CYTOTOXIC ACTIVITY OF ISOLATED ALKALOIDS FROM THE BARK OF *KOPSIA TERENGGANENSIS* (APOCYNACEAE) AND CORROSION INHIBITION STUDIES OF ITS EXTRACT

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by

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LIST OF SYMBOLS

%	Percent
°C	Degree Celcius
µM	Micromolar
M	Molar
^{13}C	Carbon NMR
^{1}H	Hydrogen NMR
br	Broad
C	Carbon
cm	Centimeter
cm^{-1}	Per centimeter
δ_C	Chemical shift carbon
δ_H	Chemical shift hydrogen
g	Gram
h	Hour
H	Hydrogen
Hz	Hertz
J	Coupling constant
kg	Kilogram
m	Meter
m/z	Mass to charge ratio
mg	Milligram
MHz	Megahertz
min	Minute
mL	Milliliter
mm	Millimeter
nm	Nanometer
O	Oxygen
ppm	Parts per million
R	Substituent group
α	Alpha
β	Beta
λ	Lambda

π	Pi
mpy	Milli per year
η	Inhibition efficiency
Fe	Iron
Fe^{2+}	Ferrous
Fe^{3+}	Ferric
eV	Electron volt
mV	Milli volt
R_{ct}	Charge transfer resistance
R_s	Solution resistance
E_{corr}	Corrosion potential
i_{corr}	Corrosion current density
CPE	Constant phase element
β_a	Anodic Tafel constant
β_c	Cathodic Tafel constant
Ω cm^2	Ohm's centimeter square
MTT	3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide
IC_{50}	Concentration required to inhibit 50% of activity
R_f	Retention factor

LIST OF ABBREVIATIONS

1D-NMR	One-dimensional nuclear magnetic resonance
2D-NMR	Two-dimensional nuclear magnetic resonance
AC	Alternating current
ASTM	American Society for Testing and Materials
CC	Column chromatography
$CDCl_3$	Deuterated chloroform
CE	Counter electrode
CH_2Cl_2/ DCM	Dichloromethane
COSY	^1H-^1H correlation spectroscopy
d	Doublet
dd	Doublet of doublets
DEPT	Distortionless enhancement by polarization transfer
dt	Doublet of triplets
EDX	Energy dispersive X-ray
EIS	Electrochemical impedance spectroscopy
FTIR	Fourier transformer infrared
HCl	Hydrochloric acid
HMBC	Heteronuclear multiple bond coherence
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear single quantum coherence
IE	Inhibition efficiency
LC-TOF-MS	Liquid chromatography time-of-flight mass spectrometer
m	Multiplet
MeOD	Deuterated methanol
MeOH	Methanol
MS	Mild steel
Na_2SO_4	Sodium sulphate
NaCl	Sodium chloride
NH_3	Ammonia
NH_4OH	Ammonium hydroxide
NMR	Nuclear magnetic resonance

OCP	Open circuit potential
OH	Hydroxy group
PD	Potentiodynamic polarization
PRC	Preparative radial chromatography
RE	Reference electrode
s	Singlet
SEM	Scanning electron microscopy
t	Triplet
td	Triplet of doublets
TLC	Thin layer chromatography
TMS	Tetramethylsilane
UV	Ultraviolet
WE	Working electrode

AKTIVITI SITOTOKSIK ALKALOID YANG DIPENCILKAN DARIPADA KULIT KAYU *KOPSIA TERENGGANENSIS* (APOCYNACEAE) DAN KAJIAN PERENCAT KARAT DARIPADA EKSTRAKNYA

ABSTRAK

Penggunaan bahan semula jadi dalam pembuatan agen kemoterapi telah menunjukkan impak positif dalam bidang onkologi. Selain itu, bahan semula jadi juga memberikan keputusan signifikan sebagai perencat karat. Justeru, penyelidikan ini bertujuan mengkaji aktiviti sitotoksik dan perencat kakisan bahan mentah dan alkaloid yang dipencilkan daripada *Kopsia terengganensis* (*K. terengganensis*). Kaedah ujikaji melibatkan pengekstrakan dan pengasingan alkaloid menggunakan teknik kromatografi, penentuan struktur kimia melalui teknik spekstroskopi dan penilaian aktiviti sitotostik menggunakan sel HT-29 kolorektal adenokarsinoma. Manakala, kajian perencat karat melibatkan eksperimen penyusutan berat, kajian elektrokimia dan analisis permukaan. Proses pengekstrakan dan pemencilan menghasilkan tujuh sebatian alkaloid: eburnamine (**90**), isoeburnamine (**58**), eburnaminol (**99**), larutensine (**100**), eburnamenine (**114**), eburnamonine (**56**) dan quebrachamin (**148**). Dalam aktiviti sitotoksik, hanya sebatian **99** yang mampu merencat pertumbuhan sel kanser dengan aktiviti moderat (IC_{50} 75.8± 3.06 µM). Dalam kajian perencatan karat, ekstrak alkaloid menunjukkan keputusan signifikan (80-90% kecekapan perencatan) dalam kedua-dua kajian penyusutan berat dan electrokimia. Perencat karat tersebut mengikuti model penjerapan isoterma Langmuir dan pembentukan lapisan perencat diatas permukaan keluli lembut dibuktikan melalui analisis permukaan. Sebagai kesimpulan, alkaloid daripada *K. terengganensis* wajar diberi perhatian dalam kajian kanser dan

perencat kakisan disebabkan keputusan positif yang ditunjukkan dalam ujian sitotoksik terhadap sel kanser dan pengurangan proses pengkaratan keluli lembut.

CYTOTOXIC ACTIVITY OF ISOLATED ALKALOIDS FROM THE BARK OF *KOPSIA TERENGGANENSIS* (APOCYNACEAE) AND CORROSION INHIBITION STUDIES OF ITS EXTRACT

ABSTRACT

The use of natural products in developing chemotherapy agents has shown a positive impact in the oncology field. Besides, they also showed a significant result as a corrosion inhibitor. Hence, this study aims to evaluate the cytotoxic activity and corrosion inhibition potential of the crude extract and isolated alkaloids from *Kopsia terengganensis* (*K. terengganensis*). The experimental involved extraction and isolation of alkaloids using different chromatographic methods, structural elucidation using various chromatographic techniques and evaluation of cytotoxic activity on HT-29 colorectal adenocarcinoma. Meanwhile, corrosion inhibition studies included weight loss studies, electrochemical studies, and surface analysis. The extraction and isolation process resulted in seven alkaloid compounds: eburnamine (**90**), isoeburnamine (**58**), eburnaminol (**99**), larutensine (**100**), eburnamenine (**114**), eburnamonine (**56**) and quebrachamine (**148**). In the cytotoxic activity, only compound **99** was able to suppress the growth of HT-29 with moderate activity (IC_{50} =75.8± 3.06 µM). For the corrosion inhibition potential, the alkaloid crude showed significant results (80-90% inhibition efficiency) in both weight loss and electrochemical studies. The inhibitor followed the Langmuir adsorption isotherm and the formation of an inhibitive film on the mild steel surface was proved in the surface analysis. In conclusion, the alkaloids from *K. terengganensis* were noteworthy to be investigated in both cancer and corrosion inhibition studies as it gives a positive result in the toxicity towards cancer cells and also mitigated the mild steel corrosion.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Malaysia, located at the heart of Southeast Asia, is a tropical country blessed with a vast array of an ecosystem. Geographically, Malaysia lies in the entirely equatorial zone which allows them to receive a high amount of rainfall, which is over 2200 mm per year, favourably for the growth of more than 15,000 species of flora (Mohamad, 1994). Malaysia forest, other than being a living habitat for various species of fauna, also popular among researchers for the growth of many types of medicinal plants (Chan *et al*., 2016a; Harun *et al*., 2015; Chan *et al*., 2016b).

Bioactive compounds or known as secondary metabolites in plants are one of the prominent studies in natural product chemistry. Secondary metabolites did not appear to play a direct function in the growth and development of the organism, in contrast to primary metabolites, which are crucial for performing metabolic roles in plants. However, the existence of secondary metabolites attracted organic chemists to investigate their chemical properties ever since the 1850s. Based on their biosynthetic pathway, these secondary metabolites are divided into three main classes (Sanchez & Demain, 2011). The first group is terpenoids which were derived from isopentyl diphosphate (IPP) and synthesized through condensation of isoprene units (C_5) (Mahmoud *et al*., 2002). For example, menthol (**1**) is a monoterpene (C_{10}) that is formed by two isoprene units (Sanchez & Demain, 2011). The second group is alkaloids which have one or more nitrogen atoms in the molecule. With a few exceptions, it is generated from amino acids, and the majority of the compounds have been reported to have active pharmacology (Croteau *et al.*, 2000). For example, ajmalicine (**2**) isolated from *Catharanthus roseus* was derived from the acid amino

tryptamine and monoterpenoid secologanin (Croteau *et al.*, 2000). The last group is phenolic compounds which were formed either by the shikimic pathway or the malonate/ acetate pathway (van der Heijden *et al*., 2004). A simple phenolic compound such as rosmarinic acid (**3**) can be found in the sage extract, a type of aromatic plant.

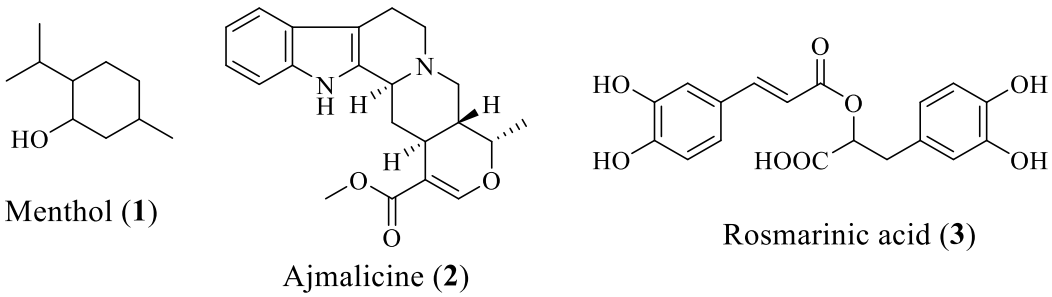


Figure 1.1 Example of secondary metabolites according to their classification: terpenoid (**1**), an alkaloid (**2**), and phenolic acid (**3**).

These chemical compounds are responsible for the desired pharmacological actions. For example, compound **2** was introduced in 1957 and has been marketed as a pharmaceutical to treat hypertension whereas compound **3** was reported among the most active antioxidant that has been extracted from butanol fractions of sage extract (van der Heijden *et al*., 2004; Wang *et al*., 1998). Progress in technology specifically before the post-genomic era showed the use of natural products has derived more than 80% of drugs directly or indirectly (Sneader, 1997). A review by Butler (2008) showed that the use of natural products is still a viable source since 1994 in clinical development, especially in oncology.

Cancer incidence has been rising over time, and efforts to lessen the burden of this illness have been continuous for decades. According to an estimation by World Health Organization (WHO) in 2019, cancer is the second leading cause of death before the age of 70 years in 112 countries, including Malaysia (Sung *et al*., 2021). In the Malaysia National Cancer Registry (MNCR) Report 2011-2016 by National

Cancer Registry (2019), colorectal cancer was ranked second after breast cancer as the most common cancer in the population of Malaysia (Figure 1.2). Colon cancer is regarded as a primary cause of mortality and the main barrier to improving life expectancy in this 21st century. Modern lifestyle and changes in diet i.e., excessive intake of animal-source products and practising a sedentary life are believed to be factors that led to colon cancer (Kuppusamy *et al*., 2014). According to GLOBOCAN 2020, it is estimated that more than 1.9 million (6.0%) out of 19.3 million new cases and 0.935 million (5.8%) out of 10.0 million new deaths were reported in 2020 for colon cancer. This brought to the statistics for every 10 new cases, one case will be represented by colon cancer (Sung *et al*., 2021).

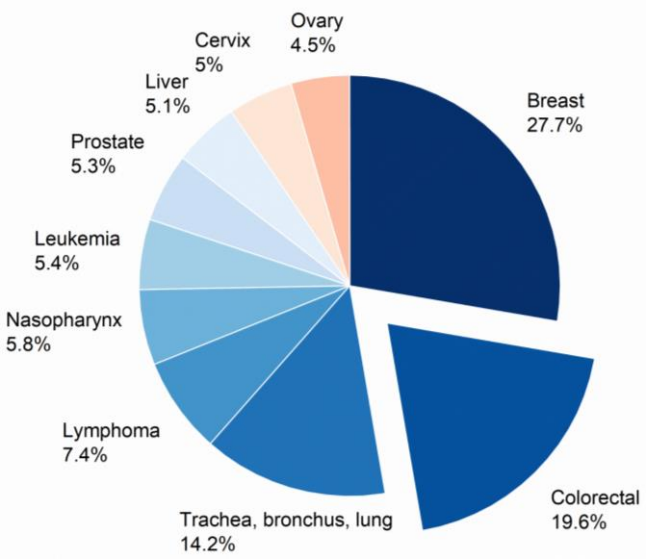


Figure 1.2 Top ten common cancers reported in Malaysia 2011-2016 (National Cancer Registry, 2019)

Another field that involves secondary metabolites in plants is the corrosion study (Raja & Sethuraman, 2008). Corrosion could be defined as a natural process through which metals and alloys attempt to return to their more stable thermodynamic forms in response to their surrounding environment (Shehata *et al*., 2018). When the steel encounters a corrosive environment, electrons in the steel will flow away from

the contacted surface causing the iron to oxidize (Goni & Mazumder, 2019). As a result, the steel becomes brittle and over time it causes the structure to break down. A common environment that experiences such detrimental situations is oilfields specially located in seawater where a high concentration of chloride ions was found.

Since crude oil and natural gas are transported mostly by metal pipelines from offshore production sites to refineries and petrochemical plants, the oil and gas industry frequently suffers from these issues. The presence of carbon dioxide (CO_2) as well as chloride in the medium increased the vulnerability of the pipelines towards corrosion (Cen & Chen, 2021). CO_2 can be found naturally in crude oil or natural gas (El-Lateef *et al*., 2012). In other circumstances, CO_2 was injected to enhance oil recovery and fracturing applications (Chen *et al*., 2021). When CO_2 dissolved in the seawater, it produced carbonic acid (H_2CO_3) which lowers the pH value and generated a condition named sweet corrosion (Xhanari *et al*., 2021). Although the pipeline is made up of low-carbon steel which gives strong durability and mechanical strength, after some years of exposure to the aggressive environment, the functionality is decreasing and wears off (Al-janabi, 2020). As a result, the country's economy could suffer greatly from the cost of maintaining the pipelines. The problem also raises concerns about the environment in the event of leakage occurring along the transportation pipeline (Papavinasam, 2013; Xhanari *et al.*, 2021).

Various methods were conducted to reduce the corrosion impact including selecting appropriate materials, using the cathodic protection technique, applying protective coatings, performing adequate corrosion inspection, and using corrosion inhibitors (Popoola *et al.*, 2013). Of all these methods, the common practice in the field is to use corrosion inhibitors. Corrosion inhibitors are chemical substances that when applied to a corrosive environment in a minimal amount, decreased the rate of

metal dissolution (Khanna, 2013). Besides their low cost and ease of application, corrosion inhibitor was found to have flexibility in term of dosage and are compatible with all type of structures (Khanna, 2013). When there is an increase in corrosion activity or wells containing hydrogen sulphide (H_2S) or carbon dioxide (CO_2), a film-forming corrosion inhibitor will be employed either by spraying the outside of the drill pipe or added to the drilling fluid during the drilling process. The commonly used corrosion inhibitors are chromates, phosphates, molybdates, arsenic, and organic (Papavinasam, 2013).

The studied plant, *Kopsia terengganensis* (*K. terengganensis*) comes from the family of Apocynaceae and genus *Kopsia*. The Apocynaceae family can be found throughout the world and most of them are diverse in the tropics. In Malaysia, there are about 34 genera and 110 species distributed mostly in Peninsular Malaysia (Qureshi, 2015). Plants from genus *Kopsia* were popular for producing alkaloids specifically indole alkaloids (Kam, 1999a). On top of that, these alkaloids also possessed various biological activities such as anti-leishmanial (Kam *et al.* 1999c), anti-hypertensive (Mok *et al.* 1998), anti-proliferative (Kam *et al.* 2004) and anti-plasmodial (Khozirah *et al.* 2011). These findings prove that alkaloids from genus *Kopsia* are worth to be studied and can be apply on different field studies.

1.2 Problem statements

The recent chemotherapy treatments for cancer are non-specific, not just focusing on cancer cells but also targeting healthy cells. Consequently, there is a need to develop chemotherapeutic agents that are more potent and selective as well as less harmful to healthy cells. Research on cancer drugs from natural resources has shown a positive result in reducing the chances of colon cancer and decelerating its

progression (Pandey *et al*., 2011). Recently, the uses of natural product-based cancer treatments have been highlighted and are presently under development (Cai *et al*., 2014; Han *et al*., 2017).

The commercial corrosion inhibitors, despite their excellent work in reducing corrosion, were inevitably causing harmful effects on human beings and environment. For example, arsenic usage might release toxic arsine gas as the product of corrosion. Chromates and their derivative, despite being the most effective inhibitor also toxic to marine species (Papavinasam, 2013). Also, developing corrosion inhibitors require high expenditure on chemicals and solvents (Popoola *et al*., 2013). To counter these problems, researchers have been working extensively to develop a green-based corrosion inhibitor. The majority of the inhibitors were derived from naturally occurring materials, such as plant extracts and chemical drugs, which have shown to be excellent corrosion inhibitors and involve a reasonable cost. (Tamalmani & Husin, 2020).

Alkaloids from *K. terengganensis* was first isolated in 1997 by Uzir *et al.* However, to the best of our knowledge, there is limited study on the biological activities and corrosion inhibition potential of alkaloids from *K. terengganensis.* Hence, the present research was conducted to explore the versatility of the alkaloids for these applications. The study included isolation, identification and characterization of alkaloids from *K. terengganensis* and the evaluation of its cytotoxic activity on colon cancer cell as well as the corrosion inhibition potential on mild steel.

1.3 Research objectives

The objectives of this research are listed as follows:

1. To isolate and elucidate alkaloids from *K. terengganensis* using various chromatographic and spectroscopic methods.

2. To evaluate the cytotoxic activity of selected alkaloids from *K. terengganensis* on HT-29 colorectal adenocarcinoma.

3. To investigate the inhibition efficiency of the alkaloid-rich extract against mild steel corrosion in a CO_2-saturated 3.5% sodium chloride (NaCl) environment.

1.4 Scope of research

The study comprises the separation, isolation, elucidation, and characterization of alkaloids from *K. terengganensis* using various chromatographic and spectroscopic methods. The isolated compounds were evaluated for their cytotoxic activity on the HT-29 adenocarcinoma. The research also includes the investigation of crude alkaloids to be proposed as a potential corrosion inhibitor on mild steel corrosion in NaCl medium saturated with CO_2. The corrosion inhibition was examined using the weight loss method, electrochemical measurement, and surface analysis technique.

CHAPTER 2

LITERATURE REVIEW

This chapter discussed the biological and chemical aspects of the genus *Kopsia,* especially for the species *K. terengganensis*. The following subchapters will discuss the botanical aspects, the type of alkaloids, and the biogenesis of selected types of alkaloids. This chapter also included the literature studies on corrosion and corrosion inhibitors.

2.1 Apocynaceae family

2.1.1 Botanical features

The family Apocynaceae which is from angiosperm families comprises about 250 genera and 2000 species of tropical trees, shrubs, woody climbers, herbs, and vines (Wiart, 2006). The plant is known as *pelai penipu paya* among the Malays (Wiart, 2006). This family falls in the order of Contortae and represents five subfamilies which are Rauvolfioideae, Apocynoideae, Asclepiadoideae, Periplocoideae, and Secamonoideae (Endress & Bruyns, 2000). In the subfamily Rauvolfioideae, there are eight genera under the Vinceae tribe as shown in Figure 2.1 (Endress & Bruyns, 2000).

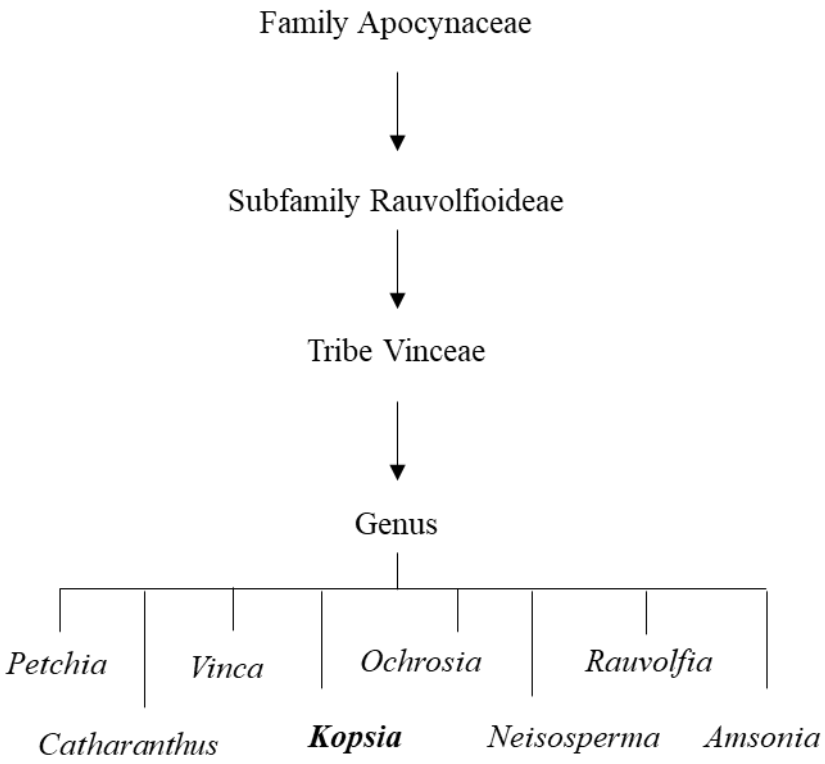


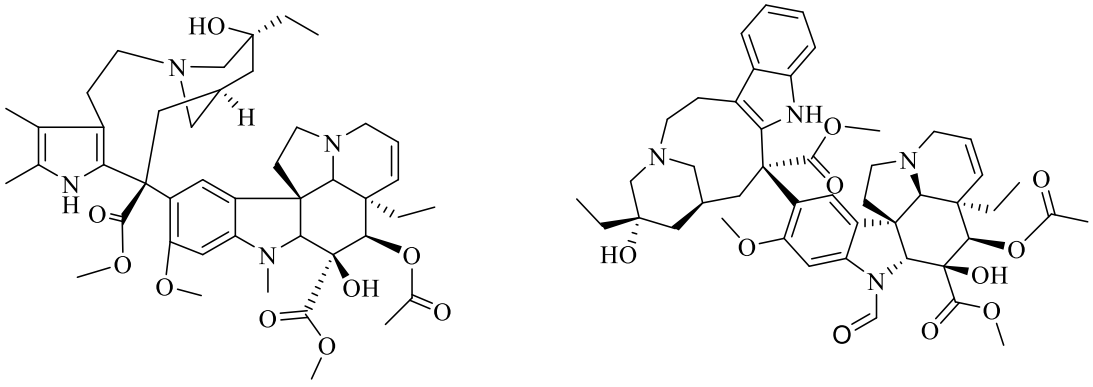
Figure 2.1 Genera from subfamily Rauvolfioideae of Vinceae tribe (Endress & Bruyns, 2000).

Some characteristics of the family were described as producing white latex in the majority of the species, the leaves are opposite or whorled and the flowers are large, colourful, and slightly fragrant with five contorted lobes (Chan *et al.*, 2016a). Most genera of Apocynaceae produced paired fruits with projections generally air-filled that serve as flotation devices (Middleton, 2004).

2.1.2 Uses

In the Asia-Pacific region, Apocynaceae species is used as a treatment for fever, malaria, pain, diabetes, and gastrointestinal ailments (Wiart, 2006). Since the family members have an abundance of metabolites for example alkaloids, triterpenoids, iridoids and cardenolides, they possessed a variety of biological and

pharmacological activities (Chan *et al*., 2016a). The first commercial anti-cancer drugs from Apocynaceae were vinblastine (**4**) and vincristine (**5**), used to treat Hodgkin's disease and lymphocytic leukaemia in children, respectively. Both drugs as shown in Figure 2.2 and their derivatives were developed from terpenoid-indole alkaloids isolated from the pantropical plant *Catharanthus roseus* (Madagascar periwinkle) from the genus *Catharanthus* (Van der Heijden *et al.*, 2004).



Vinblastine (4)

Vincristine (5)

Figure 2.2 Examples of anti-cancer drugs from the genus *Catharanthus* of the Apocynaceae family.

2.2 The genus *Kopsia*

2.2.1 Botany and distribution

The name "*Kopsia*" was published in 1823 by Blume in honour of the Dutch botanist J. Kops (1765-1849) with one species, *K. arborea* Blume. To date, there are about 23 species have been identified from Southern China and Burma to northern Australia and Vanuatu (Middleton, 2004). The majority of the species are concentrated in Southeast Asia which is Peninsular Malaysia and Borneo (Middleton, 2004).

In Malaysia, there are almost 15 species which have been particularly well-investigated. Among the Malays, *K. larutensis* King and Gamble are known for its

Malay names “chabai hutan” and “pokok karang”, *K. macrophylla* Hook. f. as “bangku”, *K. pauciflora* Hook f. as “sertong” and *K. singapurensis* Ridl. as “selada”. Table 2.1 compiled all *Kopsia* species found in Peninsular Malaysia and Borneo including its distribution.

Table 2.1 *Kopsia* species and its distribution in Peninsular Malaysia and Borneo (Middleton, 2004).

Species	Distribution
K. arborea Blume	Perak
K. dasyrachis Ridl.	Sabah (Lukan)
K. deverrei L. Allorge	Johor
K. grandifolia D. J. Middleton	Johor (Sungai Mupar)
K. larutensis	Perak
K. griffithii King & Gamble	Melaka, Selangor
K. macrophylla Hook. F	Negeri Sembilan, Johor (Gunung Angsi)
K. pauciflora Hook. F	Melaka
K. profunda Markgr	Terengganu (Belara Forest Reserve)
K. terengganensis L. Allorge & Wiart	Terengganu (Dungun)
K. rajangensis D. J. Middleton	Sarawak (Kapit)
K. singapurensis Ridl.	Johor (Mersing)
K. sleesenia Markgr.	Sarawak (Bintulu)
K. tenuis Leenh. & Steenis	Sarawak (Mattang)
K. teoi L. Allorge	Johor (Keluang)

2.2.2 Morphology

The *Kopsia* plant is a part of the forest's vegetation and can be found growing at or near the forest's main canopy. The tallest species, *K. arborea*, stands at 14 metres (Middleton, 2004). Young branchlets on the majority of plants are a little angled. Angles in certain species, like *K. lancifolia*, resemble wings. The leaves are always opposite. Many species have very short petioles which represent they may or may not

be sessile except for *K. teoi* and *K. pauciflora* where the petioles are absent. As for reproductive characters, the basic inflorescence structure would be a terminal dichasium or sometimes a trichasium. The majority of the sepals are oval, and the apex can range from acuminate to rounded. The filament is short and the corolla lobes are dexterously bent in the bud. The fruit is reported to have a little drupe, less than 4.5 cm long, and is often paired for all species (Middleton, 2004).

2.2.3 Medicinal uses

The *Kopsia* species has been suggested for several medical applications. In 1966, a report by Burkill stated that the root from *K. larutensis* King & Gamble, *K. macrophylla* Hook f., *K. singapurensis* Ridl., and *K. pauciflora* Hook f. is used by the Malays for poulticing ulcerated noses in tertiary syphilis. In Java, *K. arborea* is used against headaches. *K. officinalis* Tsiang & Li is used to treat rheumatoid arthritis and gout in Chinese traditional medicine (Sevenet *et al*., 1994). Other than that, *Kopsia* species also have been studied for their biological activities. Chan *et al*.,(2016a) reported that *K. dasyrachis* and *K. fruticosa* have anti-plasmodial properties meanwhile *K. arborea, K. grandifolia, K. singapurensis* and *K. tenuis* possessed anti-proliferative characteristics against human cancer cells. A positive result on the anti-hypertensive activity was also reported by *K. teoi* from preliminary screening of its alkaloid extracts.

2.2.4 *Kopsia terengganensis*

Kopsia terengganensis L. Allorge & Wiart is the latest species of *Kopsia* grown in Peninsular Malaysia. It was found in the lowland of evergreen forests from 230 to

330 m altitude (Middleton, 2004). It is synonym with *K. profunda* Markgr. but slightly different in its morphology (Middleton, 2004). Both species were differentiated in 1994 by Sevenet *et al*. based on where the stamens were located within the corolla tube (Sevenet *et al*., 1994). They believed *K. profunda's* stamens were at the tube's base and *K. terengganensis'* were in the centre. They are indistinguishable in other characteristics.

The tree can grow up to a 3 m height with a grey bark. The leaves have petioles that is 2-12 mm long and glabrous at above and beneath. Sometimes the leaves has central groove at above. The branchlets range in density from sparse to intense lenticellate, or they may not. The corolla is either entirely white or white with a yellow "eye," while the inflorescence is dichasial or cincinnate. The fruit has a short, blunt, hooked spur that is falcate. Figure 2.3 showed the flower, bark and leaves taken from the *K. terengganensis*. Previous study reported that the bark and the leaves extract showed significant result for cytotoxic activity against the KB cells (Uzir, 1997).

Figure 2.3 *Kopsia terengganensis* L. Allorge & Wiart.

2.3 Alkaloids

Plants, specifically in some families of flowering plants typically contained alkaloids. Due to their toxicity, alkaloids are effective defensive chemicals for plants against infections and predators (Matsuura & Fett-Neto, 2017). According to a French chemist, Pelletier (1983) alkaloids can be defined as "a cyclic organic compound containing nitrogen in a negative oxidation state, which has limited distribution in living organisms". This modern definition includes heterocyclic nitrogen-containing alkaloids as well as several alkaloids that do not incorporate nitrogen atoms with the cyclic ring, such as colchicine (**6**) and capsaicin (**7**). (Figure 2.4).

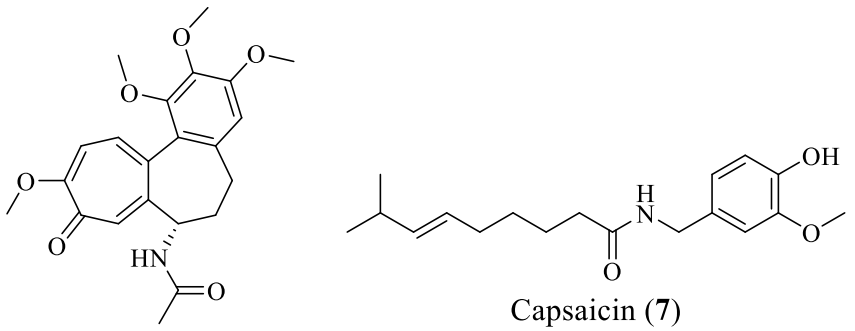


Figure 2.4 Examples of alkaloid that does not incorporate nitrogen atom in the ring structure.

In the classical definition, alkaloids are described as a chemical which has alkali-like properties and at least one nitrogen atom in a heterocyclic ring structure. Further definitions are added such as (Pelletier, 1983):

i. Compounds are large and complex molecular structure

ii. Pharmacologically actives compounds

iii. Restricted to certain genera and families of the plant kingdom

However, these definitions no longer take place as the study on the alkaloids has been made in-depth because it violated the requirements for the modern definition of alkaloids. Alkaloids do not need to have alkali-like properties nor nitrogen atoms in the heterocyclic system as in compounds **6** and **7**. The complexity of a molecule can also be denied as a different chemist would perceive each molecule differently. Finally, the plant-derived requirement is no longer valid as they also include alkaloids isolated from animal, fungal and bacterial sources.

Early in the 19th century, humans started to recognize alkaloids ever since it has been employed as pharmaceuticals, potions, medicines, teas, poultices and poisons. The dried opium poppy latex from *Papaver somniferum* is known as the first crude medicine to be chemically studied (Perkin & Robinson, 1910). Compelled by the analgesic and narcotic properties, Derosne was motivated to isolate a semi-pure alkaloid from opium in 1803. This isolation method then continued and was characterized as morphine (**8**) by Sertürner in 1805. Morphine is a naturally occurring compound used as an alternative way of treating pain caused by cancer when another analgesic is failed (Aniszeswki, 2015). He also managed to recognize the basic nature of morphine.

It is known that more than 3000 types of alkaloids have been identified from more than 4000 plant species (Kurek, 2019). Numerous findings in the study of alkaloids showed how important it is towards nature and human life. For example, an alkaloid in plants plays its role as a secondary metabolite and the bitterness which comes from the alkalinity of the nitrogen atom in the cyclic system creates a defence mechanism towards herbivorous organisms (Hopkins, 2003). As for human beings, alkaloids have been widely used for medicinal purposes for example colchicine (**6)** for gout (Paré *et al*., 2020), quinine (**9**) from *Cinchona* species for malaria, and injection

of atropine (**10**) from *Hyoscyamus niger* to treat low heart rate (Kurek, 2019). Alkaloids also can be found in the human diet for example caffeine (**11**) in coffee seeds and theophylline (**12**) in tea leaves (Kurek, 2019). Figure 2.5 shows the structure of alkaloids used in the medicinal field and human diet.

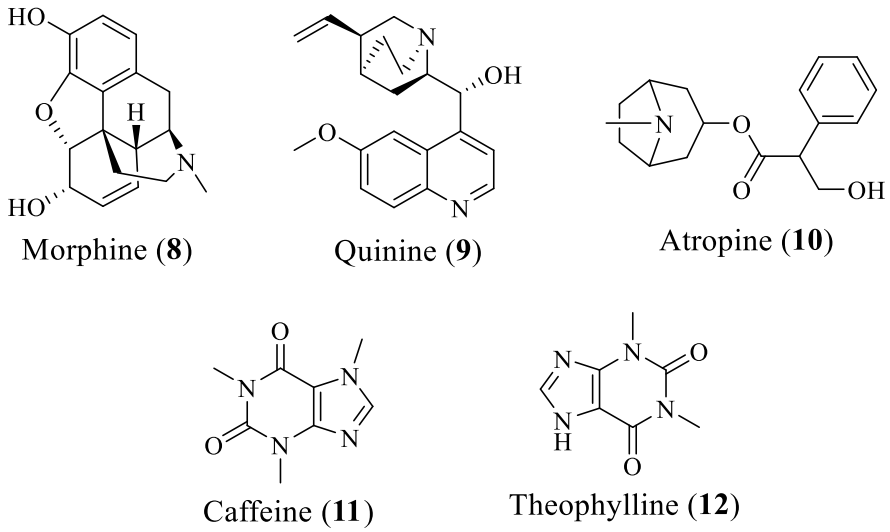


Figure 2.5 Examples of alkaloids in the medicinal field: morphine (**8**), quinine (**9**), atropine (**10**) and human diet: caffeine (**11**), theophylline (**12**).

2.3.1 Classifications and types of alkaloids

In the classifications of alkaloids, generally, the molecules were grouped based on the starting materials called precursor compounds in their biological pathway. The precursor, for instance, acid amino provides a nitrogen atom and part of the structure to construct the molecule (Aniszewski, 2015). Figure 2.6 showed some of the common ring skeletons in the classification of alkaloids (Kurek, 2019).

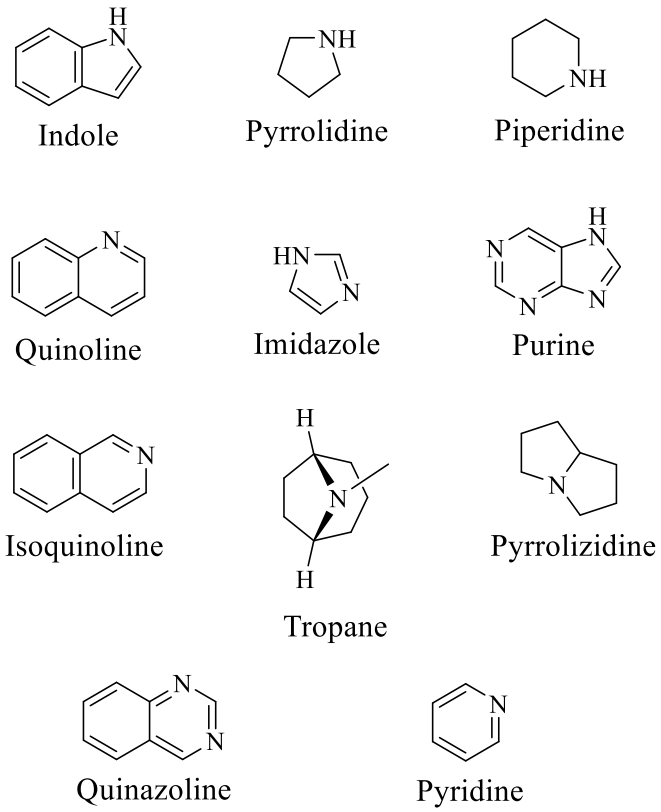


Figure 2.6 Examples of common ring skeleton for alkaloids.

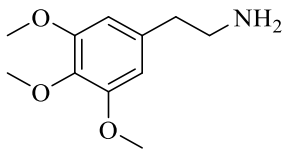
However, not all alkaloids were derived from amino acids. Thus, alkaloids can be classified as (Aniszewski, 2015):

i. **True alkaloids**: resulted from amino acid derivation and the cyclic ring contains a nitrogen atom. Known for highly reactive substances with minimal doses used in biological activity.

ii. **Protoalkaloids**: derivation of amino acid but no nitrogen atom in the cyclic ring. They form a minority of all alkaloids.

iii. **Pseudoalkaloids**: not derived from amino acids but connected with amino acid pathways. The derivation can be from amination and transamination reaction, non-amino acid precursors or acetate and phenylalanine-derived.

Table 2.2 shows the type of alkaloids and their precursors used to construct different types of alkaloid structures. Ajmalicine (**2**) is an example of a true alkaloid. It is derived from tryptophan and the nitrogen atom present in the ring system (Contin *et al*., 2018). For protoalkaloid, mescaline (**13**) (Figure 2.7), from the extraction of Peyote cactus (*Lophophora williamsii*) is a result of amino acid (*L*-tyrosine) derivation, but no nitrogen atom was present in the ring structure (Rosenberg *et al*., 1974). The compound was known to have high psychoactive and hallucinogenic properties. As for pseudoalkaloids, such an example is caffeine (**11**), a purine alkaloid derived from precursor guanine (Aniszewski, 2015).



Mescaline (**13**)

Figure 2.7 Example of alkaloid without nitrogen atom in the ring system.

Table 2.2 Type of alkaloids and their chemical groups (Aniszewski, 2015)

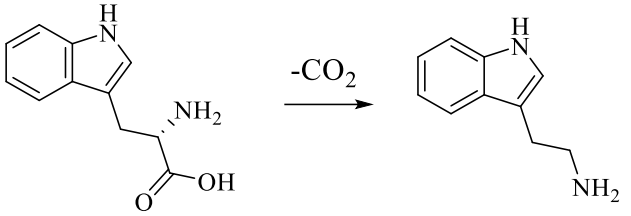
Alkaloid type	Precursor compound	Chemical group of alkaloids
True alkaloid	*L*-ornithine	Pyrroline
		Tropane
		Pyrrolizidine
	L-lysine	Piperidine
		Quinolizidine
	L-tryptophan	Indole
		Quinoline
		Pyrroloindole
	L-histidine	Imidazole
	Nicotinic acid	Pyridine
Protoalkaloids	*L*-tyrosine	Phenylethylamino
	L-tryptophan	Terpenoid indole
	L-ornithine	Pyrrolizidine
Pseudoalkaloids	Acetate	Piperidine
	Ferulic acid	Aromatic
	Geraniol	Terpenoid
	Adenine /Guanine	Purine

2.4 Biogenesis of indole alkaloid

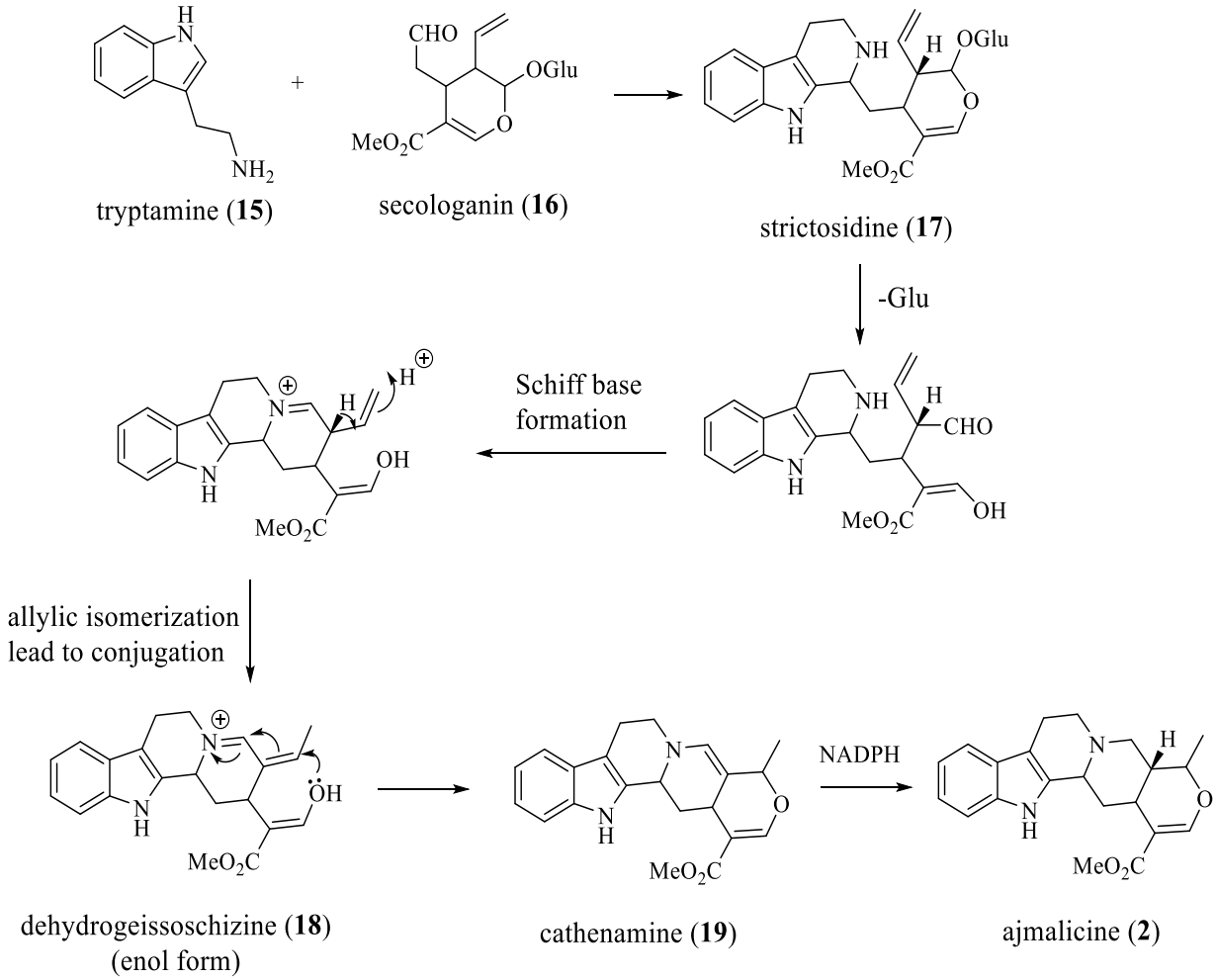
The members of the Apocynaceae family (*Kopsia, Tabernaemontana, Alstonia, Leuconotis*) are popular for bearing a large group of indole alkaloids. To date, more than 4000 compounds of indole alkaloids were identified and had been reported (Rosales *et al*., 2020). The bicyclic structure of indole alkaloid consists of a benzene

ring fused to a pyrrole ring. It is derived from *L*-amino acid tryptophan (**14**) or tryptamine (**15**) as their precursors and some in combination with a steroidal, secoiridiod such as secologanin (**16**) or other terpenoid-type moieties (Rosales *et al*., 2020; Croteau *et al*., 2000). Generally, indole alkaloids are classified into three major groups which are corynanthe, aspidosperma and iboga (Marinho *et al*., 2016). These classifications are based on their biogenic pathway.

In the biosynthesis of ajmalicine (**2**), a coryane-type alkaloid, the process started with the decarboxylation of *L*-tryptophan (**14**) as a precursor to tryptamine (**15**) by tryptophan decarboxylase (Scheme 2.1). The tryptamine stereospecifically condensed with secoiridiod secologanin (**16**) by strictosidine synthase action to form 3*α*-strictosidine (**17**), a vincosan skeleton alkaloid. Hydrolysis of glucoside produced hemiacetal and further hydrolysis opened the cyclic acetal function to give aldehyde and alcohol. The aldehyde group reacted with the secondary amine function. Then, allylic isomerization generated dehydrogeissoschizine (**18**) followed by cathenamine (**19**) via cyclization. Reduction of **19** by NADPH yielded ajmalicine (**2**) (Croteau *et al*., 2000; Dewick, 2009). The process is shown in Scheme 2.2.



Scheme 2.1 Decarboxylation of *L*-tryptophan to tryptamine



Scheme 2.2 Condensation of secologanin with tryptamine (Croteau *et al*., 2000; Dewick, 2009).

2.5 Alkaloids reported from the genus *Kopsia*

Plants belonging to this genus produce an abundance of indole alkaloids, many of which have intriguing carbon skeletons and biological activity (Kam, 1999a). The first alkaloids from the genus *Kopsia* were determined by Greshoff in 1890 who worked on the seeds of *K. flavida* Blume, *K. arborea* Blume and *K. fruticosa* A. DC. Most species in the genus *Kopsia* comprises alkaloids derived from the aspidofractinine type which can be described as C_{18} linked to C_2. There was also the aspidospermane type which refers to having an ethyl chain and the eburnane type which contained a linkage N_1-C_{16} (Sevenet *et al.*, 1994). Figure 2.8 illustrated the structure of each type of alkaloid in the genus *Kopsia*.

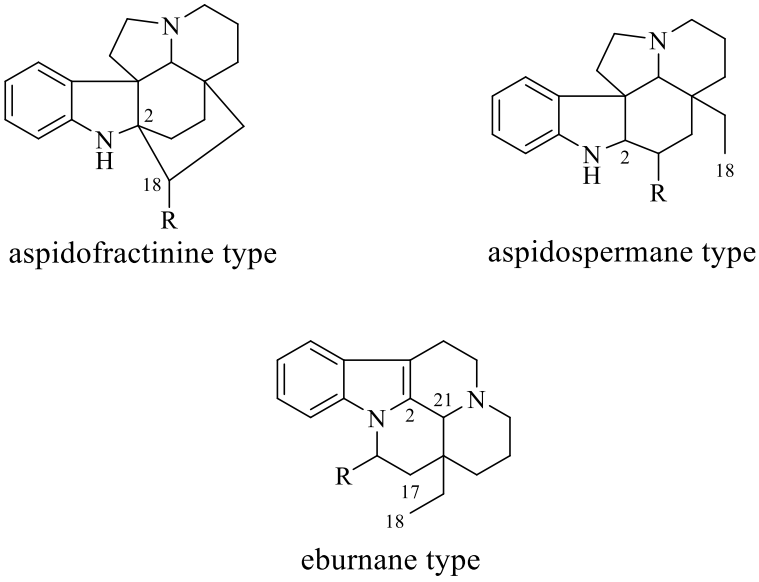


Figure 2.8 Indole alkaloid types in the genus *Kopsia*.

Researchers had preferable ways of detecting the presence of alkaloids which were through the Mayer test or using the Dragendorff reagent or using TLC followed by Dragendorff detection (Raal *et al.*, 2020). Table 2.3 showed *Kopsia* species that have been reported for their alkaloidal content and Figure 2.9 depicted the isolated alkaloids which were distributed in Malaysia.

Table 2.3 *Kopsia* species and its reported isolated alkaloids.

Plant	Plant part	Isolated alkaloid	References
Kopsia arborea Bl.	L	Methyl-12-methoxychanofruticosinate (**20**) Methyl-11,12-dimethoxychanofruticosinate (**21**) Methyl-N_1-decarbomethoxychanofruticosinate (**22**) Methyl-11,12-methylenedioxychanofruticosinate (**23**) Methyl-11,12-methylenedioxy-N_1-decarbomethoxychanofruticosinate (**24**) Methyl-11,12-methylenedioxy-N_1-decarbomethoxy-$\Delta^{14,15}$-chanofruticosinate (**25**) Arboricine (**26**) Arboricinine (**27**) Arborisidine (**28**) Arbornamine (**29**) Prunifoline A (**30**) Prunifoline B (**31**) Prunifoline C (**32**) Prunifoline D (**33**) Prunifoline E (**34**) Prunifoline F (**35**)	Lim & Kam, 2008; Lim *et al*., 2007; Wong *et al*., 2016; Wong *et al*., 2021
	SB	Arbolodinine A (**36**) Arbolodinine B (**37**) Arbolodinine C (**38**)	
Kopsia dasyrachis Ridl.	L	Kopsidasine (**39**) Kopsidasine-*N*-oxide (**40**) Kopsidasinine (**41**) Kopsirachine (**42**)	Kam, *et al*., 1999b; Kam, *et al*., 1999d; Saxton, 1998

Plant part abbreviations: L(leaves), B(barks), S(stems) and SB (stembark)

		Methyl-11,12-methylenedioxychanofruticosinate (**23**) Methyl-N_1-decarbomethoxychanofruticosinate (**22**) Methyl-11,12-methylenedioxy-N_1-decarbomethoxychanofruticosinate (**24**) 11,12-dimethoxykopsamine (**43**) Danuphylline (**44**) Kinabalurine G (**45**)	
	S	Kopsiflorine (**46**) Kopsilongine (**47**) 11-methoxykopsilongine (**43**) Kopsinine (**48**) Kopsinine-N_4-oxide (**49**) 11,12-methylenedioxykopsinaline (**50**) Tetrahydroalstonine (**51**) Pleiocarpamine (**52**) 16-hydroxymethylpleiocarpamine (**53**) Kopsine (**54**) *N*-carbomethoxy-5,22-dioxokopsane (**55**) (+)-eburnamonine (**56**) (+)-isoeburnamine (**57**) Leuconoxine (**58**) Paucidactine B (**59**) (−)-norpleiomutine (**60**) (−)-demethylnorpleiomutine (**61)** (+)-kopsoffinol (**62**) Kopsiflorine-N_4-oxide (**63**) Kopsilongine-N_4-oxide (**64**) Decarbomethoxykopsifine (**65**) Kopsinarine (**66**) 11,12-methylenedioxykopsine (**67**) Dasyrachine (**68**)	