) and human Maltase-Glucoamylase (MGAM) (PDB ID: 3TOP), thus agreed with α-amylase and α-glucosidase enzymes inhibitions. Hence, compound **143** which acts as dual inhibitor may serve as lead candidates for the drug development of new anti-hyperglycemic agents from natural products.

CHAPTER 1

INTRODUCTION

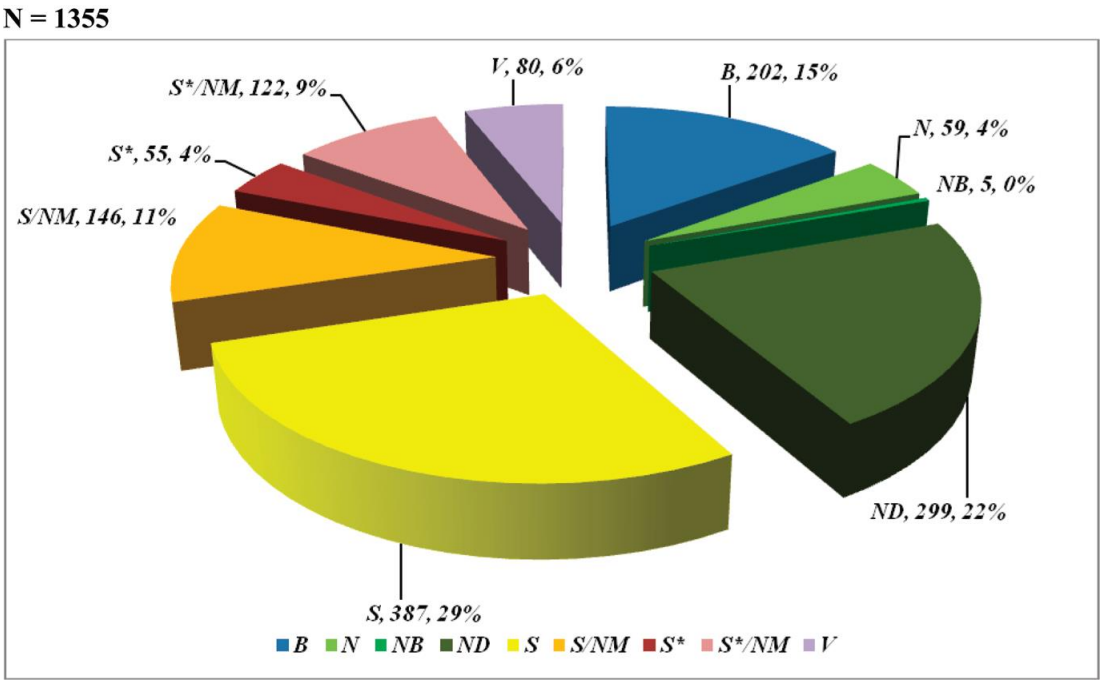
1.1 General Introduction

Malaysia, incorporating the Malayan Peninsular and Malaysian Borneo, is one of the mega diversity countries which ranks 17th globally (Tong, 2020). This index is based on the estimation of Malaysia's richness and endemism in certain classes of plants, birds, mammals, fish, reptiles, amphibians and others. The richness of Malaysian flora offers significant opportunities for bioprospecting toward discovering of bioactive compounds or natural products with remedial value.

Natural products are chemical compounds or substances isolated from living organisms such as plants, marine organisms fungi and insects (Anulika *et al.*, 2016). Interestingly, since a long time ago, humans have commonly exploited natural products as traditional medicines, food, dyes, poisons, polymers, glues, waxes, drugs, fibres and perfumes (Croteau, Kutchan & Lewis, 2000). Since it is prominent as a rich source of medicinal value, humans have used these to alleviate and treat disease. In 1805, Friedrich Sertürner, a young German pharmacist, extracted morphine from the poppy plant as the first pharmaceutically active chemical constituent (Yuan *et al.*, 2016). During that time, even a small percentage of the existing plant species were researched for their phytochemical studies, still it is proven that traditional medicines and natural products have already benefits modern medicine development (Yuan *et al.*, 2016).

As a result, a varied range of natural components have been established to provide an ongoing supply of molecular templates in the development for new and novel potent drugs and hence, contributed to drug development procedures of many pharmaceutical industries. Figure 1.1 showed the percentage of all new approved drugs

from 1981 to 2010 with all sources of categories (Newman and Cragg, 2012). Among them, only 29% were synthetic in origin and the rest were approved drug from natural origin or natural products. Scientific research of the natural products will develop from time to time till present day.



B – biologics; **N** – natural product; **NB** – natural product botanical; **ND** – natural products derivatives; **S** – synthetic; **NM** – natural product mimic; **V** – vaccines.

Figure 1.1 Percentage of all new approved drugs from 1981 to 2010 with all sources of categories (Newman and Cragg, 2012).

Previously, several plant species like tongkat ali (*Eurycoma longifolia*), dukung anak (*Phyllanthus niruri*) mas cotek (*Ficus deltoidea*) misai kucing (*Orthosiphon aristatus*), kacip fatimah (*Labisia pumila*), hempedu bumi (*Andrographis paniculata*), Roselle (*Hibiscus sabdariffa*), mengkudu (*Morinda citrifolia*), and ginger (*Zingiber officinale*) have been reported having ethnopharmacological information (Hashim *et al.*, 2018). For example, *Eurycoma longifolia* is a good anti-malarial agent against *P. falciparum, Phyllanthus niruri* is an anti-hyperalgesic, while *Ficus deltoidei* is an

anti-diabetic agent. Moreover, *Orthosiphon aristatus* and *Morinda elliptica* possess good antioxidant activity (Hashim *et al.*, 2018). These substances that played an essential role in contributing to the medicinal properties are their secondary metabolites.

Secondary metabolites are organic compounds that do not appear to play a direct role in growth and development. Examples of secondary metabolites in plants organisms that belong to several chemical classes are terpenoids, alkaloids, phenylpropanoid, flavonoids, sterols and others (Croteau, Kutchan and Lewis, 2000). Secondary metabolites are common chemical constituents generated by plants as a result that branch off from primary metabolic pathways. Secondary metabolites were reported to have a wide range of biological activities such as anti-biotics, anti-viral, anti-fungal, anti-cancer and anti-inflammatory. Hence, the secondary metabolites were beneficial act as herbs in traditional medicines (Hussein & El-Anssary, 2019). As mentioned before, *Eurycoma longifa* which contained eurycomanol (**1**) (class: quassinoids), *Phyllanthus niruri* contained corilagin (**2**) (class: tannins) and *Ficus deltoidei* contained vitexin (**3**) contributes to biological effects such as anti-malarial, anti-hyperalgesic and anti-diabetic, respectively. In addition, *Marantodes pumilum* contained fatimahol (**4**) (class: alkylphenols) (Hashim *et al.*, 2018), while *Orthosiphon aristatus* and *Morinda elliptica* both contained phenolics thus contribute to the antioxidant activity . The compounds beneficial to the plants are shown in Figure 1.2 below.

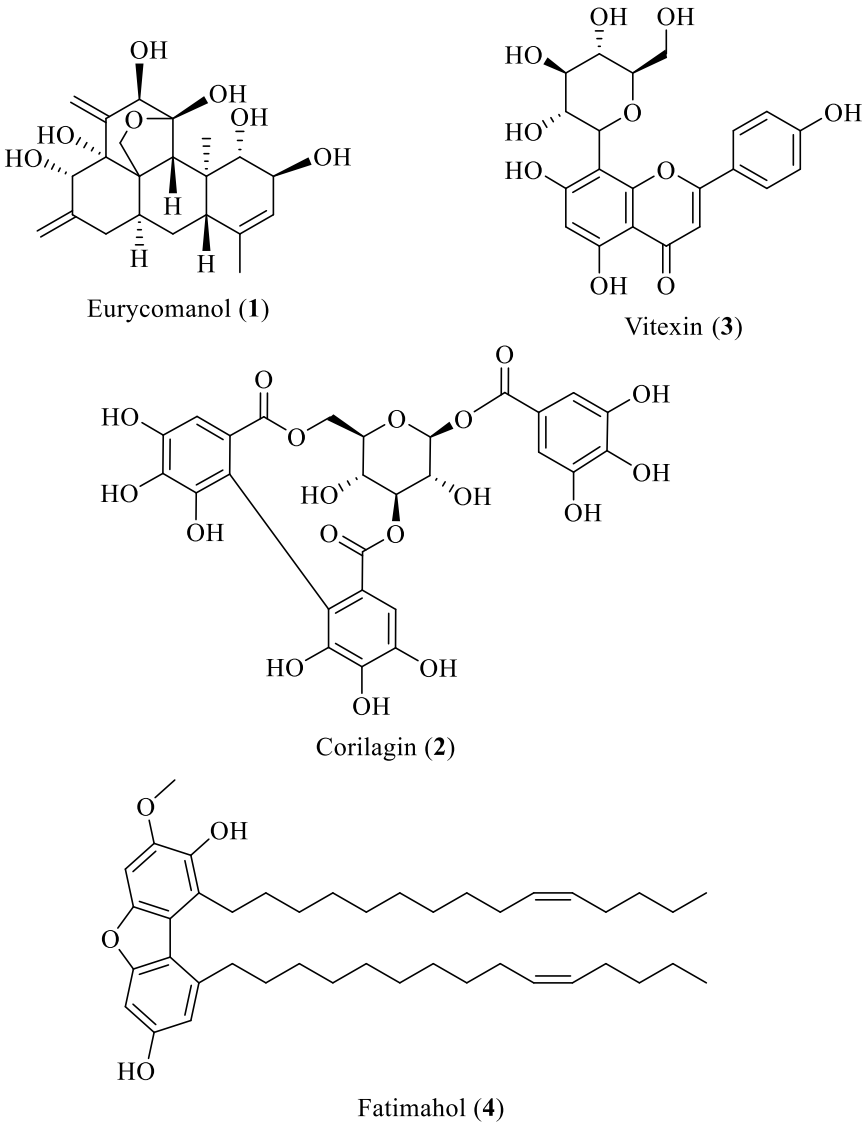


Figure 1.2 Compound isolated from *Eurycoma longifa* – eurycomanol (**1**), *Phyllanthus niruri* – corilagin (**2**), *Ficus deltoidei* – vitexin (**3**) and *Marantodes pumilum* – fatimahol (**4**).

According to the World Health Organisation (WHO) survey, there are about 80% of the populations living in the developing countries rely almost exclusively on traditional medicine for their primary health care needs (Ekor, 2014). Therefore, it is an urge for scientists to conduct research relating to the Malaysian tree flora. Extensive phytochemical investigations have been conducted upon the species from these genera; *Endiandra* and *Beilschmiedia* and successfully isolated a few classes of secondary metabolites

1.2 Problem Statement

Recently, diabetes is one of the Malaysia's most prevalent non-communicable disease. Hyperglycemia, or high blood glucose, is a symptom that characterizes diabetes. According to the National Health and Morbidity Surveys (NHMS), the number of Malaysian patients with diabetes has increased dramatically from 11.2% in 2011 to 13.4% in 2015 and 18.3% in 2019. It is a severe problem in this country with roughly one in every five persons or approximately 3.9 million individuals aged 18 years old and over suffers to have diabetes in 2019.

Common therapeutic approaches to treat diabetes is to slow down carbohydrate absorption, increase carbohydrate digestion time in the gastrointestinal tract and decrease hyperglycemia. This could be achieved by the inhibition of carbohydrates-hydrolysing enzymes including *α*-amylase and *α*-glucosidase. Currently, there are four *α*-glucosidase inhibitors (AGIs) approved for clinical use such as acarbose (**5**), miglitol (**6**), voglibose (**7**), and emiglitate (**8**). Due to side effects from the use of insulin and oral hypoglycaemic agents to treat diabetes, scientists work hard to develop alternative approaches from natural plants that can inhibit *α*-amylase and *α*-glucosidase. Taking advantage of our natural resources, Malaysia is aggressively discovering useful new compounds from plants that can benefits in drugs, nutraceuticals and agrochemicals.

To the best of our knowledge, there is limited study upon *E. kingiana* and *B. lumutensis* for anti-hyperglycemic evaluation against *α*-amylase and *α*-glucosidase yet. A preliminary study was done upon crude extracts of *E. kingiana* and *B. lumutensis.* Hence, it reported that these plant extracts showed good inhibition on both enzymes with IC_{50} values of 2.32 ± 0.0 μg mL^{-1} and 7.90 ± 1.00 μg/mL for *α*-amylase and 1.83 ± 0.03 μg mL^{-1} and 21.91 ± 3.93 *α*-glucosidase respectively. Since these plants are endemic and rare in Malaysia, it increased our interest to further study the

key sources that contributes to the biological activity. The study included isolation, purification and characterization of bioactive compounds from both plants. The *in-silico* study will help to validate the *in vitro* activity. Hence these bioactive compounds will lead to the development of potential anti-hyperglycemic agents.

1.3 Research Objectives

The objectives of this study are as follows:

1) To isolate the chemical compounds from *E. kingiana* and *B. lumutensis* extracts using various chromatographic techniques.
2) To characterize the isolated compounds from *E. kingiana* and *B. lumutensis* using various spectroscopic methods.
3) To evaluate the *in vitro* α-amylase and α-glucosidase inhibitory activity of the selected compounds from *E. kingiana*.
4) To conduct molecular docking for the active compounds on α-amylase and α-glucosidase.

CHAPTER 2

LITERATURE REVIEW

2.1 Lauraceae Family

2.1.1 Botany and Distribution

Lauraceae family consist of 68 genera and about 2978 species all over the world, mainly in most tropical regions such as Southeast Asia and tropical America (*Lauraceae — The Plant List*, 2013). The Lauraceae family which falls in the order of Laurales consists of a major group of flowering plants (Angiosperms). In Malaysia, the family of Lauraceae or Laurel family is commonly known as *Medang* or *Tejur* (Taib *et al.*, 2015). There are about 16 genera and 213 species, subspecies and varieties Lauraceae family in Malaysia (Corner, 1988).

The species in Lauraceae family can be found in a wide range of habitats, from upper-canopy species to under-canopy species (Hara *et al.*, 2003). The growth of Lauraceae plants depends on its environment which are lowland or highland. For lowland, most of the species are normally small tress of the lower canopy except for a new species which may reach up to 30 meters tall. In addition, for highland, the members of Lauraceae in the tropical montane zone of Southeast Asia becomes more abundant which normally reaching the top layer of forest canopy. This vegetation type is known as oak-laurel forest, and it is found in tropical Asia's mountains from the Himalayas to New Guinea, and it is closely connected to East Asia's temperate evergreen oak forests (Sri-Ngernyuang *et al.*, 2003)

Identification of genus and species of Lauraceae members remains difficult for some reasons. Some genera are poorly defined. The recognition required both flowers and fruits (Van der Werff, 2001). Unfortunately, the fruits of Lauraceae need a few

months before reaching their maturity. Therefore, the flowers and fruits are rarely present together in herbarium specimens. Somehow the members of the Lauraceae family were being identified by using the details of the stamen. But still, it is hard to distinguish (Van der Werff, 2001).

2.1.2 Classification of Tribes

Lauraceae family can be illustrated as below. The classification included 68 genera which mainly found in Latin America and Southeast Asia (*Lauraceae — The Plant List*, 2013).

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Order : Laurales

Family : Lauraceae

Genus :

Actinodaphne	*Aiouea*	*Alseodaphne*	*Aniba*
Apollonias	*Aspidostemon*	***Beilschmiedia***	*Camphora*
Caryodaphnopsis	*Cassytha*	*Chlorocardium*	*Cinnadenia*
Cinnamomum	*Cryptocarya*	*Dehaasia*	*Dicypellium*
Dodecadenia	***Endiandra***	*Endlicheria*	*Eusideroxylon*
Gamanthera	*Hufelandia*	*Hypodaphnis*	*Iteadaphne*
Kubitzkia	*Laurus*	*Licaria*	*Lindera*
Litsea	*Machilus*	*Malapoenna*	*Mespilodaphne*

Mezilaurus	*Misanteca*	*Mocinnodaphne*	*Mutisiopersea*
Nectandra	*Neocinanamomum*	*Neolitsea*	*Notaphoebe*
Nothaphoebe	*Ocotea*	*Oreodaphne*	*Paraia*
Parasassafras	*Parthenoxylon*	*Persea*	*Phoebe*
Phyllostemonodaphne	*Pleurothyrium*	*Polyadenia*	*Potameia*
Potoxylon	*Povedadaphne*	*Ravensara*	*Rhodostemonodaphne*
Sassafras	*Schauera*	*Sextonia*	*Sinopora*
Sinosassafras	*Syndiclis*	*Systemonodaphne*	*Tetranthera*
Umbellularia	*Urbanodendron*	*Williamodendron*	*Yasunia*

2.1.3 Medicinal Uses

In Malaysia, Lauraceae family are economically important and useful in our daily live as source of medicine, spices, perfumes, timber, nutritious fruits and others. The leaf, bark, stems and roots of members of Laurel family were reported to have remedy properties and heal wide range of ailments. The bark of some species has commercial value such as cinnamon (*Cinnamomum verum, Cinnamomum cassia*) and massoy (*Cryptocarya massoy*) (Salleh *et al.*, 2016). They are used for plywood production and decorative projects including interior designing, finishing, panelling, making furniture and cabinet.

According to Salleh *et al.* (2016) there are four genera in Lauraceae family which have been studied for their essential oil which are *Lindera, Beilschmiedia, Litsea* and *Cinnamomum*. It has been reported that the major components as shown in Figure 2.1 are mainly safrole (**9**), eugenol (**10**), linalool (**11**), camphor (**12**) benzyl benzoate

(**13**) or cinnamaldehyde (**14**) (Salleh *et al.*, 2016). The major aromatic product such as camphor (**12**) can be obtained from genus *Cinnamomum* for example *Cinnamomum camphora*, *Cinnamomum glanduliferum* and *Cinnamomum parthenoxylon* (Singh, Sharma and Sharma, 2015). Both aromatic products and essential oils are useful in medicine and making perfume.

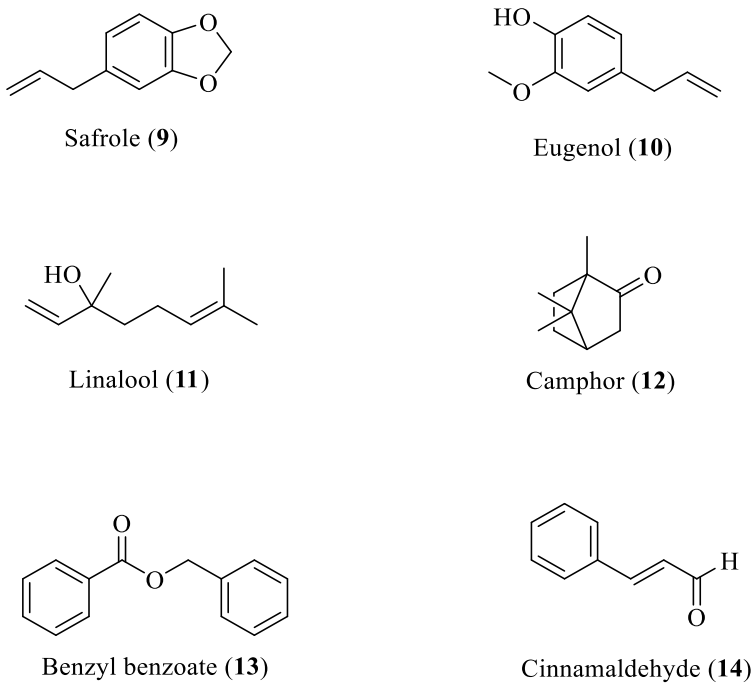


Figure 2.1 Example of major components; safrole (**9**), eugenol (**10**), linalool (**11**), camphor (**12**) benzyl benzoate (**13**) and cinnamaldehyde (**14**).

The woods of species from Laurel family are good quality material for plywood manufacture, furniture, construction and cabinet making. Avocado (*Persea americana*) which are species indigenous to tropical America is essential for its fruits. In addition, *Cinnamomum iners* has been widely planted as a shade tree in Malaysia. It also well known for their function to relieve headache, appetite and breathing problems (Wahab & Hussain, 2018). Traditional Chinese remedies include the bark of *Cinnamomum cassia* and the roots of *Lindera aggregatea* (Li *et al.*, 2008).

Based on the ethnopharmacological history of *Endiandra* and *Beilschmiedia*, it shows that some of the species from both genera have been widely utilised in remedy to cure some diseases such as tuberculosis, malaria, bacterial infections and tumours (Salleh *et al.*, 2015). The *in vivo* and *in vitro* assays revealed that *Endiandra* and *Beilschmiedia* plant extracts possess good results for those biological activities. The chemical compounds present are the key player for these properties.

2.2 Genus Endiandra

2.2.1 Botany and Distribution

Genus *Endiandra* is part of Laurel family and a major group of *Angiosperm*. According to The Plant List (2013), *Endiandra* comprises of more than 125 species distributed in tropical parts of Southeast Asia, Western Pacific Ocean and Australia (*Endiandra— The Plant List*, 2013). According to Ng and Burkill, there are ten species in genus *Endiandra* that can be found in Malaysia which are *E. holttumii, E. kingiana, E. macrophylla, E. maingayi, E. praeclara, E. rubescens, E. wrayi, E. sp.1* and *E. sp. 2* (Burkill, 1966; Ng & Whitmore, 1989).

2.2.2 Morphology

Plants in *Endiandra* genus are mostly medium to large sized evergreen trees. *Endiandra's* leaves are spirally arranged, alternating, petiolate, rarely subopposite, penninerved and rarely triplinerved (Arifiani, 2001). Inflorescences in the axils of foliage leaves or bracts and paniculate with seldom racemose (Arifiani, 2001). Flowers are bisexual, commonly 3-merous and pedicellate. *Endiandra's* ovary was superior, sessile, short and stigma inconspicuous while *Endiandra's* fruits were berries ellipsoid, cupule absent tepal cacducous and absent at the base of the fruits (Arifiani, 2001). Plants of the genus *Endiandra* have six tepals in two whorls of three or two, and four tepals in rare cases. Anthers were bilocellate, rarely unilocllate, and occasionally fused to form a disc, and sometimes absent in *Endiandra's*. In the case of staminodes, it is usually three, although it may sometimes be missing (Arifiani, 2001).

2.2.3 Medicinal Uses

Economically, plants in *Endiandra* are prominent source of woods. For example, in Australia *Endiandra* produce woods that has been widely used by local people (Salleh *et al.*, 2015). They recognized *Endiandra* as source of walnut such as rose walnut (*E. cowleyana*), Brown Walnut (*E. glauca*), Pink Walnut (*E. sieberi*) and Queensland Walnut (*E. palmerstonii*). Queensland Walnut yielded the best quality of wood among species listed above. Therefore, this species gives high quality in furniture production. In addition, others produce large logs and has been used for panelling, wood flooring and furniture (Hyland, 1989; Van der Werff & Richter, 1996).

2.2.4 *Endiandra kingiana*

Endiandra kingiana (*E. kingiana*) (Figure 2.2) was described by Gamble, a British physician and botanist on a collection from Perak, Peninsular Malaysia (*Endiandra kingiana* Gamble, 1910.). It is a sub-canopy tree that can grow up to 29 m tall and 54 cm diameter at breast height. It is mostly found in Southeast Asia specifically in Peninsular Malaysia, Borneo (Sarawak, Brunei, East-Kalimantan) and Celebes, Indonesia. It vegetates in undisturbed mixed dipterocarp forests up to 100 m altitude and on ridges. *E. kingiana* preferred to grow on sandy soils however clay and limestone may occasionally be found (*Endiandra kingiana* Gamble, 1910).

Bark of *E. kingiana* occasionally smooth, partly peeling off irregularly with grey in outer part and white in inner bark. In term of fruits, *E. kingiana* have brown, ellipsoid and base obtuse. Inflorescences in the axils of leaves, paniculate, short, condensed, ranging about 1 cm to 6 cm long and has brown indument. The flowers are white in colour, 2.5 mm to 4.0 mm diameter at anthesis. The leaves of *E. kingiana* are alternate, seldom subopposite, spirally arranged, chartaceous, lamina elliptic to broadly elliptic and base cuneate to obtuse flat at junction with petiole (Arifiani, 2001).

Figure 2.2 *E. kingiana*.

(Herbarium of the Department of Chemistry, University of Malaya, Kuala Lumpur)

2.3 Genus Beilschmiedia

2.3.1 Botany and Distribution

Beilschmiedia Nees was first described by Nees von Esenbeck in Wallich in 1831. In 1793 to 1848, it is named as *Beilschmiedia* by Karl Traugott which is a chemist and botanist who wrote a lot about plant geography (De Kok, 2016). *Beilschmiedia* is one of the largest pantropical genera in Lauraceae family which about 287 species being recognised mainly in Southeast Asia and Africa. According to Plant List (*Beilschmiedia— The Plant List*, 2013), 287 names of this genus are accepted and the remaining are either synonyms or unresolved names.

Plants in *Beilschmiedia* species mostly grow in tropical climates but still a few of them are native to the temperature regions. They are widely spread in tropical Asia, Africa, Australia, New Zealand, Central America, Caribbean, and South America (Nishida, 1999). In Southeast Asia, *Beilschmiedia* can normally be found in Vietnam, Myanmar, Thailand, Cambodia, Indonesia, Philippines, Malaysia and various island such as Sumatra and Java (Burkill, 1966).

2.3.2 Morphology

The genus *Beilschmiedia* mostly shrub, are trees which are up to 25 m to 35 m tall. According to Nishida, *Beilschmiedia* species show two different phyllotactic arrangement which one with alternate leaves and one with opposite leaves (Nishida, 1999). Spirals are very rare for this genus. Species such as *B. anay* and

B. manantlanensis prone to have crowded leaves at the branch apices. Shape of the leaves vary from one species to another. It ranges from ovate to obovate.

Beilschmiedia species mostly have axillary and paniculate inflorescences. They can appear to be subterminal because of some are situated in the axils of the leaves at the ends of the twigs. As defined by Van der Werff, *Beilschmiedia* species mostly have type three inflorescences which are paniculate-cymose and repeatedly branced with the lateral flowers of the ultimately cymes not strictly opposite (Van der Werff, 2001). According to Tetsana, a few species such as *B. glabra, B. membranacea, B. penangiana* and *B. wallichiana* have inflorescence that enclosed by large orbicular bracts at the base (Tetsana, 2005).

Flowers of *Beilschmiedia* species are said to be bisexual, small and almost suspherical except for *B. linharensis.* This species has depressed globose fruits. Erect, almost equal, six and usually ovate to elliptic are a few characteristics for tepal in *Beilschmiedia* species (Nishida, 1999). For the stamens, *Beilschmiedia* species usually have six to nine fertile stamens indicating the outer two or three whorls.

The size of fruits which is one of the vital characteristics in species identification for *Beilschmiedia* species are vary ranging from massive (50 – 88 mm long) to small (1.9 to 4.5 mm long) (De Kok, 2016). The fruit stalk such as in *B. glambra* can swell up to diameter at the range of 7.6 mm to 10 mm, differ to *B. palembanica* which does not swell at all. According to Nishida, some species have small constriction of the fruit stalk. The fruits are often ellipsoid and lack of cupules (Nishida, 1999).

2.3.3 Medicinal Uses

According to Iwu, the fruits of *B. manni, B. gabonensis* and *B. zenkeri* from *Beilschmiedia* species, function as appetite stimulants and as spices (Iwu, 1993). In addition, since *Beilschmiedia* species are rich in source of pharmacologically active chemical constituents, so they have been widely used in medicinal field. The plants listed below are the previous studies for *Beilschmiedia* species with various plants parts which function as medicine to treat some disease.

Table 2.1 Medicinal uses of several *Beilschmiedia* species.

Species (Plants parts)	Medicinal uses	References
B. anacardioides (bark)	Cure uterine tumours, rubella, rheumatisms, female genital infections	Chouna *et al.*, 2009
B. acuta (leaf)	Cancer and gastrointestinal infections	Kuete *et al.*, 2015
B. cryptocaryoides (fruits/ bark)	Treatment for infectious diseases and malaria	Talontsi *et al.*, 2013
B. gaboonesis (bark)	Analgesic and healing ointments	Iwu, 1993
B. lancilimba (bark)	Cure skin bacterial infections	Efouet & Pépin, 2012
B. madang (wood)	Decoction as an anti-malarial preparation	Kitagawa *et al.*, 1993
B. manni (fruits)	Treatment for dysentery and headache	Iwu, 1993
B. sphaerocarpa (bark)	Herbs to cure skin disease such as scabies, acne, pustule	Perry & Metzger, 1980
B. pahangensis (bark)	As drink after childbirth, assuage stomachache, diarrhea and dysentery	Wiart, 2006
B. obscura (bark)	Treatment for gastrointestinal infections	Fankam *et al.*, 2014

2.3.4 *Beilschmiedia lumutensis*

Beilschmiedia lumutensis (B. lumutensis) is endemic to Peninsular Malaysia and Cambodia. The trees or shrub are 3 to 15 m tall. *B. lumutensis* vegetate in lowland and hill forests at 150 m to 200 m altitude and sometimes on sandstone or near streams.

The bark of *B. lumutensis* are smooth and light or greenish grey to yellowish brown in colour. *B. lumutensis's* fruits are ellipsoid 18 to 30 by 8.5 to 15 mm, apex rounded, glabrous and have smooth surface (De Kok, 2016). It has open and lax inflorescence which correspond to type 2. This is the first species that have unequivocal record of type 2 inflorescence (De Kok, 2016). In terms of flowers, *B. lumutensis* are glabrous, perianth lobes elliptic to orbicular with pale yellowish green. *B. lumutensis* have subopposite, blades elliptic to oblong, shiny when dried, glabrous at the upper surface and thinly leathery (De Kok, 2016).

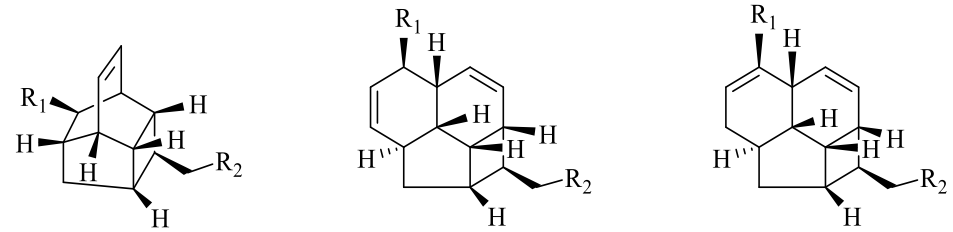
Figure 2.3 *B. lumutensis.*

(Herbarium of the Department of Chemistry, University of Malaya, Kuala Lumpur)

2.4 Chemical Constituents

2.4.1 Endiandric Acids

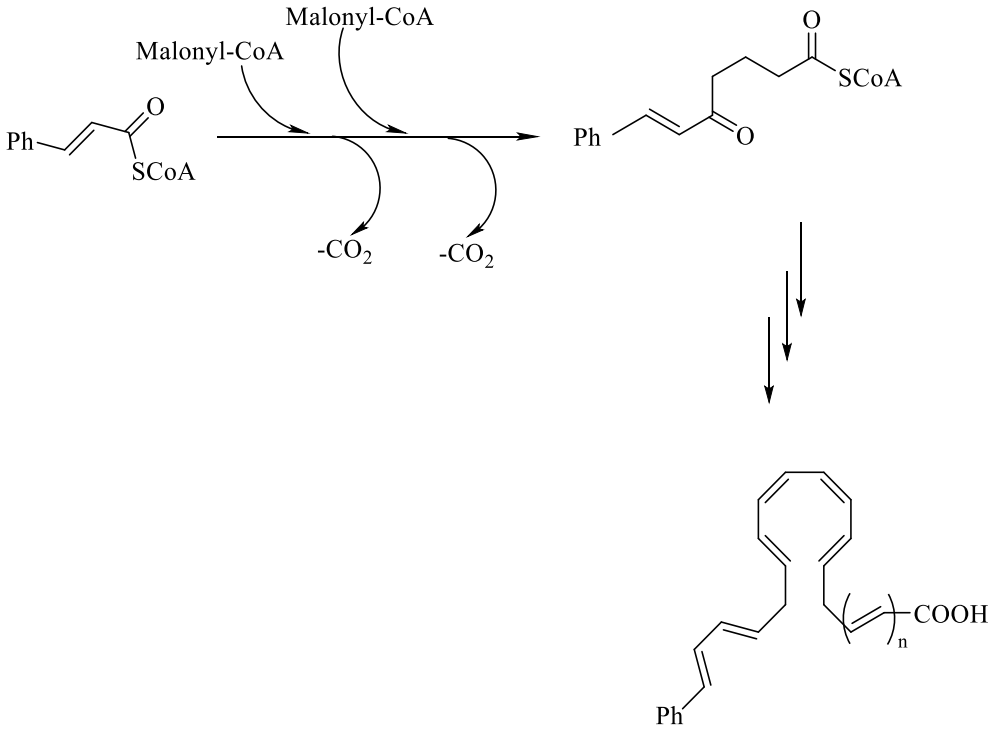
Endiandric acids are formed exclusively by the *Beilschmiedia* and *Endiandra* species which have a distinctive tetracyclic carbon skeleton. Type A, type B, and type B′ (Figure 2.4) are three primary skeletal groups of these cyclic polyketides, which have eight chiral centres and normally isolated as a racemic mixture [αD] = 0°. In general, this type of compounds is containing with two cyclohexanes, one cyclopentane, and one cyclobutane ring, and commonly substituted with a phenyl ring and a carboxylic acid chain.



Endiandric acid type A Endiandric acid type B Endiandric acid type B'

Figure 2.4 Endiandric acids main skeleton.

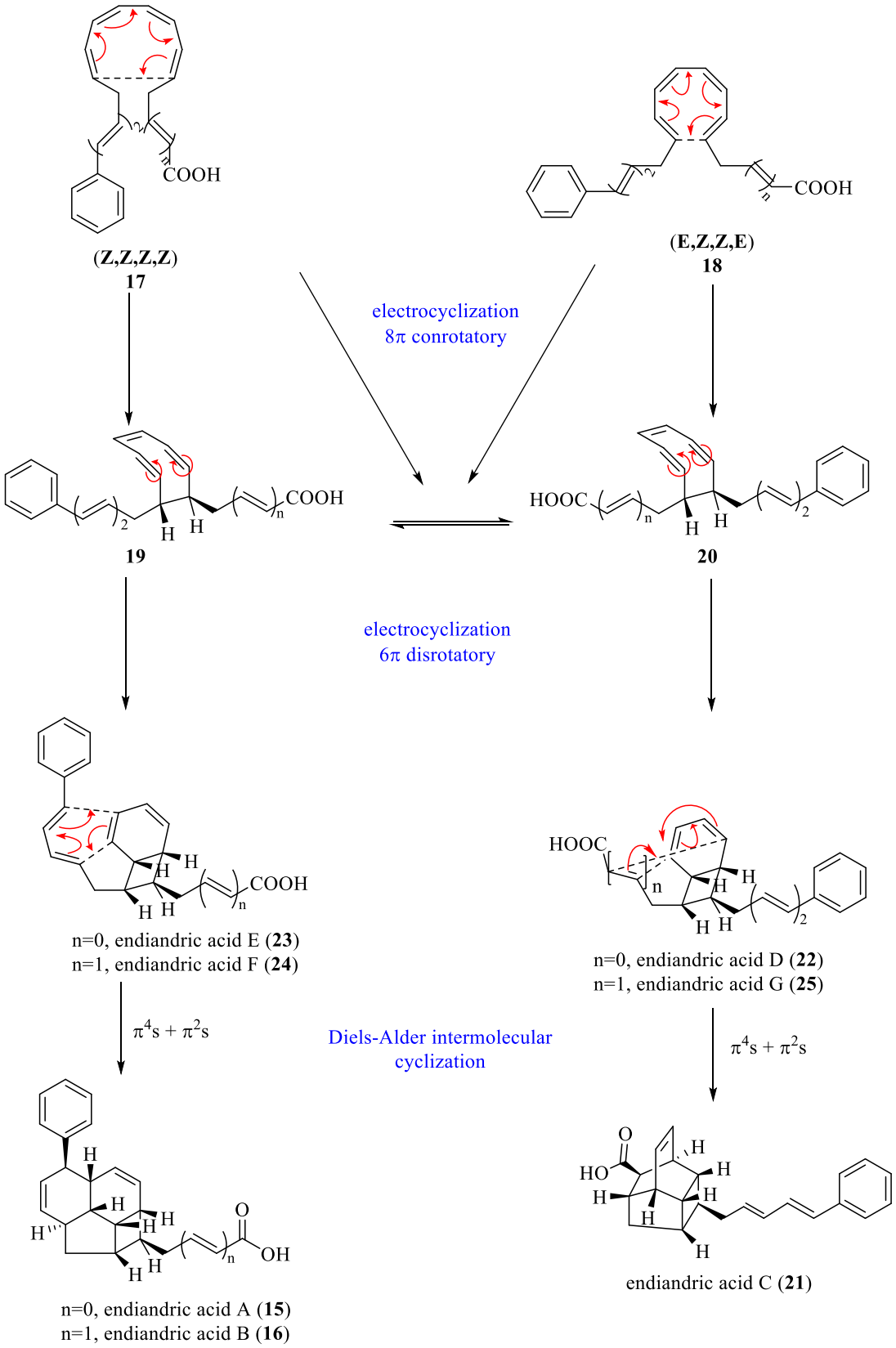
Endiandric acids which are polycyclic compounds, generally possess eight asymmetric centres. It occurs as a racemic mixture rather than enantiomeric form. This is a rather unusual observation for naturally occurring compounds resulting from both shikimate and acetate pathways (Lenta *et al.*, 2015). This observation led Black *et al.* to propose a hypothetical "biogenesis" pathway for these compounds from achiral precursors by a series of non-enzymatic electrocyclization (Bandaranayake, Banfield & Black, 1980; Banfield *et al.*, 1982, 1983). Black's hypothesis suggests a cascade of reactions. Scheme 2.1 shows the general biosynthesis of polyketides.



Scheme 2.1 General biosynthesis of polyketides (Lenta *et al.*, 2015).

Based on this biomimetic hypothesis, Nicolaou and his team reported a total synthesis of endiandric acid A and its analogues (Nicolaou *et al.*, 1982; Nicolaou and Petasis, 1984). It is specifically proposed that these polycyclics are formed from phenyl polyenes, which contain a central conjugated tetraene unit. For the formation of endiandric acid type B such as endiandric acid A (**15**) and endiandric acid B (**16**), which are all-*cis*-isomers **17**, or the *trans*-, *cis*-, *cis*-, *trans*-isomer **18**, the polyenes have undergone two continuous non-enzymatic electrocyclization reactions which are 8π conrotatory and 6π disrotatory, to form intermediate precursors of endiandric acids E (**23)** and F (**24)**. The intermediates then underwent an intramolecular π^4s + π^2s cycloaddition (known as intramolecular Diels-Alder cyclization) which led to the *α*, *β*–unsaturated acids of endiandric acid A (**15**) and endiandric acid B (**16**).

Meanwhile, the polyenes **17** and **18** could act as precursors of cyclo-octatriene **20**, which is a ring-invertomer of **19**. The conformer **20** should then undergo the same electrocyclization process to afford endiandric acid D (**22**) and G (**25**), which on intramolecular Diels-Alder cyclization would yield endiandric acid C (**21**), i.e., the cage-like structure (endiandric acid type A) with a free phenyl butadiene unit. In conclusion, the example of biosynthesis for both endiandric acid type A and type B is basically through conrotatory 8π electron cyclization, disrotatory 6π electron cyclization and Diels-Alder intramolecular cyclization. These conversions are shown in Scheme 2.2.



Scheme 2.2 Biosynthesis of endiandric acids A (**15**), B (**16**) and C (**17**).

2.4.2 Kingianins

A pentacyclic carbon skeleton (bicyclo[4.2.0] backbone) (Figure 2.5) was the common key features for kingianins. In spectroscopic data of kingianins, there were sixteen skeletal signals involving twelve methines and four *cis* double bonds representing the backbone. As a rule, the structure of kingianins were divided into two fragments; western part comprising of H-1, H-2, H-3, H-4, H-5, H-6, H-7 and H-8 and eastern part made up of H-1', H-2', H-3', H-4', H-5', H-6', H-7' and H-8'. The position of four substituents bonded at C-1, C-8, C-1' and C-8' were the characteristic to distinguish kingianin series from one another. The substituents encomposed of two methylenedioxyphenyl groups with a singlet signal around δ_H 5.89. Other substituents might be *N*-ethylacetamide (**27**) , butyric acid (**28**) and acetic acid (**29**) chain (Leverrier *et al.*, 2010).

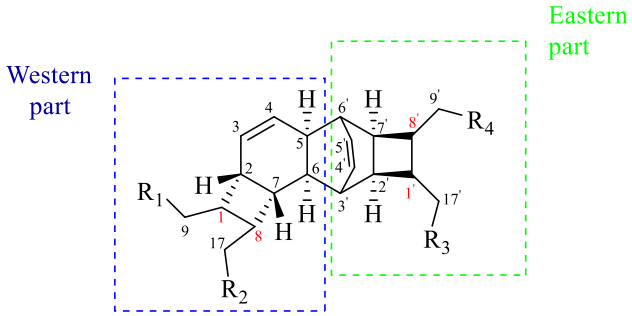
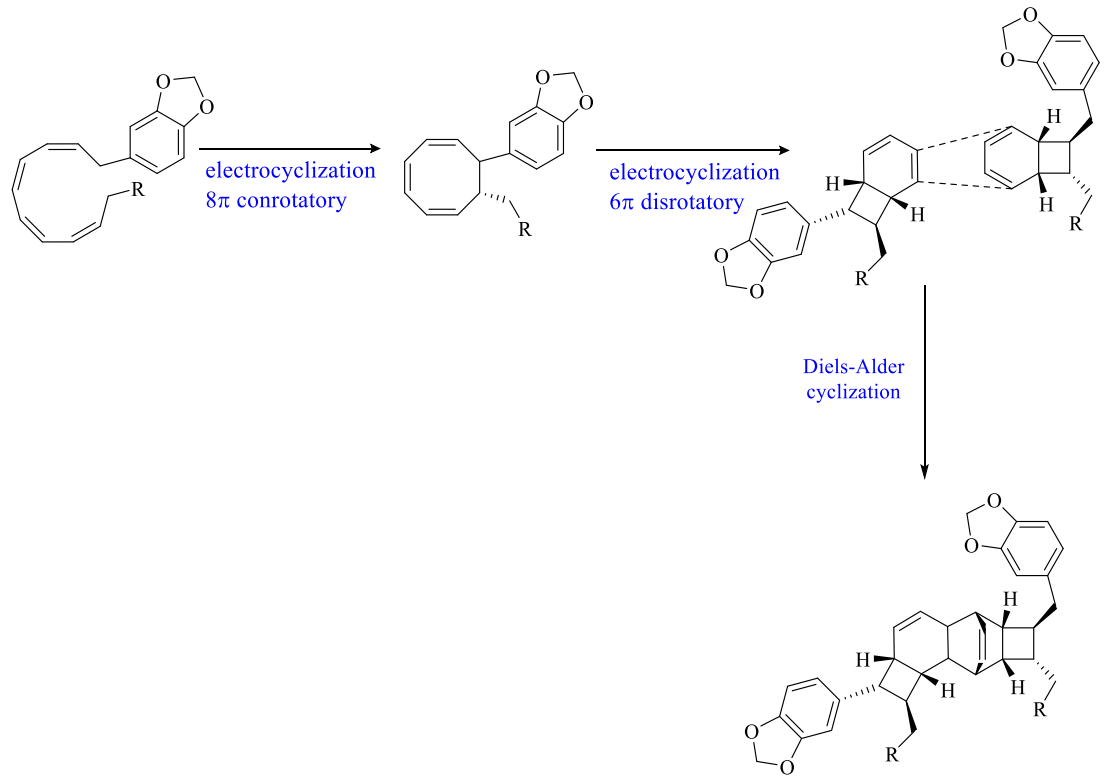


Figure 2.5 Kingianin pentacyclic skeleton.

Apart from that, kingianins are distinctive, complicated and stereochemically rich pentacyclic core frameworks which are specifically extracted from *Endiandra kingiana* bark. Scheme 2.3 shows the general biosynthesis of kingianin derivatives beginning from arylpolyene which undergoes a conrotatory 8π electrocyclization and disrotatory 6π electrocyclization to form cyclooctatriene (*E,Z,Z,E*-, or *Z,Z,Z,Z*-tetraene). Then, Diels-Alder cyclization follows, forming a complex and unique pentacyclic derivative.



Scheme 2.3 General biosynthesis of kingianin derivatives.