VOLATILE CONSTITUENTS AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS OF SELECTED *Boesenbergia* SPECIES

NOOR ATIEKAH BINTI MD NOR

FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
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NOOR ATIEKAH BINTI MD NOR

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VOLATILE CONSTITUENTS AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS OF SELECTED *Boesenbergia* SPECIES

ABSTRACT

Essential oils from the rhizomes and leaves of three wild *Boesenbergia* species from Sabah, namely *Boesenbergia armeniaca* Cowley, *Boesenbergia stenophylla* R. M. Sm., *Boesenbergia* sp. *nova,* and two wild species from Peninsular Malaysia, namely *Boesenbergia plicata* (Ridl.) Holttum var. *plicata*, and *Boesenbergia prainiana* (King ex Baker) Schltr: were obtained by hydrodistillation. The volatile constituents and their compositions in the oils were identified by Gas Chromatography-Flame Ionization Detector (GC-FID), Gas Chromatography-Mass Spectrometry (GC-MS) and Kovats index analysis. The analysis revealed the presence of monoterpene hydrocarbons, sesquiterpene hydrocarbons, oxygenated monoterpenes, oxygenated sesquiterpenes, diterpene hydrocarbons and oxygenated diterpenes. Five major compounds were identified: methyl cinnamate (55.42%-83.17%) and nerolidol (22.77%-42-55%) from rhizome and leaf oils of *Boesenbergia stenophylla* and *Boesenbergia armeniaca* respectively; γ-maaliene (22.82%) from leaf oil of *Boesenbergia* sp. *nova*, isocamphene (28.07%) and (-)-β-pinene (21.33%) from rhizome and leaf oil of *Boesenbergia plicata,* respectively. The antibacterial activity of essential oils against food-borne pathogens namely, *Staphylococcus aureus, Bacillus cereus, Salmonella enteritidis* and *Escherichia coli,* were evaluated by disc diffusion assay and their minimum inhibitory concentration (MIC) values were determined. Almost all the essential oils displayed relatively moderate level of MIC values (2.5 mg/mL) against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*). However, the most potent essential oil was the leaf oil of *Boesenbergia plicata* (26.5 mm zone inhibition) against Gram-positive bacteria, *Bacillus cereus* with MIC value of 1.25 mg/mL. Whereas, all the

essential oils tested against the Gram-negative bacteria (*Salmonella enteritidis* and *Escherichia coli*) exhibited low antibacterial activity with MIC value of 5 mg/mL.

Keywords: *Boesenbergia*, antibacterial activity, essential oil, food-borne pathogens, *Bacillus cereus*

KONSTITUEN VOLATIL DAN AKTIVITI ANTIBAKTERIA MINYAK PATI BEBERAPA SPESIES *Boesenbergia* YANG TERPILIH

ABSTRAK

Minyak pati rizom dan daun yang diperolehi daripada tiga spesies liar *Boesenbergia*, iaitu *Boesenbergia armeniaca* Cowley, *Boesenbergia stenophylla* R. M. Sm., *Boesenbergia* sp. *nova* dari Sabah dan dua spesies liar dari Semenanjung Malaysia iaitu *Boesenbergia plicata* (Ridl.) Holttum var. *plicata*, dan *Boesenbergia prainiana* (King ex Baker) Schltr diekstrak melalui proses penyulingan hidro. Konstituen volatil dan komposisinya dikenalpasti melalui kromatografi gas-pengesan pengionan nyala (GC-FID), kromatografi gas-spektrometer jisim (GC-MS) dan analisis Kovats indeks. Analisis tersebut menunjukkan kehadiran hidrokarbon monoterpen, hidrokarbon seskuiterpen, monoterpen beroksigen, seskuiterpen beroksigen, hidrokarbon diterpen dan diterpen beroksigen. Lima konstituen volatil utama telah dikenal pasti iaitu: metil sinamat (55.42%-83.17%) dan nerolidol (22.77%-42-55%) masing-masing diperolehi dari minyak pati rizom dan daun *Boesenbergia stenophylla* dan *Boesenbergia armeniaca*; γ-maaliena (22.82%) daripada minyak pati daun *Boesenbergia* sp. *nova*; dan isokamfana (28.07%) dan (-)-β-pinena (21.33%) masing-masing diperolehi daripada minyak pati rizom dan daun *Boesenbergia plicata.* Aktiviti antibakteria minyak pati terhadap patogen bawaan makanan iaitu *Staphylococcus aureus, Bacillus cereus, Salmonella enteritidis* dan *Escherichia coli,* dinilai dengan menggunakan kaedah ujian penyebaran cakera dan kaedah nilai kepekatan perencatan minimum (MIC). Hampir kesemua minyak pati yang diuji menunjukkan nilai perencatan yang sederhana dalam melawan bakteria Gram-positif (*Staphylococcus aureus* dan *Bacillus cereus*) dengan nilai MIC 2.5 mg/mL. Minyak pati daripada daun *Boesenbergia plicata* (26.5 mm zon perencatan) menunjukkan perencatan yang paling tinggi terhadap bakteria Gram-positif, *Bacillus cereus* dengan nilai MIC 1.25 mg/mL. Manakala, secara keseluruhan, minyak

pati yang diuji ke atas bakteria Gram-negatif (*Salmonella enteritidis* dan *Escherichia coli*) menunjukkan perencatan yang rendah dengan nilai MIC 5 mg/mL.

Kata kunci: *Boesenbergia,* aktiviti antibakteria, minyak pati, patogen bawaan makanan, *Bacillus cereus*

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Thank you.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

>	:	greater than
<	:	less than
≥	:	greater than or equal to
≤	:	less than or equal to
%	:	percent
a.s.l	:	above sea level
α	:	alpha
β	:	beta
γ	:	gamma
δ	:	delta
cfu	:	colony-forming unit
°C	:	degree celcius
ft.	:	feat
g	:	gram
L	:	liter
ml	:	mililiter
m	:	meter
mm	:	milimeter
µL	:	microliter
µm	:	micrometer
µg/mL	:	microgram/mililiter
mg/mL	:	milligram/mililiter
ATCC	:	American Type Culture Collection
BORH	:	Borneensis Herbarium
GC	:	Gas Chromatography
GC-MS	:	Gas Chromatography-Mass Spectrometer
GC-FID	:	Gas Chromatography-Flame Ionization Detector
MeOH	:	Methanol
MIC	:	Minimum Inhibitory Concentration
MHA	:	Mueller Hinton Agar
MHB	:	Mueller Hinton Broth
NA	:	Nutrient Agar
NB	:	Nutrient Broth

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

Medicinal plants can be defined as plants that contain properties or compounds that can be used for therapeutic purposes or those that synthesize metabolites to provide useful drugs. Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years around the world (Sen & Batra, 2012). The most important compounds of these bioactive constituents of plants are alkaloids, flavonoids and phenolic compounds (Balunas & Kinghorn, 2005). Natural products have proven to be the richest source of medicinal compounds, derived from natural sources such as plants, animals and micro-organisms (Li Ji *et al.,* 2016).

The World Health Organization (WHO) statistics showed that 80% of the people living in rural areas depend on the medicinal herbs as primary healthcare system, mostly plant-based drugs for their primary health care needs (Suran & Eapen, 2013). Malaysia had an extensive variety of plant species and they are widely valued for their aromas, tastes and also as medicinal agents to treat various human illnesses (Alsarhan *et al*., 2014).

Essential oils, also known as essences or volatile oils are natural products formed by volatile compounds. These oils have been used for thousands of years mainly in medical practice, beauty treatment, food preparation and religious ceremonies (Saxena & Patil, 2014). Essential oils are usually terpenoids responsible for aroma and flavour associated with herbs, spices and perfumes. In addition, essential oils have been known to demonstrate pharmacological effects such as anti-inflammatory, antioxidant, cytotoxic and act as biocides against a broad range of organisms such as bacteria, fungi, viruses and protozoas (Sivasothy *et al.,* 2012).

The food-borne pathogens which lead to food spoilage is encountered as one of the most important issue concerning both consumers and the food industry. The common foodborne illnesses caused by food-borne pathogens are vomiting, diarrhea and nausea. Many pathogenic microorganisms such as *Escherichia coli, Staphylococcus aureus, Bacillus cereus, Listeria monocytogens* and *Salmonella enterica* have been reported as the causal agents of food-borne diseases (Oonmetta-aree *et al*., 2006; Voravuthikunchai *et al*., 2006; Natta *et al*, 2008). The essential oil of oregano (*Origanum vulgare*), ginger (*Zingiber officinale*), kaempferia (*Kaempferia pandurata*) and bastard cardamom (*Amomum xanthioides*) have been demonstrated to be effective against these problematic bacteria (Natta *et al*, 2008; Mazzarrino *et al*., 2015). Hintz *et al*., (2015) reported that preservatives are commonly used to reduce the risk of foodborne illnesses. The compounds that are found in the plant oils and plant extracts in some spices and produced by herbs act as self-defence mechanisms to protect the plant against infectious organisms and food-borne pathogens. Thus, the natural preservative from the essential oils could be explored as these may develope as a food preservative agent to control the food-borne pathogens.

Zingiberaceae is one of the largest monocotyledonous family and one of the most important herbaceous group found in tropical forest with approximately 50 genera and over 1000 species (Larsen *et al*., 1999). They are rich sources of essential oils that consist of numerous complex terpenoid mixture and well-known for their medicinal uses and economic significance. Essential oils of Zingiberaceae family have great potential in the field of biomedicine as they effectively destroy several bacterial, fungal, and viral pathogens (Voravuthikunchai, 2007). Some of the important medicinal Zingiberaceous species belong to the *Curcuma, Alpinia, Zingiber, Hedychium, Kaempferia,* and *Boesenbergia.*

The genus *Boesenbergia* comprises of small forest plants, with approximately 80 species worldwide (Larsen *et al*., 1999; Techaprasan *et al*., 2006). To date, 33 species of *Boesenbergia* are found in Borneo while 10 species in Peninsular Malaysia. Of these species, only *Boesenbergia rotunda* is cultivated commercially and its rhizome has been used for medicinal and culinary purposes. The rhizomes of *Boesenbergia pulchella*, *Boesenbergia kingii, Boesenbergia rotunda* are reported to exhibit antioxidant, anti-bacterial and anti-inflammatory activity (Kirana *et al*., 2007; Jing *et al*., 2010; Sudsai *et al*., 2014). Due to these properties, *Boesenbergia* species has gained attention as important source of active constituents for medicinal treatment, biological activity as well as for other purposes.

Problem statement

Except for the leaf oil of *Boesenbergia plicata* from Langkawi, and the rhizome and leaf oils of *Boesenbergia stenophylla* from Sarawak, the chemical composition of the rhizome and leaf oil of five wild *Boesenbergia* species namely, *Boesenbergia armeniaca* Cowley, *Boesenbergia stenophylla* R. M. Sm. and *Boesenbergia* sp. *nova* collected from Sabah and *Boesenbergia plicata* (Ridl.) Holttum var. *plicata* and *Boesenbergia prainiana* (King ex Baker) Schltr. collected from Taman Negara Endau-Rompin have not been studied before. Therefore, there is a need to study further these five *Boesenbergia* species, with the hope of discovering more variety of compounds and their potential uses. Hence this study will also investigate for the first time the antibacterial activity of the five *Boesenbergia* species.

1.2 Research objectives

The objectives of this present study are as follows:

1. To identify the volatile constituents of essential oils from different parts (leaves and rhizomes) of *Boesenbergia armeniaca* Cowley*, Boesenbergia stenophylla* R. M. Sm.*, Boesenbergia* sp. *nova, Boesenbergia plicata* (Ridl.) Holttum var. *plicata* and *Boesenbergia prainiana* (King ex Baker) Schltr.
2. To determine the antibacterial activity and the minimum inhibitory concentration (MIC) of the essential oils of the five *Boesenbergia* spp. against selected food-borne pathogens.

CHAPTER 2: LITERATURE REVIEW

2.1 The family Zingiberaceae

Zingiberaceae is one of the largest families of the order Zingiberales found in tropical and subtropical forests, with comprises about 1200 species of which about 1000 occur in Tropical Asia. The classification of Zingiberaceae family is shown in Figure 2.1. The centre of distribution is in Southeast Asia and the greatest concentration of genera and species are in the Malesian region including Indonesia, Malaysia, Singapore, Brunei, the Philippines and Papua New Guinea with 24 genera and about 600 species (Larsen *et al.,* 1999).

It is estimated that there were 150 species of ginger belonging to 23 genera found in Peninsular Malaysia (Sukari *et al*., 2008). Meanwhile, 19 genera and over 200 species have been reported so far from Borneo (Julius *et al*., 2010). Many of the species recorded in Sabah were predominantly wild, growing in various habitats ranging from riverine to montane regions (Larsen *et al*., 1999).

Zingiberaceous species are known to have important natural resources that provide man with many useful products for food, spices, medicines and as source for certain dyes (Burkill, 1966). Zingiberaceae species have been reported to possess biological activities such as antimicrobial, antioxidant and antifungal activity, and thus might be effective as anticancer agents (Habsah *et al*., 2000; Jantan *et al*., 2003; Jing *et al*., 2010).

Detailed ethnobotanical studies have found that numerous species from the ginger family (Zingiberaceae) are beneficial to the local communities and horticulturally important for some of them. Some of the commercial importance of selected species from Zingiberaceae is shown in Table 2.1.

Table 2.1: Uses of selected Zingiberaceae species

Species	**Parts**	**Uses**	**Reference**
Alpinia conchigera Griff.	Rhizome	To treat bronchitis, jaundice.	Sirirugsa (1999)
Alpinia galanga (L.) Willd.	Rhizome	To treat indigestion, dysentery and cancer of the stomach.	Sirirugsa (1999)
Amomum subulatum Roxb. (Bari ilaichi)	Seed	Act as an antidote to scorpion and snake venom.	Pruthi (1979)
Boesenbergia pulchella var. *attenuata* R. M. Sm.	Rhizome	Sap is used to cure the skin diseases.	Kulip (2007)
Curcuma longa L.	Rhizome	Rhizomes are chewed for relief from asthma.	Tushar *et al.*, (2010)
Curcuma parviflora Wall.	Rhizome	Pulp of the rhizome is scraped and applied to cuts.	Sirirugsa (1999)
Elettaria cardamomum (L.) Maton	Rhizome	Exported as cardamom ("buah pelaga"), treatment for eye inflammation, kidney and urinary disorder.	Tushar *et al.*, (2010)
Etlingera elatior (Jack) R. M. Sm.	Leaves	Reduce fever, the inflorescence used as vegetable, food flavour.	Kulip (2007)
Etlingera fimbriobracteata (K. Schum.) R. M. Sm.	Leaves	Used to make hat and roof for forest hut.	Kulip (2007)
Globba clarkei Baker	Rhizome	To cure cough	Tushar *et al.*, (2010)
Hedychium coronarium J. Koenig	Flower	Consumed as vegetable and also as a source of perfume.	Sirirugsa (1999)
Hedychium spicatum Sm.	Rhizome	To treat vomiting, diarrhoea, and inflammation.	Tushar *et al.*, (2010)
Kaempferia galangal L.	Leaves	For poultice and lotion, reduce stomachache.	Kulip (2007)
Zingiber montanum (J. Koenig) Link ex A. Dietr.	Rhizome	Act as an antidote to snake venom and antidiarrheal	Tushar *et al.*, (2010)
Zingiber ottensii Valeton	Rhizome	Rhizomes are included in a sedative lotion as a remedy for convulsions.	Sirirugsa (1999)
Zingiber purpureum Roscoe	Rhizome	To treat fever and intestinal disorder	Sirirugsa (1999)
Zingiber zerumbet (L.) Roscoe ex Sm.	Rhizome	Consumed as salad and tonic	Kulip (2007)

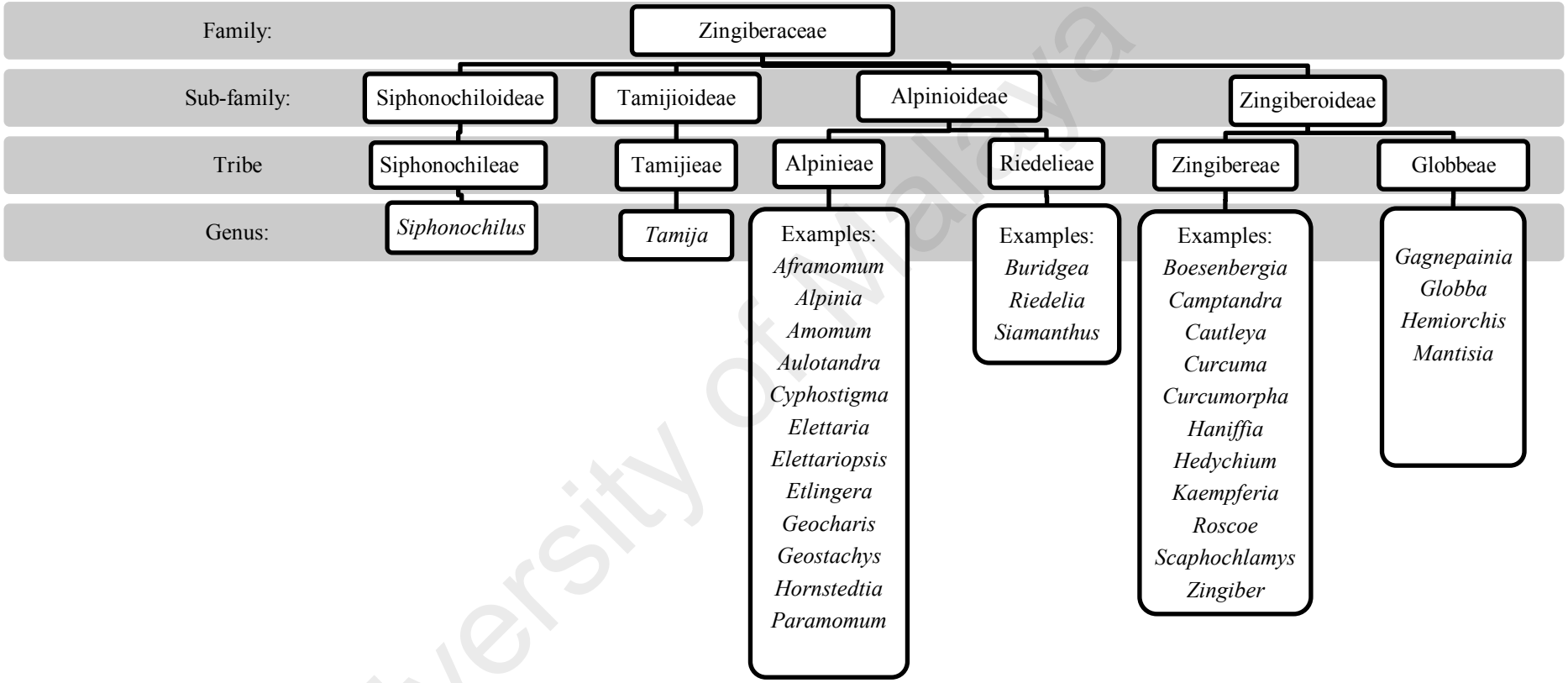


Figure 2.1: The classification of Zingiberaceae by Kress *et al*., (2002).

2.2 The genus *Boesenbergia*

The genus *Boesenbergia* belongs to the tribe Zingibereae (Zingiberaceae) comprises approximately 80 extant species distributed throughout tropical Asia (Saensouk & Larsen, 2001). The centre of diversity for the genus *Boesenbergia* is mainly in Borneo with more than 21 species followed by Thailand. Hence, Borneo and Thailand were proposed to be the center of origin for the genus *Boesenbergia* (Techaprasan *et al*., 2006).

Boesenbergia species are rare compared to other genera within Zingiberaceae. Habitats of *Boesenbergia* are mainly the undergrowth areas of the tropical forests, in particular damp and humid shady places. *Boesenbergia* spp. also grow in mixed deciduous and evergreen forests and on limestone hills (Jing *et al*., 2010). Most *Boesenbergia* species were easy to identify by their basipetalous flowering sequence, that is the first flower opens near the inflorescence apex followed by subsequent flowers closer to the base (Mood *et al*., 2014).

In some parts of Borneo, some *Boesenbergia* species were known to tolerate poor soil condition where many other plants do not grow such as limestone soil area, for instance *Boesenbegia pulchella* is found abundantly at the Dagat limestone area in Tabin Wildlife Reserve, Sabah (Gobilik & Limbawang, 2010) and Mulu limestone area, Sarawak (Smith, 1982). Growing *Boesenbergia* species outside its natural habitat such as a garden may be difficult but possible (Gobilik & Limbawang, 2010). To date, only *B. rotunda* is widely cultivated commercially among the *Boesenbergia* species (Techaprasan *et al.,* 2006).

Table 2.2: Properties of selected *Boesenbergia* species

Species	Parts	Biological activity	References
Boesenbergia rotunda (L.) Mansf.	Rhizomes	• As a condiment in food (curry and soup) • To treat rheumatism, muscle pain and gout. • The rhizome is used to prepare "jamu" for women in Indonesia after childbirth	Chong *et al*., (2012)
	Leaves	• Consumption of leaves is to alleviate food allergies and poisoning.	
Boesenbergia kingii Mood & L. M.	Rhizomes	• Used in the treatment of inflammatory bowel diseases. • Also to treat abscess and dysentery.	Chuakul & Boonpleng (2003)
Boesenbergia longiflora (Wall.) Kuntze.	Rhizomes	• As a treatment of inflammatory bowel diseases. • As a tonic • Also to treat aphthous ulcer and ulcerative colitis	Chuakul & Boonpleng (2003)
Boesenbergia stenophylla R. M. Sm.	Rhizomes	• As a protection against convulsions and prevention of intoxication • A mixture of crushed rhizomes of *B. stenophylla* and *Zingiber cassamunar* is used as a poultice or lotion. • Decoction of rhizomes is used for treating stomach-ache	Chai (2006) Poulsen (2006)

2.3 *Boesenbergia* species used in this study

In this present study, five wild *Boesenbergia* species from the family Zingiberaceae were investigated for their essential oil components and their antibacterial activity. The five *Boesenbergia* species investigated are *Boesenbergia armenica, Boesenbergia stenophylla, Boesenbergia* sp. *nova*, *Boesenbergia plicata a*nd *Boesenbergia prainiana.* Below are the descriptions on the five *Boesenbergia* species.

2.3.1 *Boesenbergia armeniaca* Cowley

Description: Perennial herb to 0.75 m tall. The fibrous root formed from shortly spreading rhizome. Shoots are several-leaved with up to 4 (sometimes pink or purple), clasping. Leaves, 3-7, distichously arranged. Staminodes are linear-oblong, apex obtuse and emarginated. Inflorescence branched, pedunculate; peduncle is about 3-5 cm long. The ovary is shortly hairy and stigmas are whitish, truncate and hairy at apex. Nearly all the species of *Boesenbergia* are mainly white flowers with splashes of yellow sometimes with red at the base of the labellum. However, in *B. armeniaca* the flower has been variously described as yellow, orange or apricot. Other Bornean species which have yellow to orange flowers are *B. aurantiaca, B. oligosperma and B. ornate* (Cowley, 2000).

Distribution: Brunei and Western Sabah.

Habitat: Secondary forest, dipterocarp forest, valley floors, shaded riverside vegetation: 40-440 m a.s.l. and also at sandy alluvial soil.

Figure 2.2: *Boesenbergia armeniaca* Cowley.

Figure 2.3: The flower of *Boesenbergia armeniaca* Cowley.

2.3.2 *Boesenbergia stenophylla* R. M. Sm.

Description: *Boesenbergia stenophylla*, known locally as '*jerangau merah*' is a perennial rhizomatous herb. The petiole is not exceeding 6 cm and many flowers on an inflorescence (Sakai & Nagamasu, 2006). They are found thriving under heavy shaded forest floor, preferring slopes nearby streams. It was never found on altitude less than 3000 ft. and therefore may requires low temperature for optimum growth.

Distribution: Sarawak and Sabah

Habitat: In kerangas and mixed dipterocarp forest floor of highland areas

Figure 2.4: *Boesenbergia stenophylla* R. M. Sm.

Figure 2.5: The flower of *Boesenbergia stenophylla* R. M. Sm.

2.3.3 *Boesenbergia* sp. *nova*

Description: *Boesenbergia* sp. *nova* is a new species found at the highland area of Sabah (Serinsim sub-station). The scientific name of this species is still under identification process.

Distribution: Sabah

Habitat: Secondary forest, dipterocarp forest and shaded riverside vegetation

Figure 2.6: *Boesenbergia* sp. *nova*

2.3.4 *Boesenbergia plicata* (Ridl.) Holttum var. *plicata*

Description: Erect stem of *Boesenbergia plicata* is very short and bearing 3 - 5 leaves. Leaf-blade is green and plicate by 14 cm. Apex is shortly pointed and petiole above sheath to about 12 cm. Inflorescence from stem apex is emerging from between the leaf-sheaths and elongating to nearly 30 cm. Primary bracts alternate in two ranked and the bracts in each rank is about 1 cm apart which is facing one way. According to Holttum, (1950) this species was apparently not uncommon in lowland forests in Peninsular Malaysia, at least on the eastern side of the country. There seems to have variation in colour pattern of the flower and distribution of red colour on the lip part, and also some variation in the length of the connective at the apex of the anther.

Distribution: Peninsular Malaysia, Peninsular Thailand and Myanmar

Habitat: This species is found in lowland forest up to 800m a.s.l.

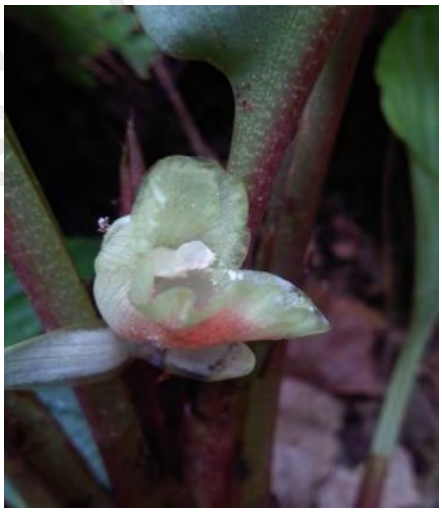


Figure 2.7: The flower of *Boesenbergia plicata* (Ridl.) Holttum var. *plicata*

2.3.5 *Boesenbergia prainiana* (King ex Baker) Schltr.

Description: The stem is short, rarely 10 cm to base of last leaf and bearing 1 to 3 leaves or rarely more. Leaves blade to about 25 by 12 cm, nearly elliptic, apex shortly pointed, base cuneate to rounded and slightly decurrent. The lower surface is purplish towards apex and bearing rather sparse to very fine hairs, petiole to about 9 cm long. Inflorescence apical, appearing from within the innermost leaf-sheaths, elongating when fully grown to about 18 cm. The bracts are in two alternating rows, folded down the middle, pale green more or less mottled with fine dull red spots like other sheaths, 2.5 cm to 3.5 cm long. *B. prainiana* has a habit closely similar to *B. plicata* but shorter wider bracts more widely spaced, much narrower bracteoles, smaller flowers with relatively shorter staminodes and deeply basin-shaped lip. It has been collected at several lowland localities on the east side of the Peninsular, twice in Perak and once in Langkawi (Holttum, 1950).

Distribution: Peninsular Malaysia

Habitat: common in evergreen forest and can be found along streams (50-400m a.s.l)

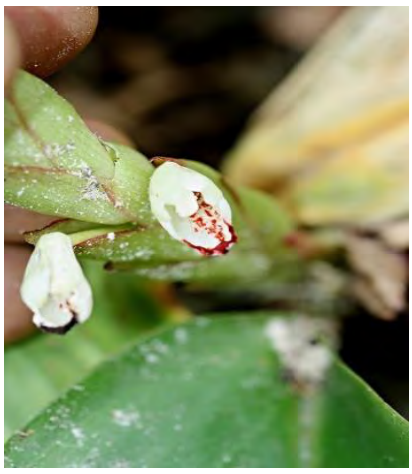


Figure 2.8: *Boesenbergia prainiana* (King ex Baker) Schltr.

2.4 Essential oil

In recent years, the utilization and studies of plant essential oils have become increasingly important in scientific research and industrial applications including pharmaceutical, nutritional and cosmetic uses due to the oils possessing various potent biological activities including antimicrobial, antioxidant and anti-inflammatory activities (Tsai *et al*, 2011).

Essential oils are also known as volatile oils because they easily diffuse into the air (Vidyasagar & Tabassum, 2013). It can be found in aromatic plants and are accumulated in secretion ducts of leaves, stems, flowers, rhizomes or wood (Kumar, 2014). It has a high commercial value due to its potential therapeutic properties. Essential oil compounds and their derivatives are considered to be alternative means of controlling many bacterial and fungal pathogens.

According to Tripathi *et al*., (2013), the major essential oil components identified in various species of Zingiberaceae broadly belong to monoterpenes, sesquiterpenes, and few phenols. Zingiberaceae species are aromatic due to the presence of essential oils that can be found in highly specialized secretory structures such as β-zingiberene, linalool and 1,8-cineole (Joseph *et al*., 2001; Bickers *et al*., 2003; Tripathi *et al*., 2013). The pungency of this family is provided by non-volatile phenolic compounds such as oleoresin, gingerols, shogaols and zingerone (Wohlmuth *et al*., 2006; Mashhadi *et al.*, 2013). Some of the chemical constituents of some plants of Zingiberaceae family are shown in Table 2.3.

2.5 Methods of extraction of essential oils

Essential oils are generally derived from one or more plant parts such as fruits (*Amomum subulatum*), flowers (*Alpinia purpurata* and jasmine), rhizomes (*Alpinia galanga* and *Zingiber officinale*), leaves (*Amomum gagnepainii* and *Alpinia zerumbet*), roots (angelica and vetiver), bark (cinnamon and cassia) and seeds (coriander and nutmeg).

Essential oil can be extracted from plant materials by using various methods such as hydrodistillation, steam distillation, solvent extraction, soxhlet extraction and so on. Therefore, the composition of the extracted oil may vary from one extraction method to another. However, based on Charles & Simon (1990), hydrodistillation appears to be an ideal method for extracting the essential oil as it resulted in a good yield, good recovery of its constituents and slightly low cost effective than other methods.

Factors that determine the composition and yield of the essential oil obtained are numerous. For instance, geographical divergence and ecological conditions of samples may contribute to the variation of volatile constituents of the oil. Other than that, the yield of the oil also depend on several factors such as the methods of extraction, drying process of material, parts of plant utilized and storage of the samples (Wohlmuth *et al*., 2006; Rusenova & Parvanov, 2009; Aziz *et al*., 2012; Kamaliroosta *et al*., 2013).

2.6 Chemistry of essential oils

The chemical components of the essential oils are separated into several classes such as monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated hydrocarbons and others (Wink, 1999).

2.6.1 Terpenes

Terpenes form structurally and functionally different classes. They are formed from combinations of several 5-carbon base (C_5) units called isoprene. The main terpenes are monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), triterpenes (C_{30}) and tetraterpenes (C_{40}). A terpene containing oxygen is called a terpenoid. Terpenes are amongst the chemicals responsible for the medicinal, culinary and fragrant uses of aromatic and medicinal plants (De Sousa, 2015).

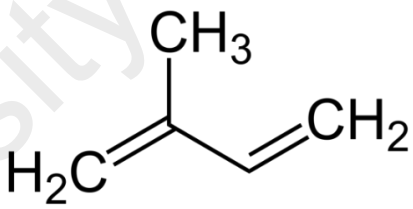


Figure 2.9: Isoprene unit

2.6.2 Monoterpenes

The monoterpenes are formed from the coupling of two isoprene units (C_{10}) that can be found in acyclic, monocyclic forms, and in various state of oxidation. They are the most representative molecules containing 90% of the essential oils (Bakkali *et al*., 2008). Many of them are able to exhibit biological activities and also give pleasant smell for the oils.

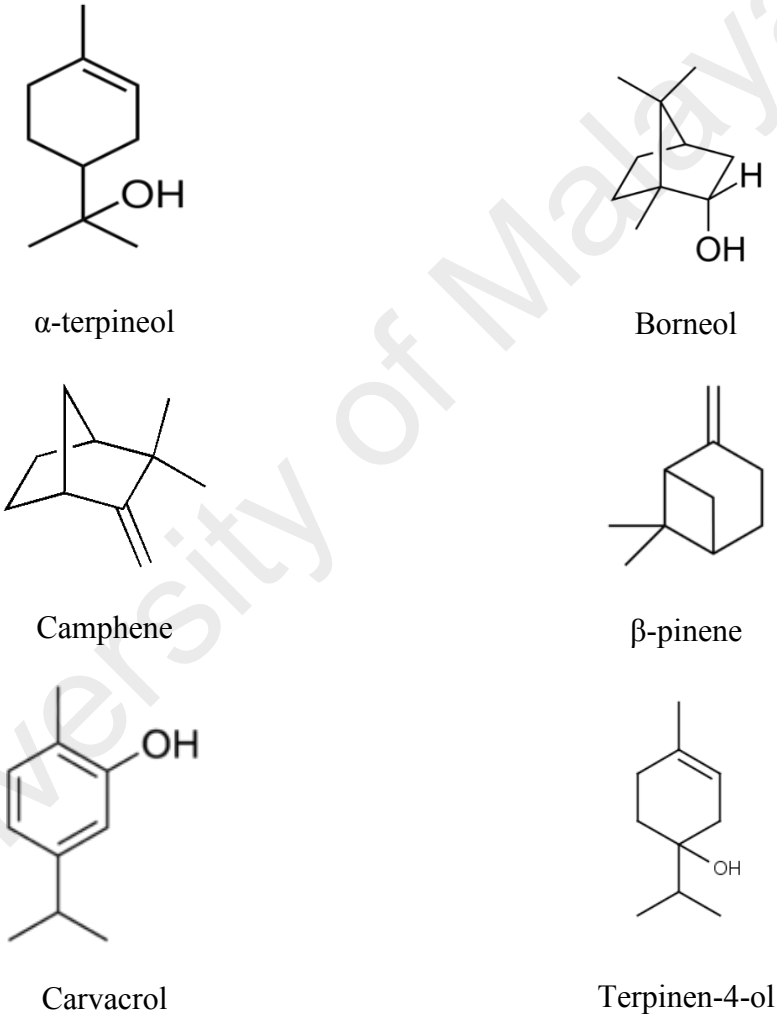
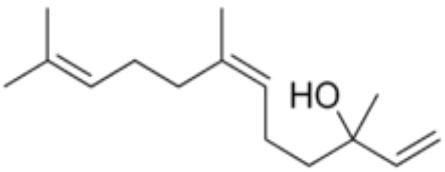


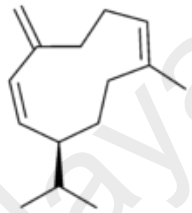
Figure 2.10: Structure of some components of essential oils for monoterpenes

2.6.3 Sesquiterpenes

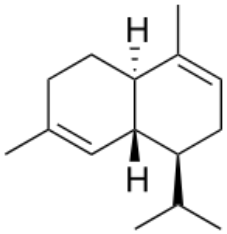
Sesquiterpenoids are C_{15} compounds ($C_{15}H_{24}$) containing three isoprene units, occurring in simple acyclic, as well as simple and complex bicyclic and tricyclic forms. Sesquiterpenes are reported to display anti bacterial, antifungal and anti-inflammatory activities (Bermejo *et al*., 2002; Neerman, 2003).



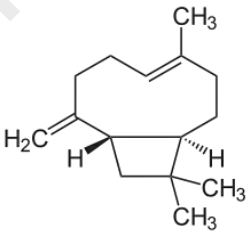
cis-nerolidol



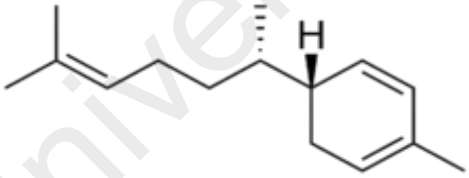
Germacrene D



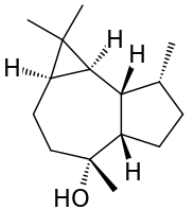
α-cadinene



Caryophyllene



Zingiberene

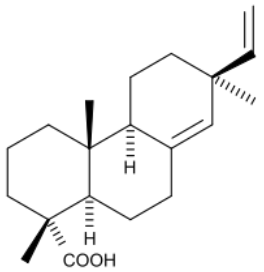


Ledol

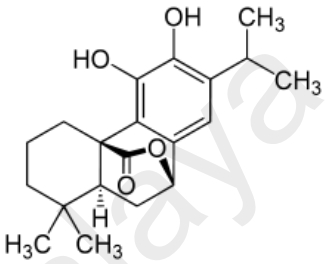
Figure 2.11: Structure of some components of essential oils for sesquiterpenes

2.6.4 Diterpenes

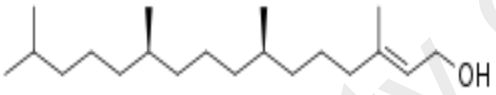
Diterpenoids are C_{20} compounds containing four isoprene units ($C_{20}H_{32}$). Their structural diversity range from simple acyclic to complex polycyclic rings. Diterpenes are responsible for essential physiological or ecological functions, particularly as growth hormones or defence compounds (Alan, 2006).



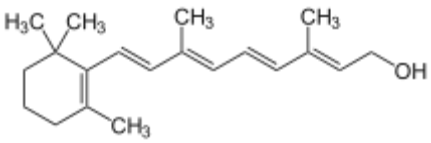
Pimaric acid



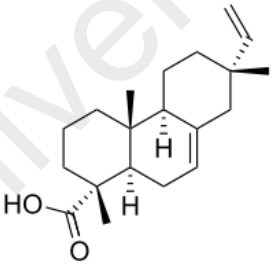
Carnosol



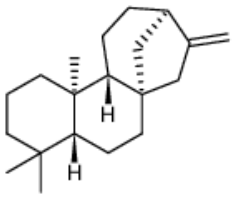
Phytol



Retinol



Isopimaric acid



ent-kaurene

Figure 2.12: Structure of some components of essential oils for diterpenes

Table 2.3: Properties of selected chemical compounds from selected essential oils of Zingiberaceae species.

Compounds	**Species**	**Properties**	**References**
β-phellandrene	*Zingiber mioga* (Thunb.) Roscoe	Anti-allergic activity	Kurobayashi et al., (1991)
β-pinene	*Aframomum daniellii* (Hook.f.) K. Schum. and *Etlingera elatior* (Jack) R. M. Sm.	Antioxidant activity	Essien *et al*., (2017) Abdelwahab *et al*., (2010)
1,8-Cineole	*Alpinia galanga* Willd. and *Alpinia calcarata* Roscoe	Give pleasant spicy aroma of the plant	Raina & Abraham, (2017)
1,8-Cineole and Linalool	*Aframomum daniellii* (Hook.f.) K. Schum.	Exhibit higher scavenging efficacy for antioxidant activity.	Dongmo *et al*., (2008)
Caryophyllene	*Zingiber zerumbet* (L.) Roscoe ex Sm.	Contributes to the spiciness taste of the plant	Baby *et al.,* (2009)
Curcumin	*Curcuma longa* L.	Anti-inflammatory and antimicrobial activity	Srimal, (1997)
Eucalyptol	*Elettaria cardamomum* (L.) Maton	Antibacterial activity	Batubara *et al*., (2016)
Germacrone	*Curcuma longa* L.	Anti-tumor activity	Zhong *et al*., (2011)
Gingerol	*Zingiber officinale* Roscoe	Pungent smell	Ghosh *et al*., (2011)
Humulene	*Zingiber zerumbet* (L.) Roscoe ex Sm.	Anti-allergic activity	Rogerio *et al.,* (2009)
Limonene	*Zingiber zerumbet* (L.) Roscoe ex Sm.	Anti-inflammatory activity	Hirota *et al*., (2012)
Linalool	*Zingiber zerumbet* (L.) Roscoe ex Sm.	Give an aromatic scent of the plant	Baby *et al.,* (2009)
Methyl cinnamate	*Alpinia malaccensis* Roscoe and *Keampferia galanga* L.	Give pleasant and aromatic smell	Silva *et al*., (2014)
Sabinene	*Zingiber cassumunar* Roxb. (Syn. *Zingiber montanum* (J. Koenig) Link ex A. Dietr.)	Anti-allergic activity	Sharifii-Rad *et al.,* (2017)
Terpinen-4-ol	*Zingiber cassumunar* Roxb. (Syn. *Zingiber montanum* (J. Koenig) Link ex A. Dietr.)	Antimicrobial activity	Cb *et al*., (2015)
Turmerone	*Curcuma longa* L.	Antifungal activity	Ferreira *et al*., (2013)
(-)-zingiberene	*Zingiber officinale* Roscoe	Fragrant smell	Ghosh *et al*., (2011)

2.7 Essential oils of *Boesenbergi*a species

Many researchers have demonstrated several pharmacological properties of Zingiberaceae species. However, studies on antibacterial, anti-allergic and anti-viral activities of *Boesenbergia* species are limited especially those activities that are related to essential oil properties.

The essential oil of *Boesenbergia rotunda*, also known as *temu kunci* has been extensively reported to exhibit biological activities such as antimicrobial, antioxidant, anti-inflammatory, anticancer and anti-HIV activities. Baharudin *et al*., (2015) reported that the major compounds present in the rhizome oil of *B. rotunda* from Pahang were nerol (39.56%) and *L*-camphor (36.01%).

According to Omar *et al*., (2015), the leaf oil of *Boesenbergia plicata* collected from Langkawi, Kedah, obtained by hydrodistillation revealed several major components such as linoleic acid (35.2%), palmitic acid (32%) and β-pinene (11.4%).

Ahmad and Jantan (2003) revealed that the most abundant compound in the rhizome and leaf oils of *Boesenbergia stenophylla* collected from Sarawak, was (E)-methyl cinnamate with 53.4% and 49.9% respectively. The other components present in the rhizome oil were δ-elemene (7.4%) and γ-muurolene (5.1%). β-calacorene (7.7%) and α-humulene (5.3%) were the major components in the leaf oil.

Kar *et al.,* (2014) revealed that longipinocarvone was the major compound in the rhizome oil of *Boesenbergia longiflora* from India with 81.69%. The other components present in the rhizome oil were β-cis-caryophyllene (3.41%), patchoulene (2.97%), borneol (2.32%). Table 2.4 summarize the essential oil constituents of *Boesenbergia* species from previous studies.

Table 2.4: Summary of chemical compositions from the essential oils of selected *Boesenbergia* species.

Species	Locality	Parts	Major compounds	References
Boesenbergia longiflora	Odisha, India	Rhizomes	Longipinocarvone (81.69%)	Kar *et al*., (2014)
Boesenbergia plicata	Langkawi, Kedah.	Leaves	Linoleic acid (35.2%), palmitic acid (32.0%) and β-pinene (11.4%)	Omar *et al.*, (2015)
Boesenbergia rotunda	Kuantan, Pahang	Rhizomes	Nerol (39.56%), L-camphor (36.01%), cineole (9.47%), trans-methyl cinnamate (6.84%)	Baharudin *et al.,* (2015)
	Bangkok, Thailand	Rhizomes	γ-terpinene (44.0%), geraniol (20.6%), 6-camphenone (18.7%), 1,8-cineole (12.8%)	Natta *et al*., (2008)
	Wet wholesale market, Selangor	Rhizomes	Camphor (57.97%), trans-geraniol (6.24%), trans-2-hexanyl-n-propionate (5.59%)	Sukari *et al.,* (2008)
	Bangkok, Thailand	Rhizomes	Trans-β-ocimene (27.0%), camphor (24.0%), 1,8-cineole (17.0%), geraniol (11.0%)	Phanthong *et al.,* (2013)
	Bangkok, Thailand	Rhizomes	Camphor (23.71) 1,8-cineole (16.92), geraniol (10.91)	Kitphati *et al.,* (2012)
Boesenbergia stenophylla	Bario, Sarawak	Rhizomes	(E)-methyl cinnamate (53.4%), δ-elemene (7.4%) and γ-muurolene (5.1%)	Ahmad & Jantan, (2003)
		Leaves	(E)-methyl cinnamate (49.9%), β-calacorene (7.7%) and spathulenol (5.6%)	

Legend:
Components (%) : Major (≥20) (Bakkali *et al.,* 2008)
: Main (≥5)

2.8 *In vitro* tests of antibacterial activity

The active components that were commonly found in the essential oil of Zingiberaceae species have a wide spectrum of antimicrobial activity, against food-borne pathogens and spoilage bacteria (Tripathi *et al*., 2013).

Several bioassays that are widely used and the most known are the disc-diffusion, broth dilution or agar dilution. Nevertheless, disc-diffusion assay offers many advantages over other methods due to its simplicity, low cost, the ability to test enormous numbers of microorganisms and antimicrobial agents and the ease to interpret results provided (Balouiri *et al*., 2016). The concept of this assay was; the wider the inhibition zone, the greater the antibacterial activity occurred (Bauer *et al*., 1966).

In this study, the antibacterial activity of the essential oils has been tested against four foodborne pathogens namely *Staphylococcus aureus, Bacillus cereus, Salmonella enteritidis* and *Escherichia coli* using disc-diffusion assay and their minimum inhibitory concentration (MIC) were determined. MIC was defined as the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation (Andrews, 2001).

Staphylococcus aureus is a non-spore forming Gram-positive cocci that occur in grape-like clusters, which in some strains were able to produce an enterotoxin (Vanderzant & Splittstoesser, 1992; Lowy, 1998). Common infections caused by *S. aureus* include endovascular disorders, respiratory, bone and soft-tissue (Lowy, 1998). According to Oonmetta-aree *et al.,* (2006), ethanol extract of the rhizome of *Alpinia galanga* showed the greatest inhibitory effect against *S. aureus* compared to *Zingiber officinale, Curcuma longa* and *Boesenbergia rotunda* with the minimum inhibitory concentration (MIC) value of 0.325 mg/mL.

Bacillus cereus belongs to the taxonomically complex genus *Bacillus* and basically they are aerobic, endospore-forming and Gram-positive rods that were commonly found in soil and water (Adley, 2006). It spread easily to many types of food especially from plant origin such as rice and pasta, and also frequently isolated from meat, eggs and dairy products (Miksusanti *et al*., 2009). *B. cereus* is responsible for an increasing number of foodborne diseases in industrial countries since it can cause diarrheal and emetic types of food poisonings (Kotiranta *et al*., 2000).

Salmonellae are facultative anaerobic Gram-negative rod shaped bacteria generally 2-5 microns long and motile by peritrichous flagella (Andino & Hanning, 2015). *Salmonella enterica* subspecies *enterica* serovar *enteritidis*, also known as *S. enteritidis* has been identified as a significant cause of salmonellosis in humans. *S. enteritidis* is transmitted to human through poultry meat contaminated at the time of slaughter, consumption of contaminated water and also via consumption of raw or partially cooked eggs (Wisner *et al*., 2010). It frequently causes nausea, headache, abdominal cramps and diarrhea.

Escherichia coli is a facultative anaerobic Gram-negative and non-spore forming rod. It can be found in soil and water as the result of fecal contamination. This species can cause diarrhea, gastrointestinal tract disease, nausea and also loss of appetite (Welch, 2006). Ideally, the researcher found out that most of Gram-positive bacteria tend to be susceptible to inhibition by plant essential oils compared to Gram-negative bacteria due to the presence of thick cell wall in Gram-negative bacteria which block other chemical compounds from penetration.

CHAPTER 3: METHODOLOGY

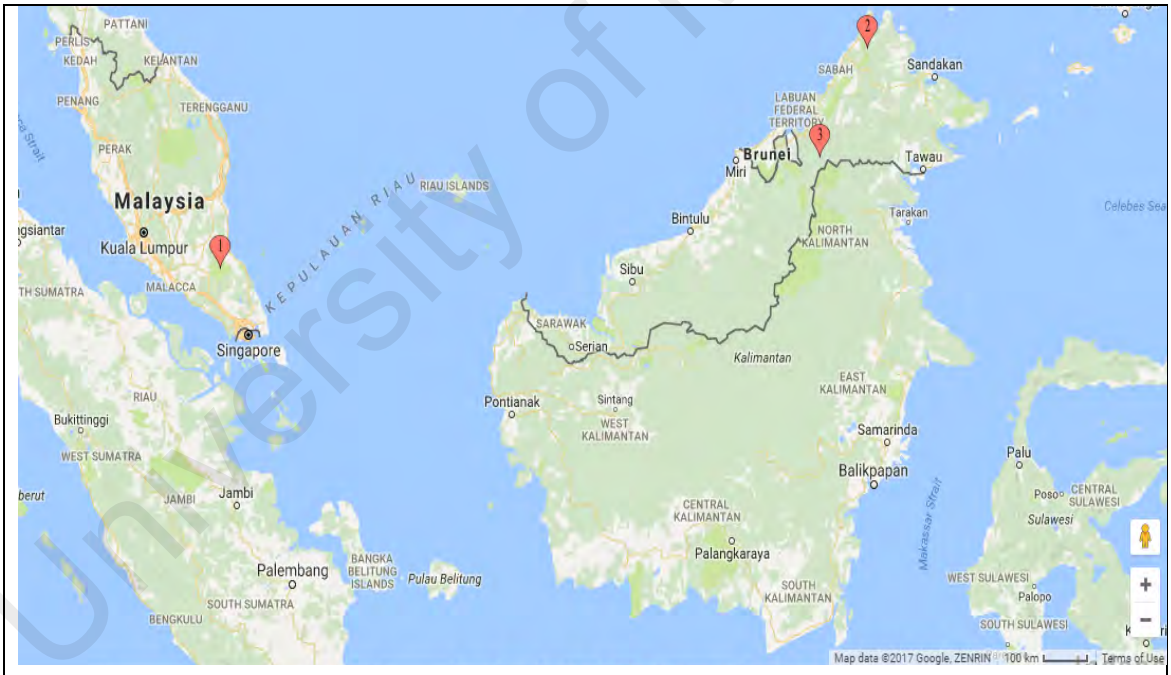
3.1 Plant material

The fresh rhizomes and leaves of five *Boesenbergia* species, namely *Boesenbergia armeniaca, Boesenbergia stenophylla*, *Boesenbergia* sp. *nova, Boesenbergia plicata* and *Boesenbergia prainiana* were studied for their essential oil content. *B. armeniaca* and *Boesenbergia* sp. *nova* were collected at Serinsim, while *B. stenophylla* was collected at Long Pasia, Sabah. *B. plicata* and *B. prainiana* were collected at Taman Negara Endau Rompin, Johor (Table 3.1; Figure 3.1).

B. armeniaca, B. stenophylla and *Boesenbergia* sp. *nova* were identified by a taxonomist at the Institute for Tropical and Conservation Biology of Universiti Malaysia Sabah, Malaysia (UMS). Meanwhile, *B. plicata* and *B. prainiana* were authenticated by Professor Dr. Halijah Ibrahim from University of Malaya. The voucher specimens were prepared as listed in Table 3.1. *B. armeniaca, B. stenophylla* and *Boesenbergia* sp. *nova* were deposited in the BORNEENSIS Herbarium (BORH), at the Institute of Tropical Biology and Conservation, University of Malaysia Sabah (UMS) while *B. plicata* and *B. prainiana* were deposited in the herbarium of University of Malaya (KLU) at Rimba Ilmu Botanic Garden, University of Malaya (UM).

Table 3.1: List of *Boesenbergia* spp. used in this study.

Species (rhizomes and leaves)	Locality	Reference number
Boesenbergia armeniaca	Serinsim, Sabah	BORH3519
Boesenbergia sp. *nova*	Serinsim, Sabah	BORH3520
Besenbergia stenophylla	Long Pasia, Sabah	BORH3521
Boesenbergia plicata	Taman Negara Endau-Rompin, Selai, Johor	KLU 49453
Boesenbergia praininana	Taman Negara Endau-Rompin, Peta, Kahang, Johor	KLU 49071



Legend:

1.Taman Negara Endau-Rompin
2. Serinsim, Sabah
3. Long Pasia, Sabah

Figure 3.1: Location of sample collections for *Boesenbergia* spp. (https://www.google.com/maps/d/)

3.2 Extraction of essential oils

The fresh samples of rhizomes and leaves of *B. armeniaca, B. stenophylla, Boesenbergia* sp. *nova, B. plicata* and *B. prainiana* were washed to remove dirt, chopped into small pieces and ground. Then, they were added with distilled water (5L) and subjected to hydrodistillation using modified Clevenger apparatus (Appendix A) for about 8 hours. About 5-10 mL of pentane was added through the top of the condenser to trap the condensed oil. Later, the mixtures of water and pentane were dried over anhydrous sodium sulphate (Na_2So_4) and the pentane solution was then evaporated by nitrogen blower to give yellowish essential oils. The oils were labelled and stored in dark vials at 4°C for further use.

3.3 GC-MS analyses

The purpose of GC-FID and GC-MS is to analyse and identify the volatile constituents that are present in the essential oils. The essential oils obtained was analyzed by GC using an Agilent GC model 7890 A, equipped with flame ionization detector (FID) (Figure 3.3) and a CBP-5 capillary column (30 m length x 250 µm internal diameter x 0.25 µm film coating). Helium was used as a carrier gas at the flow rate of 1.3 mL/minute. The oven temperature was programmed from 50ºC to 250ºC at 3ºC/minutes with an initial hold time of 1 minute at 50ºC, followed by final hold time of 3 minutes at 230ºC. Detector temperature was maintained at 250ºC. The sample (1 µL) was injected in split ratio (20:1) at 250ºC.

GC/MS analyses were performed on Agilent MSD detector 5975C and a HP-5MS capillary column (30 m length x 250 µm internal diameter x 0.25 µm film coating). The operating conditions were as follows: injection and detector temperatures were set at 250ºC respectively. A series of n-alkanes, C8-C20 and C21-

C40 (Figure 3.4) were subjected to GC-FID to calculate the retention indices (RIs) of samples.

3.4 Identification of components

Identification of essential oil constituents were done on the basis of their retention indices (RI) determined with reference to homologous series of *n*-alkanes, comparison with MS library search (NIST) and confirmed by comparison of retention indices with those of authentic compounds as well as literature data. The preparation of samples and essential oil is shown in Figure 3.2**.**

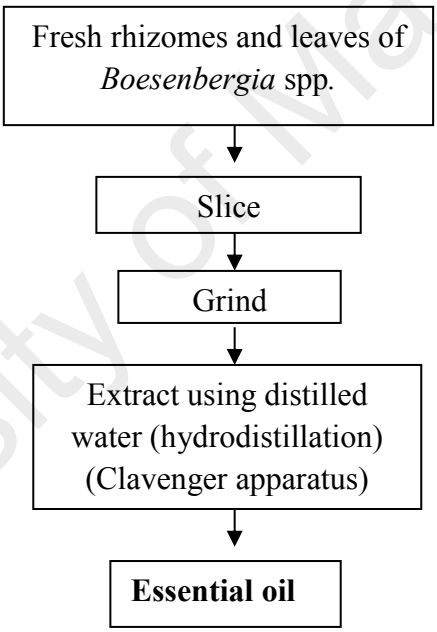


Figure 3.2: Preparation of samples and essential oil

3.4.1 Calculation of percentage yield of essential oil

Mass of vial	= (B)
Mass of vial + essential oil	= (A)
Mass of essential oil	= A– B
	= C
Weight of samples	= D
% yield of essential oil	= C/D × 100%
	= **% yield**

3.4.2 Calculation of Kovat indices

Kovats Index = $100\,[\text{Log}\,(Tx - Tm) - \text{Log}\,(Tn - Tm)] + 100\,(N)$

$$\frac{}{[\text{Log}\,(Tn + 1 - Tm) - \text{Log}\,(Tn - Tm)]}$$

Where:

Tm = Mobile phase retention time

Tx = Sample component retention time

Tn = Standard hydrocarbon containing carbon retention time

N = Lowest carbon value

Figure 3.3: GC-FID for analysis of essential oils

Figure 3.4: Standard carbon for essential oil analysis

3.5 Antibacterial activity

3.5.1 Overview

In this study, antibacterial activity and its minimum inhibitory concentration (MIC) were determined by using disc-diffusion methods against four food-borne pathogens including two Gram-positive bacteria namely *Staphylococcus aureus* (ATCC 6538) and *Bacillus cereus* (ATCC 33109) and two Gram-negative, *Escherichia coli* (ATCC 8739) and *Salmonella enterica* serovar *enteritidis* (ATCC 49223). All the methods are described below.

3.5.2 Chemicals and bacterial strains

Mueller Hinton Agar (MHA), Mueller Hinton Broth (MHB), Nutrient Agar (NA), Nutrient Broth (NB), pentane, methanol (MeOH), were purchased from Choice Care Sdn. Bhd. McFarland standard 0.5 and 6 mm diameter filter paper discs were purchased from Natural Scientific Sdn. Bhd. Kanamycin was purchased from Next Gene Scientific Sdn. Bhd.

Four bacterial strains of *Staphylococcus aureus* (ATCC 6538) and *Bacillus cereus* (ATCC 33109) and two Gram-negative, *Escherichia coli* (ATCC 8739) and *Salmonella enterica* serovar *enteritidis* (ATCC 49223) were purchased from Choice Care Sdn. Bhd.

3.5.3 Inoculum preparation

Four different bacterial strains were used. Two species of Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus cereus* and two Gram-negative bacteria, *Escherichia coli and Salmonella enteritidis* were sub-cultured on nutrient agar at 37°C prior to being grown in nutrient broth overnight. All overnight cultures were

standardised by matching to the McFarland 0.5 turbidity standard using sterile saline to produce approximately 1.5 x 10^8 colony forming units (cfu) per mL.

3.5.4 Antibacterial screening

The effectiveness of the antibacterial activity of the tested essential oils was carried out by disc diffusion assay. The Muller-Hilton agar (MHA) plates were prepared by pouring 20 mL of molten MHA into strile 90-mm Petri plates and allowed to solidify. Sterile 6 mm diameter filter paper discs were impregnated with essential oils that were diluted with methanol (10 mg/mL). Negative control was prepared using the same solvent employed to dissolve the essential oils. Therefore, methanol (MeOH) is used as negative control and kanamycin (50 µg/mL) was used as positive control to determine the sensitivity of one strain in each bacterial species tested. After 18-20 hours of incubation at 37°C, the diameter of the zone of inhibition of bacterial growth around each disc was measured in milimeter (mm) (Sivasothy *et al*., 2012). The results were shown as mean ± standard deviation. All experiments were carried out in three replicates.

In disc-diffusion tests, the level of inhibition of the extracts or oils applied on the microorganisms concerned depend on the growth inhibition diameter (mm) which can be classified as follows (Ponce *et al*., 2003):

- ≥20 mm zone of inhibition = extremely sensitive (+++)
- 15-19 mm zone of inhibition = very sensitive (++)
- 9-14 mm = sensitive (+)
- <8 mm = not sensitive (-)

3.5.5 Determination of minimum inhibitory concentration (MIC)

The MIC value of the essential oils at different concentrations was evaluated according to the method of Bauer *et al*., (1966) with reference to CSLI standard. The inoculated bacteria as prepared from 24 hours nutrient broth cultures and suspensions were adjusted to 0.5 McFarland turbidity standards.

Rhizome oils and leaf oils dissolved in methanol (MeOH) were first diluted to the highest concentration of 5mg/mL to be tested, and then serial two-fold dilutions were made in a concentration range of 5 mg/mL 2.5 mg/mL, 1.25 mg/mL and 0.625 mg/mL. The discs were impregnated in sterile 6-mm paper discs and allowed to dry completely. The discs were then placed on the surface of the agar and the extract was allowed to diffuse for 15 minutes prior to incubation. After 18-20 hours of incubation at 37^{o}C, the diameter of the zone of inhibition around each disc was measured (mm). Discs were impregnated with methanol were used as negative controls and each test was run in triplicate. The lowest concentrations without visible growth were defined as MICs.

CHAPTER 4: RESULTS

4.1 Essential oils of five wild *Boesenbergia* species

In this study, five species of *Boesenbergia* namely *Boesenbergia armeniaca, Boesenbergia stenophylla, Boesenbergia* sp. *nova*, *Boesenbergia plicata* and *Boesenbergia prainiana* were investigated for their chemical constituents of the essential oils from the rhizomes and leaves. The yield of the essential oils was calculated based on the fresh weight of each sample. The percentage of the yield varies from 0.01% (leaf of *Boesenbergia armenica*) to 0.14% (rhizome of *Boesenbergia* sp. *nova*), as shown in Table 4.1 and Figure 4.1.

Table 4.1: Yield and colour of essential oils of five wild *Boesenbergia* species.

Boesenbergia species	Parts	Essential oil (g)	Sample used (g)	Description
Boesenbergia. armeniaca	Rhizome	0.73	800	Golden yellow in colour
	Leaves	0.24	2600	Yellowish in colour
Boesenbegia stenophylla	Rhizome	0.85	1300	Light yellowish in colour
	Leaves	0.59	700	Light yellowish in colour
Boesenbergia sp. *nova*	Rhizome	0.20	140	Yellowish in colour
	Leaves	0.10	230	Whitish in colour
Boesenbergia plicata	Rhizome	0.09	400	Whitish in colour
	Leaves	0.12	500	Yellowish in colour
Boesenbergia prainiana	Rhizome	0.22	400	Light yellow in colour
	Leaves	0.23	450	Yellowish in colour

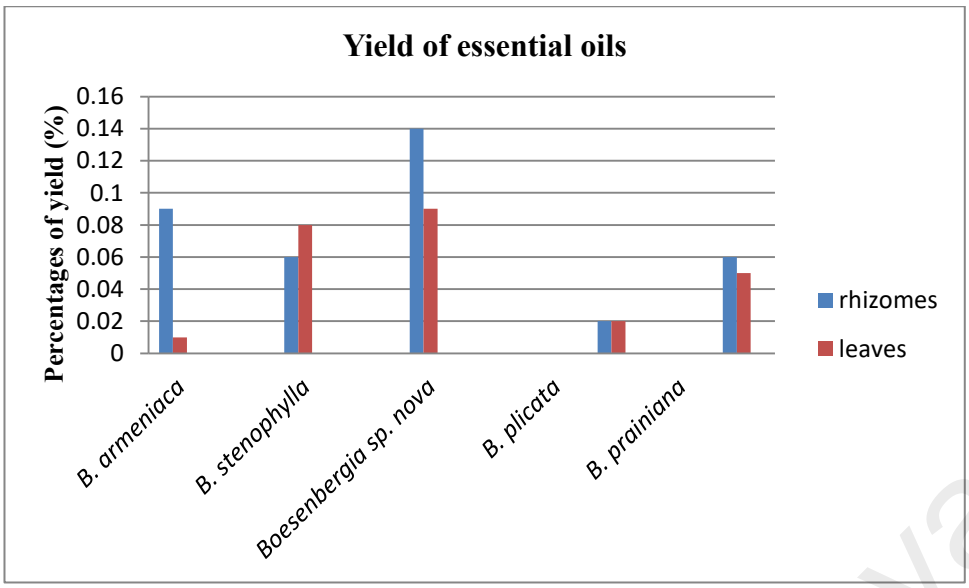


Figure 4.1: Yields of essential oil from five *Boesenbergia* species: *Boesenbergia armeniaca, Boesenbergia stenophylla, Boesenbergia* sp. *nova*, *Boesenbergia plicata* and *Boesenbergia prainiana.*

As can be seen in Figure 4.1, yield of the rhizome oils of *B. armeniaca*, *Boesenbergia* sp. *nova* and *B. prainiana* are higher than the leaf oils. Only yield of the leaf oil of *B. stenophylla* showed a higher percentage than the rhizome oil (more than 0.02%) while *B. plicata* showed the same yield of the rhizome and the leaf oil (0.02%). The yield of the essential oils depends on the significant effects of the extraction type, the duration of the extraction as well as the age the plants of harvesting time (Mejdoub & Katsiotis, 1998: Onyenekwe & Hashimoto, 1999).

In general, the colour of the leaf and rhizome oils range from lighter yellow to intense yellow for instance golden yellow (Table 4.1). All the leaf oils impart a pleasant odour while the rhizome oils emit a woody odour.

4.2 Volatile constituents and composition of the rhizome and leaf oils of five wild *Boesenbergia* species

The identification of volatile constituents and their composition of *Beosenbergia armeniaca, Boesenbergia stenophylla, Boesenbergia* sp. *nova, Boesenbergia plicata* and *Boesenbergia prainiana* will be described in this present study. To the best knowledge of the author, there are no chemical profiling and biological activities reported on the essential oils of this five species except for the chemical composition of essential oils of *Boesenbergia stenophylla* and *Boesenbergia plicata* collected from Sarawak and Langkawi, Kedah respectively. The GC chromatograms of five *Boesenbergia* species are attached in Appendix B – K.

4.2.1 Volatile constituents of the rhizome oil of *Boesenbergia armeniaca* Cowley.

The result of the rhizome oil of *Boesenbergia armeniaca* is listed in Table 4.2. Thirty-four compounds were identified, representing 96.93% of the total oils. The oil comprised of seventeen sesquiterpene hydrocarbons (41.76%), followed by seven oxygenated sesquiterpenes (29.75%), four oxygenated monoterpenes (20.55%), three monoterpene hydrocarbons (3.77%), oxygenated non-terpenes (0.9%) and non-terpene hydrocarbons (0.2%).

The major component was nerolidol (22.77%), while main components comprised of linalool (19.83%), α-gurjunene (14.27%), α-santalene (5.78%) and β-bisabolene (5.40%). Linalool may be responsible for the pleasant odour of this oil and it has been reported to have a relaxing effect (Beier *et al*., 2014; De Sousa, 2012).

Sesquiterpene hydrocarbons form the most abundant class in this oil which include α-gurjunene (14.27%), α-santalene (5.78%) and β-bisabolene (5.40%), 1,4,7 -cycloundecatriene 1599-tetramethyl- z z z- (1.97%), γ-gurjunene (1.86%), (Z)-β-farnesene (1.88%), α-cubebene (1.64%), β-elemene (1.53%), (+)-aromadendrene (1.4%), isosativene (1.34%), 2-isopropyl-5-methyl-9-methylenebicyclo[4.4.0]dec-1-ene (0.99%), β-selinene (0.94%), δ-elemene (0.91%), α-selinene (0.69%) and (-)-aristolene (0.63%).

Oxygenated sesquiterpenes were the second most abundant type of compounds identified; comprising of nerolidol (22.77%), followed by α-eudesmol (2.27%), γ-eudesmol (1.82%), β-eudesmol (1.55%), cis-α-santalol (0.83%), 7R,8R-8-hydroxy-4-isopropylidene-7-methylbicyclo[5.3.1]undec-1-ene (0.3%) and humulene epoxide II (0.21%).

Table 4.2: Chemical components of the rhizome oil of *Boesenbergia armeniaca*

No	Compounds	Group	KI	Composition (%)	RT	Method of ID
1	Methylcyclohexane	NTH	800	0.20	4.719	MS, KI
2	(R)-α-Pinene	MH	937	0.47	8.526	MS, KI
3	β-Pinene	MH	979	2.69	10.109	MS, KI
4	1,8-Cineole	OM	1035	0.14	12.326	MS, KI
5	β-Ocimene	MH	1053	0.61	13.081	MS, KI
6	**Linalool**	**OM**	**1104**	**19.83**	**15.411**	**MS, KI**
7	2-Methyl-3-butyn-2-ol	ONT	1120	0.53	16.055	MS, KI
8	Borneol	OM	1170	0.3	18.270	MS, KI
9	α-Terpineol	OM	1194	0.28	19.414	MS, KI
10	(-)-Aristolene	SH	1380	0.63	27.398	MS, KI
11	β-Elemene	SH	1394	1.53	28.004	MS, KI
12	β-Caryophyllene	SH	1417	0.29	28.933	MS, KI
13	**α-Santalene**	**SH**	**1423**	**5.78**	**29.132**	**MS, KI**
14	δ-Elemene	SH	1437	0.91	29.680	MS, KI
15	α-Guaiene	SH	1441	0.24	29.870	MS, KI
16	α-Cubebene	SH	1446	1.64	30.064	MS, KI
17	Isosativene	SH	1452	1.34	30.273	MS, KI
18	1,4,7 - Cycloundecatriene 1 5 9 9-tetramethyl- z z z-	SH	1457	1.97	30.482	MS
19	(Z)-β-Farnesene	SH	1460	1.88	30.625	MS, KI
20	2-Isopropyl-5-methyl-9-methylenebicyclo[4.4.0]dec-1-ene	SH	1483	0.99	31.574	MS
21	β-Selinene	SH	1489	0.94	31.794	MS, KI
22	α-Selinene	SH	1497	0.69	32.146	MS, KI
23	**α-Gurjunene**	**SH**	**1510**	**14.27**	**32.634**	**MS, KI**
24	γ-Gurjunene	SH	1560	1.86	34.508	MS, KI
25	**Nerolidol**	**OS**	**1570**	**22.77**	**34.879**	**MS, KI**
26	(+)-Aromadendrene	SH	1591	1.40	35.712	MS, KI
27	Humulene epoxide II	OS	1601	0.21	36.136	MS, KI
28	n-Butyl cinnamate	ONT	1612	0.37	36.517	MS, KI
29	γ-Eudesmol	OS	1636	1.82	37.363	MS, KI
30	β-Eudesmol	OS	1655	1.55	38.048	MS,KI
31	α-Eudesmol	OS	1658	2.27	38.160	MS, KI
32	**β-Bisabolene**	**SH**	**1675**	**5.40**	**38.758**	**MS,KI**
33	*cis*-α-Santalol	OS	1701	0.83	39.725	MS, KI
34	7R,8R-8-Hydroxy-4-isopropylidene-7-methylbicyclo[5.3.1]undec-1-ene	OS	1718	0.30	40.295	MS
			Total	**96.93**		

Legend:

MH	: Monoterpene hydrocarbons
OM	: Oxygenated monoterpenes
SH	: Sesquiterpene hydrocarbons
OS	: Oxygenated sesquiterpenes
NTH	: Non-terpene hydrocarbons
ONT	: Oxygenated non-terpenes
Composition (%)	: Obtained by using CBP-5 capillary column
MS	: Mass fragmentation
KI	: Kovats retention indices
RT	**:** Retention time (minutes)
Components (%)	: Major (≥20) (Bakkali *et al.,* 2008)
	: Main (≥5)
	: Trace (≤0.1) (Nampoothiri *et al*., 2012)

4.2.2 Volatile constituents of the leaf oil of *Boesenbergia armeniaca* Cowley.

Table 4.3 lists the volatile constituents from the leaf oil of *Boesenbergia armeniaca.* Thirty-eight compounds were obtained representing 99.24% of the total oil. GC and GC-MS analyses revealed that the major volatile compound of the oil was nerolidol (42.55%), while main compounds which include of linalool (11.63%) and β-caryophyllene (6.25%).

The oils were dominated by oxygenated sesquiterpenes (47.95%). The most abundant compound of this group is nerolidol and it was reported to give floral odour (Chan *et al.*, 2016). The other oxygenated sesquiterpenes are elemol (2.67%), α-eudesmol (0.85%), γ-eudesmol (0.70%), β-eudesmol (0.64%) and cis-α-santalol (0.54%). The oils also comprised significant amount of sesquiterpene hydrocarbons which consist of compounds amounting to 20.69% of the total oil. Those with concentration greater than one percent were β-caryophyllene (6.25%), β-bisabolene (3.96%), α-gurjunene (3.13%), 1,4,7-cycloundecatriene 1599-tetramethyl-zzz- (1.58%) and β-sesquiphellandrene (1.46%).

Three compounds of monoterpene hydrocarbons (5.99%) and six compounds belong to oxygenated monoterpene group(14.49%) made up 20.48% of the total oil. β-pinene was revealed as the most abundant monoterpene hydrocarbon with 4.83% while the most abundant oxygenated monoterpene was linalool with 11.63%.

The other compounds detected in this oil were one diterpene hydrocarbon (0.56%), two oxygenated diterpenes (4.18%), two non-terpene hydrocarbons (1.76%) and four oxygenated non-terpenes (3.62%).

Table 4.3: Chemical components of the leaf oil of *Boesenbergia armeniaca*

No	Compounds	Group	KI	Composition (%)	RT	Method of ID
1	Methylcyclohexane	NTH	800	0.22	4.720	MS, KI
2	(R)-α-Pinene	MH	937	0.84	8.528	MS, KI
3	β-Pinene	MH	979	4.83	10.112	MS, KI
4	1,8-Cineole	OM	1035	0.78	12.324	MS, KI
5	β-Ocimene	MH	1053	0.32	13.086	MS, KI
6	**Linalool**	**OM**	**1103**	**11.63**	**15.396**	**MS, KI**
7	(E)-2-Butenoic acid, 2-(methylenecyclopropyl) prop-2-yl ester	ONT	1120	1.15	16.057	MS
8	Borneol	OM	1170	0.22	18.274	MS, KI
9	L-4-Terpineol	OM	1181	0.42	18.786	MS, KI
10	α-Terpineol	OM	1194	0.91	19.415	MS, KI
11	α-Copaene	SH	1394	0.95	28.004	MS, KI
12	β-Elemene	SH	1417	0.21	28.936	MS, KI
13	**β-Caryophyllene**	**SH**	**1422**	**6.25**	**29.115**	**MS, KI**
14	α-Bergamotene	SH	1439	0.66	29.765	MS, KI
15	α-Elemene	SH	1446	0.32	30.067	MS, KI
16	epi-β-Santalen	SH	1451	0.48	30.247	MS, KI
17	1,4,7 - Cycloundecatriene 1 5 9 9-tetramethyl- z z z-	SH	1457	1.58	30.481	MS, KI
18	β-Sesquiphellandrene	SH	1460	1.46	30.62	MS, KI
19	Isocaryophyllene	SH	1464	0.32	30.772	MS, KI
20	Germacrene D	SH	1484	0.51	31.585	MS, KI
21	α-Gurjunene	SH	1509	3.13	32.619	MS, KI
22	δ-Cadinene	SH	1536	0.46	33.582	MS, KI
23	Hotrienol	OM	1539	0.53	33.721	MS, KI
24	Elemol	OS	1555	2.67	34.302	MS, KI
25	**Nerolidol**	**OS**	**1570**	**42.55**	**34.905**	**MS, KI**
26	4-Methyl-1,5-heptadiene	NTH	1582	1.54	35.383	MS, KI
27	β-Gurjunene	SH	1586	0.40	35.532	MS, KI
28	Isobutyl cinnamate	ONT	1612	0.85	36.505	MS, KI
29	γ-Eudesmol	OS	1637	0.70	37.368	MS, KI
30	β-Eudesmol	OS	1655	0.64	38.047	MS, KI
31	α-Eudesmol	OS	1658	0.85	38.155	MS, KI
32	β-Bisabolene	SH	1675	3.96	38.759	MS, KI
33	*cis*-α-Santalol	OS	1701	0.54	39.728	MS, KI
34	Hexadecanal	ONT	1718	1.07	40.294	MS, KI
35	Isophytol	OD	1952	1.03	48.056	MS, KI
36	Kaurene	DH	2033	0.56	50.574	MS, KI
37	1,6,10,14,18,22 Tetracosahexaen-3-ol,2,6,10,15,19,23-hexamethyl-,(all-E)-	ONT	2039	0.55	50.741	MS
38	Phytol	OD	2117	3.15	53.077	MS, KI
			Total	**99.24%**		

Legend:

MH	: Monoterpene hydrocarbons
OM	: Oxygenated monoterpenes
SH	: Sesquiterpene hydrocarbons
OS	: Oxygenated sesquiterpenes
DH	: Diterpene hydrocarbons
OD	: Oxygenated diterpenes
NTH	: Non-terpene hydrocarbons
ONT	: Oxygenated non-terpenes
Composition (%)	: Obtained by using CBP-5 capillary column
MS	: Mass fragmentation
KI	: Kovats retention indices
RT	**:** Retention time (minutes)
Components (%)	: Major (≥20) (Bakkali *et al.,* 2008)
	: Main (≥5)
	: Trace (≤0.1) (Nampoothiri *et al*., 2012)

4.2.3 Volatile constituents of the rhizome oil of *Boesenbergia stenophylla* R. M. Sm.

Twenty-nine volatile constituents from the rhizome oil of *Boesenbergia stenophylla* were identified and comprising 99.63% of the total oil (Table 4.4). The major compound present in this oil is methyl cinnamate (55.42%). The other main volatile compounds present in this oil are δ-elemene (9.25%) and β-caryophyllene (6.86%).

Oxygenated monoterpene was detected as the most abundant group present in this oil (58.22%) comprising three compounds namely, methyl cinnamate (55.42%), borneol (2.44%) and linalool (0.36%). This oil could be a good source of fragrant ingredient such as fine fragrances and shampoos since methyl cinnamate is reported to have a pleasant and strong aromatic constituent (Sharma & Kanwar, 2012).

Sixteen sesquiterpene hydrocarbons were detected in this oil which comprised of 34.02%. δ-elemene (9.25%), β-caryophyllene (6.86%), germacrene D (3.68%), α-santalene (3.49%) where the compounds are present in appreciable amounts, while the other twelve compounds were present in low concentrations.

Monoterpene hydrocarbons comprised of seven volatile compounds (5.92%) which include of β-pinene (2.64%), R-α-pinene (1.16%), cis-β-ocimene (0.71%), camphene (0.57%), α-terpinene (0.39%), myrcene (0.25%) and limonene (0.2%). The rest of the oils were made up of one oxygenated sesquiterpene (1.06%), one diterpene hydrocarbon (0.29%) and one non-terpene hydrocarbon (0.12%).

Table 4.4: Chemical components of the rhizome oil of *Boesenbergia stenophylla*

No	Compounds	Group	KI	Composition (%)	RT	Method of ID
1	Methylcyclohexane	NTH	800	0.12	4.718	MS, KI
2	(R)-α-Pinene	MH	937	1.16	8.523	MS, KI
3	Camphene	MH	952	0.57	9.055	MS, KI
4	β-Pinene	MH	979	2.64	10.106	MS, KI
5	Myrcene	MH	993	0.25	10.694	MS, KI
6	Limonene	MH	1032	0.20	12.218	MS, KI
7	*cis*-β-Ocimene	MH	1053	0.71	13.077	MS, KI
8	Linalool	OM	1103	0.36	15.371	MS, KI
9	Borneol	OM	1170	2.44	18.264	MS
10	**δ-EIemene**	**SH**	**1341**	**9.25**	**25.717**	**MS, KI**
11	α-Copaene	SH	1378	1.51	27.303	MS, KI
12	**Methyl cinnamate**	**OM**	**1391**	**55.42**	**27.872**	**MS, KI**
13	**β-Caryophyllene**	**SH**	**1395**	**6.86**	**28.039**	**MS, KI**
14	α-Santalene	SH	1422	3.49	29.129	MS, KI
15	α-Bergamotene	SH	1439	0.80	29.761	MS, KI
16	Epizonarene	SH	1446	0.32	30.062	MS, KI
17	Isosativene	SH	1451	0.71	30.267	MS, KI
18	1,4,7 - Cycloundecatriene 1 5 9 9-tetramethyl- z z z-	SH	1457	2.47	30.481	MS, KI
19	Allo-aromadendrene	SH	1460	0.34	30.625	MS, KI
20	(Z)-β-Farnesene	SH	1463	0.31	30.754	MS, KI
21	Aromadendrene	SH	1479	0.69	31.402	MS, KI
22	Germacrene D	SH	1484	3.68	31.59	MS, KI
23	α-Cadinene	SH	1498	0.47	32.174	MS, KI
24	β-Bisabolene	SH	1509	0.53	32.61	MS, KI
25	α-Terpinene	SH	1511	0.39	32.679	MS, KI
26	δ-Cadinene	SH	1527	1.02	33.248	MS, KI
27	γ-Elemene	SH	1560	1.57	34.504	MS, KI
28	Ledene oxide	OS	1634	1.06	37.272	MS, KI
29	Kaurene	DH	2038	0.29	50.724	MS, KI
			Total	**99.63**		

Legend:

MH : Monoterpene hydrocarbons
OM : Oxygenated monoterpenes
SH : Sesquiterpene hydrocarbons
OS : Oxygenated sesquiterpenes
DH : Diterpene hydrocarbons
NTH : Non-terpene hydrocarbons
Composition (%) : Obtained by using CBP-5 capillary column
MS : Mass fragmentation
KI : Kovats retention indices
RT **:** Retention time (minutes)
Components (%) : Major (≥20) (Bakkali *et al.,* 2008)
: Main (≥5)
: Trace (≤0.1) (Nampoothiri *et al*., 2012)

4.2.4 Volatile constituents of the leaf oil of *Boesenbergia stenophylla* R. M. Sm.

The volatile constituents from the leaf oil of *Boesenbergia stenophylla* are presented in Table 4.5. Twenty-three compounds were identified and comprising 100% of the total oil. The essential oil mainly consist of oxygenated monoterpenes (85.12%), followed by monoterpene hydrocarbons (6.78%) and sesquiterpene hydrocarbons (6.44%).

The oil is dominated by oxygenated monoterpenes representing 85.12%. This oil is rich in methyl cinnamate (83.17%). As mentioned before, methyl cinnamate was reported to have pleasant fragrance, hence responsible for the pleasant odour of this oil (Sharma & Kanwar, 2012).

Monoterpene hydrocarbons were the second abundant type of compounds identified representing 13.22% of the total oil. Six compounds of this group are β-pinene (4.84%), (R)-α-pinene (1.22%), β-ocimene (0.27%), camphene (0.23%), limonene (0.11%) and β-phellandrene (0.11%).

The rest of the oil was made up of non-terpene hydrocarbons (0.75%), oxygenated sesquiterpenes (0.51%) and diterpene hydrocarbons (0.4%).

Table 4.5: Chemical components of the leaf oil of *Boesenbergia stenophylla*

No	Compounds	Group	KI	Composition (%)	RT	Method of ID
1	Methylcyclohexane	NTH	800	0.14	4.717	MS, KI
2	(R)-α-Pinene	MH	937	1.22	8.522	MS, KI
3	Camphene	MH	952	0.23	9.055	MS, KI
4	**β-Pinene**	**MH**	**979**	**4.84**	**10.107**	**MS, KI**
5	β-Phellandrene	MH	993	0.11	10.694	MS, KI
6	Limonene	MH	1032	0.11	12.218	MS, KI
7	β-Ocimene	MH	1053	0.27	13.077	MS, KI
8	Linalool	OM	1103	1.18	15.37	MS, KI
9	Borneol	OM	1170	0.55	18.263	MS, KI
10	α-Terpineol	OM	1194	0.22	19.409	MS, KI
11	δ-Elemene	SH	1341	0.31	25.698	MS, KI
12	α-Copaene	SH	1378	0.21	27.302	MS, KI
13	**Methyl cinnamate**	**OM**	**1392**	**83.17**	**27.903**	**MS, KI**
14	β-Caryophyllene	SH	1322	0.82	29.11	MS, KI
15	1,4,7 - Cycloundecatriene 1 5 9 9-tetramethyl- z z z-	SH	1357	3.59	30.486	MS
16	Germacrene D	SH	1384	0.58	31.583	MS, KI
17	γ-Elemene	SH	1526	0.21	33.244	MS, KI
18	δ-Cadinene	SH	1560	0.22	34.505	MS, KI
19	Elixene	SH	1568	0.50	34.827	MS, KI
20	Nerolidol	OS	1582	0.19	35.37	MS, KI
21	Humulene epoxide II	OS	1612	0.32	36.508	MS, KI
22	1,7,7-Trimethyl-2-vinylbicyclo[2.2.1]hept-2-ene	NTH	1634	0.61	37.272	MS, KI
23	Kaurene	DH	2038	0.40	50.727	MS, KI
			Total	**100.00**		

Legend:

MH : Monoterpene hydrocarbons
OM : Oxygenated monoterpenes
SH : Sesquiterpene hydrocarbons
OS : Oxygenated sesquiterpenes
DH : Diterpene hydrocarbons
NTH : Non-terpene hydrocarbons
Composition (%) : Obtained by using CBP-5 capillary column
MS : Mass fragmentation
KI : Kovats retention indices
RT **:** Retention time (minutes)
Components (%) : Major (≥20) (Bakkali *et al.,* 2008)
: Main (≥5)
: Trace (≤0.1) (Nampoothiri *et al*., 2012)

4.2.5 Volatile constituents of the rhizome oil of *Boesenbergia* sp. *nova*

The rhizome oil of *Boesenbergia* sp. *nova* contains forty-one constituents comprising of 97.73% of the total oil (Table 4.6).

The major group was dominated by sesquiterpene hydrocarbons representing 77.77% of the total oil such as γ-maaliene (18.43%), 4,11-selinadiene (10.18%), α-panansinene (8.9%), α-cubebene (7.27%), β-cadinene (6.67%), β-selinene (4.08%), β-bisabolene (3.62%), γ-muurolene (2.91%), δ-cadinene (2.62%) and β-caryophyllene (2.11%).

Oxygenated sesquiterpenes constitute the second abundant group with 9.9% of the total oils. The most abundant compound of this group is palustrol (4.21%). This is followed by ledene oxide (I) (1.62%), spathulenol (1.24%), humulene epoxide II (1.16%), longifolenaldehyde (0.71%), nootkatone (0.54%).

The oil also comprised three oxygenated non-terpenes (2.98%), one oxygenated monoterpene (2.63%), four monoterpene hydrocarbons (2.06%), two non-terpene hydrocarbons (0.73%) and miscellaneous (1.66%).

Table 4.6: Chemical components of the rhizome oil of *Boesenbergia* sp. *nova*

No	Compounds	Group	KI	Composition (%)	RT	Method of ID
1	Methylcyclohexane	NTH	800	0.16	4.717	MS,KI
2	(R)-α-Pinene	MH	937	0.48	8.523	MS, KI
3	Camphene	MH	952	0.46	9.056	MS, KI
4	β-Pinene	MH	979	0.59	10.107	MS, KI
5	Borneol	OM	1170	2.63	18.264	MS
6	**α-Cubebene**	**SH**	**1379**	**7.27**	**27.316**	**MS, KI**
7	β-Cubebene	SH	1394	0.45	28.000	MS, KI
8	β-Elemene	SH	1417	0.33	28.930	MS, KI
9	β-Caryophyllene	SH	1422	2.11	29.099	MS, KI
10	α-Santalene	SH	1439	0.28	29.760	MS, KI
11	(+)-Aromadendrene	SH	1446	1.86	30.056	MS, KI
12	1,4,7-Cycloundecatriene 1 5 9 9-tetramethyl- z z z-	SH	1457	1.47	30.477	MS, KI
13	(E)-β-Farnesene	SH	1461	2.02	30.643	MS, KI
14	**4,11-Selinadiene**	**SH**	**1486**	**10.18**	**31.684**	**MS, KI**
15	β-Selinene	SH	1489	4.08	31.809	MS, KI
16	**γ-Maaliene**	**SH**	**1496**	**18.43**	**32.090**	**MS, KI**
17	α-Muurolene	SH	1502	0.39	32.348	MS, KI
18	β-Bisabolene	SH	1512	3.62	32.695	MS, KI
19	**α-Panansinene**	**SH**	**1521**	**8.90**	**33.028**	**MS, KI**
20	δ-Cadinene	SH	1527	2.62	33.253	MS, KI
21	1,1,6-Trimethyl-1,2-dihydronaphthalene	NTH	1547	0.57	34.007	MS, KI
22	Chloro(ethyl)diisopropylsilane	OT	1569	0.49	34.840	MS
23	Spathulenol	OS	1582	1.24	35.356	MS, KI
24	11-Oxatetracyclo[5.3.2.0(2,7).0(2,8)]dodecan-9-one	ONT	1586	1.93	35.525	MS
25	(E)-Ocimene	MH	1608	0.53	36.360	MS, KI
26	Humulene epoxide II	OS	1612	1.16	36.517	MS, KI
27	Allo-aromadendrene	SH	1616	1.85	36.652	MS, KI
28	Ledene oxide (I)	OS	1631	1.62	37.187	MS, KI
29	**1-{2-[2-Methyl-2-(5-methyl-2-furyl)propyl]cyclopropyl}ethanone**	ONT	1642	0.71	37.581	MS
30	γ-Muurolene	SH	1660	2.91	38.228	MS, KI
31	**β-Cadinene**	**SH**	**1664**	**6.67**	**38.347**	**MS**
32	Palustrol	OS	1674	4.21	38.745	MS, KI
33	α-Guaiene	SH	1682	1.55	39.043	MS, KI
34	2(1H)-Quinolinone, 1-methyl-	OT	1718	0.45	39.775	MS, KI
35	Diepi-α-cedrene epoxide	OS	1726	0.16	40.118	MS, KI

Table 4.6, Continued,

No	Compounds	Group	KI	Composition (%)	RT	Method of ID
36	Longifolenaldehyde	OS	1731	0.71	40.300	MS, KI
37	7R,8R-8-Hydroxy-4-isopropylidene-7-methylbicyclo[5.3.1]undec-1-ene	OS	1735	0.26	40.464	MS
38	Valencene	SH	1781	0.78	42.334	MS, KI
39	Nootkatone	OS	1809	0.54	43.458	MS, KI
40	5,6,7,8-Tetrahydro-2-methyl-1,4-naphthoquinone	ONT	1820	0.34	43.808	MS
41	1,3,5-Triphenyl-4,5-dihydro-1H-pyrazole	OT	2490	0.72	63.219	MS
			Total	**97.73**		

Legend:

MH : Monoterpene hydrocarbons
OM : Oxygenated monoterpenes
SH : Sesquiterpene hydrocarbons
OS : Oxygenated sesquiterpenes
NTH : Non-terpene hydrocarbons
ONT : Oxygenated non-terpenes
OT : Other
Composition (%) : Obtained by using CBP-5 capillary column
MS : Mass fragmentation
KI : Kovats retention indices
RT **:** Retention time (minutes)
Components (%) : Major (≥20) (Bakkali *et al.,* 2008)
: Main (≥5)
: Trace (≤0.1) (Nampoothiri *et al*., 2012)

4.2.6 Volatile constituents of the leaf oil of *Boesenbergia* sp. *nova*

Table 4.7 lists the volatile constituents of the leaf oil of *Boesenbergia* sp. *nova*. Forty compounds representing 96.16% of the oil were identified. The essential oil consisted mainly sesquiterpene hydrocarbons (74.25%), followed by oxygenated sesquiterpenes (5.02%), monoterpene hydrocarbons (3.87%), oxygenated monoterpenes (3.02%) and oxygenated diterpene (0.64%).

Sesquiterpene hydrocarbons formed the most abundant group in this oil. The oils were rich in γ-maaliene with 22.82%, α-panansinene (9.12%) and α-cubebene (8.98%). Oxygenated sesquiterpenes with the total yield of 5.02% showed the presence of compounds such as caryophyllene oxide (1.9%), ledene oxide-(II) (1.29%), isoaromadendrene epoxide (1.3%) and nootkatone (0.53%).

Monoterpene hydrocarbons and oxygenated monoterpenes made up of 6.89% of this oil. Three monoterpene hydrocarbons comprised of 3.87% of the total oil with the presence of β-pinene (2.81%), (-)-α-pinene (0.93%) and camphene (0.13%). Meanwhile, five compounds of oxygenated monoterpenes were borneol (1.05%), myrtenol (0.79%), α-cyclocitral (0.64%), L-trans-pinocarveol (0.28%) and pinocarvone (0.26%).

The other volatile constituents that were detected in this oil comprised of oxygenated non-terpene (3.86%), non-terpene hydrocarbons (0.74%), one oxygenated diterpene, phytol (0.64%), and miscellaneous (4.76%).

Table 4.7: Chemical components of the leaf oil of *Boesenbergia* sp. *nova*

No	Compounds	Group	KI	Composition (%)	RT	Method of ID
1	Methylcyclohexane	NTH	800	0.17	4.718	MS,KI
2	(-)-α-Pinene	MH	934	0.93	8.524	MS, KI
3	Camphene	MH	948	0.13	9.058	MS, KI
4	β-Pinene	MH	972	2.81	10.108	MS, KI
5	L-*trans*-pinocarveol	OM	1143	0.28	17.047	MS, KI
6	Pinocarvone	OM	1166	0.26	18.077	MS, KI
7	Borneol	OM	1170	1.05	18.265	MS
8	Myrtenol	OM	1199	0.79	19.637	MS, KI
9	**α-Cubebene**	**SH**	**1379**	**8.98**	**27.322**	**MS, KI**
10	β-Bourbonene	SH	1387	2.29	27.685	MS, KI
11	β-Elemene	SH	1394	0.64	28.002	MS, KI
12	β-Caryophyllene	SH	1422	2.97	29.097	MS, KI
13	β-Cubebene	SH	1432	0.57	29.491	MS, KI
14	α-Bergamotene	SH	1439	0.14	29.76	MS, KI
15	Allo-aromadendrene	SH	1454	0.29	30.354	MS, KI
16	α-Caryophyllene	SH	1457	0.62	30.478	MS, KI
17	**γ-Selinene**	**SH**	**1486**	**6.28**	**31.682**	**MS, KI**
18	**β-Selinene**	**SH**	**1489**	**6.31**	**31.807**	**MS, KI**
19	**γ-Maaliene**	**SH**	**1496**	**22.82**	**32.096**	**MS, KI**
20	β-Bisabolene	SH	1511	1.83	32.692	MS, KI
21	**α-Panansinene**	**SH**	**1521**	**9.12**	**33.03**	**MS, KI**
22	δ-Cadinene	SH	1527	1.96	33.251	MS, KI
23	1,1,6-Trimethyl-1,2-dihydronaphthalene	NTH	1547	0.57	34.009	MS, KI
24	α-Calacorene	SH	1569	0.45	34.84	MS, KI
25	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene	SH	1582	0.66	35.354	MS, KI
26	Caryophyllene oxide	OS	1586	1.90	35.524	MS, KI
27	Pentafluoropropionic acid, 1-adamantyl methyl ester	OT	1592	1.79	35.749	MS
28	Isoaromadendrene epoxide	OS	1613	1.30	36.53	MS, KI
29	Mayurone	ONT	1616	1.34	36.658	MS, KI
30	α-Cyclocitral	OM	1626	0.64	36.995	MS, KI
31	*Ethanone,1-(1,3a,4,5,6,7-hexahydro-4-hydroxy-3,8-dimethyl-5-azulenyl)-*	ONT	1633	2.52	37.24	MS, KI
32	Phenacetic acid, 2-carbmethoxy-	OT	1642	0.93	37.56	MS
33	Viridiflorene	SH	1647	0.82	37.761	MS, KI

Table 4.7, Continued,

No	Compounds	Group	KI	Composition (%)	RT	Method of ID
34	**Patchoulene**	**SH**	**1664**	**5.83**	**38.347**	**MS, KI**
35	2,4-Dimethylquinoline	OT	1674	2.04	38.716	MS, KI
36	*1R,3Z,9S-2,6,10,10 Tetramethylbicyclo[7. 2.0]undeca-2,6-diene*	SH	1735	0.58	38.914	MS, KI
37	α-Guaiene	SH	1781	1.09	39.041	MS, KI
38	Ledene oxide-(II)	OS	1809	1.29	39.347	MS, KI
39	Nootkatone	OS	1820	0.53	42.334	MS, KI
40	Phytol	OD	2490	0.64	53.065	MS, KI
			Total	**96.16**		

Legend:

MH : Monoterpene hydrocarbons
OM : Oxygenated monoterpenes
SH : Sesquiterpene hydrocarbons
OS : Oxygenated sesquiterpenes
OD : Oxygenated diterpenes
NTH : Non-terpene hydrocarbons
ONT : Oxygenated non-terpenes
OT : Other
Composition (%) : Obtained by using CBP-5 capillary column
MS : Mass fragmentation
KI : Kovats retention indices
RT **:** Retention time (minutes)
Components (%) : Major (≥20) (Bakkali *et al.,* 2008)
: Main (≥5)
: Trace (≤0.1) (Nampoothiri *et al*., 2012)

4.2.7 Volatile constituents of the rhizome oil of *Boesenbergia plicata* (Ridl.) Holttum var. *plicata*

The volatile constituents of the rhizome oil of *Boesenbergia plicata* is presented in Table 4.8. Thirty-three volatile compounds were identified, representing 71.25% of the total oil. Monoterpene hydrocarbons were detected as the most abundant group present in this oil. The oil consisted of isocamphene as the major compound (28.07%).

Six compounds of sesquiterpene hydrocarbons (4.57%) and three compounds of oxygenated sesquiterpenes (8.89%) with the total yield of 13.46% were identified in this oil. Six compounds of sesquiterpene hydrocarbons were identified namely, thujopsene (1.81%), allo-aromadendrene (1.11%), α-longipinene (0.64%), γ-selinene (0.40%), (Z)-α-farnesene (0.31%) and 6,10-dimethyl-3-(1-methylethylidene)-1-cyclodecene (0.30%), while, oxygenated sesquiterpenes comprising of longiborneol (6.90%), 1-pentadecanal (1.77%) and p-heptylacetophenone (0.22%) were detected.

The other components discovered in this oil were oxygenated non-terpenes (13.09%), non-terpene hydrocarbons (7.83%), one oxygenated diterpene; retinal (1.06%) and miscellaneous (6.96%). No oxygenated monoterpenes were detected.

Table 4.8: Chemical components of the rhizome oil of *Boesenbergia plicata*

No	Compounds	Group	KI	Composition (%)	RT	Method of ID
1	Cyclohexane, 1,2,4 tris (methylene)-	NTH	1363	0.19	29.027	KI,MS
2	γ-Selinene	SH	1398	0.40	30.621	KI,MS
3	(+)-2-Carene, 4-.alpha.-isopropenyl-	NTH	1460	1.21	31.173	MS
4	L-β-Pinene	MH	1489	0.78	33.764	KI,MS
5	α-Longipinene	SH	1492	0.64	34.046	KI,MS
6	(Z)-α-Farnesene	SH	1495	0.31	34.29	KI,MS
7	Bicyclo[4.1.0]heptane, 7-methylene-	NTH	1593	0.69	37.68	MS
8	p-Heptylacetophenone	OS	1594	0.22	37.835	KI,MS
9	Tricyclo[3.1.0.0(2,4)]hexane, 3,6-diethyl-3,6-dimethyl-, trans-	NTH	1597	0.74	38.315	MS
10	6,10-Dimethyl-3-(1-methylethylidene)-1-cyclodecene	SH	1682	0.30	38.873	MS
11	Neoisolongifolene, 8-bromo-	OT	1685	0.33	39.583	KI,MS
12	Benzene, 1,2-bis(1-buten-3-yl)-	NTH	1687	0.96	39.82	KI,MS
13	1-Pentadecanal	OS	1787	1.77	42.651	KI,MS
14	**Longiborneol**	**OS**	**1792**	**6.90**	**43.831**	**KI,MS**
15	1-Ethyldecalin, trans	NTH	1799	2.57	45.627	KI,MS
16	Pentadecanoic acid	ONT	1895	0.80	47.725	KI,MS
17	Thujopsene	SH	1896	1.81	48.181	MS
18	Epirizole	OT	1898	0.44	48.79	KI,MS
19	Podocarpan-12-ol	ONT	1900	1.37	49.295	MS
20	Butyl 2-ethylhexylphthalated	OT	1995	0.34	50.653	KI,MS
21	Hexadecanoic acid	ONT	1997	0.36	51.55	KI,MS
22	Alloaromadedrene	SH	2093	1.11	52.879	KI,MS
23	4-Hydroxy-3a,7a-dimethly-4,5-dihydro-3H-2-benzofuran-1-one	OT	2095	0.38	53.435	MS
24	2,2,7-Trimethyl-3-octyne	NTH	2097	0.90	54.524	MS
25	Retinal	OD	2194	1.06	55.839	KI,MS
26	**Linoleic acid**	ONT	2195	**5.99**	**56.389**	**KI,MS**
27	9,17-Octadecadienal	ONT	2196	2.58	56.549	KI,MS
28	2H-Phenanthro[9,10-b]pyran	ONT	2197	1.77	57.2	MS
29	Octadecanoic acid	ONT	2198	0.22	57.57	KI,MS
30	1H-Indene	NTH	2199	0.57	57.973	KI,MS
31	Tricyclo[3.2.1.0(2,4)] octane-3-carboxamide, N-(4-ethoxyphenyl)-	OT	2397	1.01	62.194	MS

Table 4.8, Continued,

No	Compounds	Group	KI	Composition (%)	RT	Method of ID
32	Trichloroacetic acid, 2-methyloct-5-yn-4-yl ester	OT	2397	4.46	62.427	KI,MS
33	**Isocamphene**	**MH**	**2399**	**28.07**	**63.596**	**KI,MS**
			Total	**71.25**		

Legend:

MH : Monoterpene hydrocarbons
SH : Sesquiterpene hydrocarbons
OS : Oxygenated sesquiterpenes
OD : Oxygenated diterpenes
NTH : Non-terpene hydrocarbons
ONT : Oxygenated non-terpenes
OT : Other
Composition (%) : Obtained by using CBP-5 capillary column
MS : Mass fragmentation
KI : Kovats retention indices
RT **:** Retention time (minutes)
Components (%) : Major (≥20) (Bakkali *et al.,* 2008)
: Main (≥5)
: Trace (≤0.1) (Nampoothiri *et al*., 2012)

4.2.8 Volatile constituents of the leaf oil of *Boesenbergia plicata* (Ridl.) Holttum var. *plicata*

Thirty-nine components representing 99.97% of the leaf oil of *Boesenbergia plicata* were identified (Table 4.9). The main components were monoterpene hydrocarbons; (-)-β-pinene (21.33%), oxygenated diterpenes; phytol (14.52%) and sesquiterpene hydrocarbons; β-caryophyllene (9.85%).

The leaf oil was dominated by five compounds of monoterpene hydrocarbons (26.65%), comprising of (-)-β-pinene (21.33%), (E)-ocimene (2.53%), D-limonene (1.94%), isodurene (0.59%) and terpinolene (0.26%).

There were eight sesquiterpene hydrocarbons with the total yield of 19.08%. The most abundant compound in this group is β-caryophyllene (9.85%). This is followed by Selinene (3.41%), 4,11-selinadiene (1.56%), bergamotene (1.19%), 6,10-dimethyl-3-(1-methylethylidene)-1-cyclodecene (0.95%), β-bisabolene (0.80%), β-elemene (0.79%) and β-sesquiphellandrene (0.53%).

This oil also comprised significant amount of oxygenated diterpenes. There are two compounds amounting to 15.29% of the total oil; phytol (14.52%) and 3,7,11,15-tetramethylhexadec-1-en-3-ol (0.77%). The other group of compounds detected in this oil were five oxygenated monoterpenes (8.76%) such as (-)-myrtenol (3.84%) and linalool (3.21%), oxygenated non-terpenes (14.69%), oxygenated sesquiterpenes (8.1%), non-terpene hydrocarbons (1.86%) and miscellaneous (5.54%).

Table 4.9: Chemical components of the leaf oil of *Boesenbergia plicata*

No	Compounds	Group	KI	Composition (%)	RT	Method of ID
1	(E)-Ocimene	MH	937	2.53	10.035	KI,MS
2	**(-)-β-Pinene**	**MH**	**981**	**21.33**	**11.856**	**KI,MS**
3	D-Limonene	MH	1033	1.94	14.058	KI,MS
4	Linalool	OM	1100	3.21	17.269	KI,MS
5	L-Borneol	OM	1176	1.05	20.676	KI,MS
6	(-)-Myrtenol	OM	1198	3.84	21.744	KI,MS
7	6,6-Dimethylspiro[2,3-diazabicyclo[3.1.0]hex-2-ene-4,1'-cyclopropane]	OT	1296	0.57	26.204	MS
8	Myrtenyl acetate	ONT	1325	0.36	27.397	KI,MS
9	6,8-Nonadien-2-one, 6-methyl-5-(1-methylethylidene)-	ONT	1331	0.30	27.647	KI,MS
10	Terpinolene	MH	1337	0.26	27.891	KI,MS
11	2-Oxa-1,3-disilacyclohexane,1,1,3,3-tetramethyl-	OT	1387	2.84	30.088	MS
12	β-Elemene	SH	1390	0.79	30.228	KI,MS
13	Isodurene	MH	1410	0.59	31.079	KI,MS
14	**β-Caryophyllene**	**SH**	**1421**	**9.85**	**31.529**	**KI,MS**
15	Bergamotene	SH	1434	1.19	32.032	KI,MS
16	β-Ionone	ONT	1479	0.84	33.915	KI,MS
17	Selinene	SH	1490	3.41	34.376	KI,MS
18	4,11-Selinadiene	SH	1496	1.56	34.643	KI,MS
19	β-Bisabolene	SH	1508	0.80	35.086	KI,MS
20	β-Sesquiphellandrene	SH	1525	0.53	35.724	KI,MS
21	Nerolidol	OS	1562	2.65	37.175	KI,MS
22	3-Undecen-5-yne, (E)-		1583	0.59	38.031	MS
23	6,10-Dimethyl-3-isopropylidene-1-cyclodecene	SH	1634	0.95	39.939	MS
24	5-Methylene-1,3a,4,5,6,6a-hexahydropentalen-1-ol	ONT	1640	0.73	40.133	KI,MS
25	1,3,4-Trimethyl-3-cyclohexen-1-carboxaldehyde	OM	1660	0.37	40.904	KI,MS
26	8-Heptadecene	NTH	1678	0.93	41.582	KI,MS
27	Bicyclo[3.1.1]hept-3-ene, 2-formylmethyl-4,6,6-trimethyl-	ONT	1691	0.40	42.048	KI,MS
28	**n-Pentadecanal**	**OS**	**1716**	**5.45**	**42.966**	**KI,MS**
29	6.beta.Bicyclo[4.3.0]nonane, 5.beta.-iodomethyl-1.beta.-isopropenyl-4.alpha.,5.alpha.-dimethyl-	OT	1746	1.72	43.987	MS

Table 4.9, Continued,

No	Compounds	Group	KI	Composition (%)	RT	Method of ID
30	2-Butyl-2-ethyl-5-methyl-3,4-hexadienal	ONT	1800	1.55	45.952	MS
31	Hexahydrofarnesyl acetone	ONT	1841	1.48	47.303	KI,MS
32	6-Dodecyne	NTH	1886	0.34	48.828	KI,MS
33	3,7,11,15-tetramethylhexadec-1-en-3-ol	OD	1945	0.77	50.737	KI,MS
34	Dibutyl phthalate	OT	1953	0.41	50.976	KI,MS
35	**Hexadecanoic acid**	**ONT**	**1964**	**7.71**	**51.339**	**KI,MS**
36	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane	ONT	1979	0.61	51.834	MS
37	α-Ionene	ONT	2074	0.71	54.790	KI,MS
38	**Phytol**	**OD**	**2107**	**14.52**	**55.814**	**KI,MS**
39	*cis*-4-Decenal	OM	2174	0.29	57.755	KI,MS
			Total	**99.97**		

Legend:

MH : Monoterpene hydrocarbons
OM : Oxygenated monoterpenes
SH : Sesquiterpene hydrocarbons
OS : Oxygenated sesquiterpenes
OD : Oxygenated diterpenes
NTH : Non-terpene hydrocarbons
ONT : Oxygenated non-terpenes
OT : Other
Composition (%) : Obtained by using CBP-5 capillary column
MS : Mass fragmentation
KI : Kovats retention indices
RT **:** Retention time (minutes)
Components (%) : Major (≥20) (Bakkali *et al.,* 2008)
: Main (≥5)
: Trace (≤0.1) (Nampoothiri *et al*., 2012)

4.2.9 Volatile constituents of the rhizome oil of *Boesenbergia prainiana* (King ex Baker) Schltr.

The volatile constituents of the rhizome oil of *Boesenbergia prainiana* is listed in Table 4.10. A total of sixty-six compounds were identified from this oil amounting to 97.73% of the total oil. GC and GC-MS analyses revealed that the main compounds of the oils were isolimonene (10.09%), L-β-pinene (9.51%), borneol (7.52%), 3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)- (5.05%) and β-elemene (5.00%).

Hydrocarbons were the principal constituents of this oil (54.04%). It comprised of ten monoterpenes (27.4%) and twenty-two sesquiterpenes (26.66%). Isolimonene (10.09%) and L-β-pinene (9.51%) were the compounds that are present in appreciable amounts while the other eight compounds were present in low concentrations. Meanwhile, the compounds for sesquiterpenes were β-elemene (5.00%), α-selinene (2.64%), 4,5-di-epi-aristolochene (2.02%), β-caryophyllene (1.86%), α-farnesene (1.59%), β-selinene (1.44%), γ-elemene (1.41%), β-bisabolene (1.26%), patchoulene (1.16%), β-patchoulene (1.11), γ-muurolene (1.09%) and thujopsene (1.04%), while the other ten compounds were present with concentrations lower than one percent.

Oxygenated monoterpenes were made up of seven compounds (12.53%): borneol (7.52%), (E)-pinocamphone (1.67%), pinocarvone (1.29%), terpinen-4-ol (0.89%), fenchol (0.57%), p-Menth-2-en-9-ol, trans- (0.39%) and dihydrocarveol (0.20%).

The rest of the oil were made up of nine compounds of oxygenated sesquiterpenes (9.12%), oxygenated non-terpenes (10.47%), non-terpene hydrocarbons (5.84%) and miscellaneous (5.71%).

Table 4.10: Chemical components of the rhizome oil of *Boesenbergia prainiana*

No	Compounds	Group	KI	Composition (%)	RT	Method of ID
1	α-Pinene	MH	937	1.64	10.031	MS,KI
2	**L-β-Pinene**	**MH**	**981**	**9.51**	**11.862**	**MS, KI**
3	D-Limonene	MH	1033	1.00	14.061	MS, KI
4	β-Ocimene	MH	1049	0.99	14.797	MS, KI
5	2-Nonanone	ONT	1092	0.78	16.856	MS, KI
6	2-Nonanol	ONT	1100	0.83	17.267	MS, KI
7	Fenchol	OM	1103	0.57	17.358	MS, KI
8	Sabinyl acetate	ONT	1146	4.58	19.235	MS, KI
9	Pinocarvone	OM	1167	1.29	20.229	MS, KI
10	**Borneol**	**OM**	**1177**	**7.52**	**20.704**	**MS, KI**
11	(E)-Pinocamphone	OM	1181	1.67	20.888	MS, KI
12	Terpinen-4-ol	OM	1184	0.89	21.054	MS, KI
13	**Isolimonene**	**MH**	**1198**	**10.09**	**21.771**	**MS, KI**
14	1,7-Octadiene, 3-methylene-	NTH	1286	0.31	25.711	MS
15	Bornyl acetate	ONT	1294	1.04	26.069	MS, KI
16	Tetracyclo[3.3.1.1(1,8).0(2,4)]decane	MH	1296	2.38	26.205	MS
17	2-Undecanol	ONT	1303	0.19	26.5	MS, KI
18	Myrtenyl acetate	ONT	1325	0.42	27.4	MS, KI
19	1,5,5-Trimethyl-6-methylene-cyclohexene	MH	1333	0.51	27.747	MS, KI
20	δ-EIemene	SH	1337	0.55	27.893	MS, KI
21	**β-Elemene**	**SH**	**1390**	**5.00**	**30.241**	**MS, KI**
22	Caryophyllene	SH	1421	1.86	31.518	MS, KI
23	γ-Elemene	SH	1430	1.41	31.885	MS, KI
24	*trans*-α-Bergamotene	SH	1434	0.76	32.037	MS, KI
25	β-Patchoulene	SH	1443	1.11	32.398	MS, KI
26	γ-Muurolene	SH	1451	1.09	32.727	MS, KI
27	(E)-β-Famesene	SH	1454	0.69	32.837	MS, KI
28	epi-β-Santalene	SH	1461	0.57	33.141	MS, KI
29	4,11-Selinadiene	SH	1474	0.56	33.704	MS, KI
30	Germacrene D	SH	1482	0.42	34.035	MS, KI
31	4,5-di-epi-aristolochene	SH	1486	2.02	34.184	MS, KI
32	β-Selinene	SH	1490	1.44	34.379	MS, KI
33	Valencene	SH	1493	0.63	34.487	MS, KI
34	α-Selinene	SH	1496	2.64	34.636	MS, KI
35	α-Farnesene	SH	1504	1.59	34.937	MS, KI
36	β-Bisabolene	SH	1508	1.26	35.095	MS, KI
37	(-)-β-Cadinene	SH	1519	0.33	35.497	MS, KI
38	β-Sesquiphellandrene	SH	1520	0.29	35.57	MS, KI

Table 4.10, Continued,

No	Compounds	Group	KI	Composition (%)	RT	Method of ID
39	2(1H)-Benzo cyclooctene,decahydro-10a-methyl-,trans-	OT	1527	0.42	35.824	MS, KI
40	Naphthalene, 1,2-dihydro-1,1,6-trimethyl-	NTH	1534	0.48	36.079	MS, KI
41	Nerolidol 2	OS	1562	2.71	37.183	MS, KI
42	7-Hydroxyfarnesen	OS	1584	0.71	38.035	MS, KI
43	Caryophyllene oxide	OS	1599	0.19	38.672	MS, KI
44	Neoisolongifolene, 8-bromo-	OT	1625	0.63	39.612	MS, KI
45	Ledol	OS	1629	0.65	39.759	MS, KI
46	Trichloroacetic acid, 2-methyloct-5-yn-4-yl ester	OT	1634	1.03	39.94	MS, KI
47	1,4-Dimethyl-8-isopropylidene tricyclo[5.3.0.0(410)]decane	OT	1640	0.45	40.154	MS, KI
48	Patchoulene	SH	1657	1.16	40.783	MS, KI
49	1-Isopropyl12-oxatetracyclo[5.2.1.1(2,6).1(9,11)]dodecane	OT	1661	2.17	40.923	MS
50	Thujopsene	SH	1667	1.04	41.155	MS, KI
51	Cyclopentanone, 3-[3,5-decadienyl]-,(Z,Z)-	OS	1671	0.89	41.301	MS, KI
52	Endo-tricyclo[5.2.1.0(2.6)]decane	MH	1674	0.54	41.428	MS, KI
53	Tricyclo[3.1.0.0(2,4)]hexane, 3,3,6,6-tetramethyl-, (1.alpha.,2.beta.,4.beta., 5.alpha.)-	MH	1678	0.52	41.549	MS
54	Viridiflorol	OS	1698	1.56	42.327	MS, KI
55	2-Pentadecanone	OS	1708	0.30	42.687	MS, KI
56	n-Pentadecanal	OS	1716	1.51	42.959	MS, KI
57	(7a-isopropenyl-4,5-dimethyloctahydroinden-4-yl)methanol	OS	1746	0.60	43.987	MS, KI
58	Dihydrocarveol	OM	1754	0.20	44.294	MS, KI
59	**3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)-**	**NTH**	**1801**	**5.05**	**45.979**	**MS**
60	1,2-Dimethylcyclooctene	MH	1886	0.22	48.833	MS, KI
61	ε-Muurolene	SH	1909	0.24	50.882	MS, KI

Table 4.10, Continued,

No	Compounds	Group	KI	Composition (%)	RT	Method of ID
62	Butyl cyclohexyl phthalate	OT	1909	0.28	50.975	MS, KI
63	Hexadecanoic acid	ONT	1911	2.63	51.285	MS, KI
64	3,9-Dimethylbicyclo [4.2.1.1(2,5)]decan-9-ol	OT	2076	0.34	54.859	MS
65	Difluoro-(2-hydroxynaphthalen-1-yl sulfonyl)-acetic acid methyl Ester	OT	2120	0.39	56.173	MS
66	p-Menth-2-en-9-ol, *trans*-	OM	2139	0.39	56.728	MS, KI
			Total	**97.73**		

Legend:

MH : Monoterpene hydrocarbons
OM : Oxygenated monoterpenes
SH : Sesquiterpene hydrocarbons
OS : Oxygenated sesquiterpenes
NTH : Non-terpene hydrocarbons
ONT : Oxygenated non-terpenes
OT : Other
Composition (%) : Obtained by using CBP-5 capillary column
MS : Mass fragmentation
KI : Kovats retention indices
RT **:** Retention time (minutes)
Components (%) : Major (≥20) (Bakkali *et al.,* 2008)
: Main (≥5)
: Trace (≤0.1) (Nampoothiri *et al*., 2012)

4.2.10 Volatile constituents of the leaf oil of *Boesenbergia prainiana* (King ex Baker) Schltr.

The leaf oil of *Boesenbergia prainiana* contains fifty-nine constituents comprising of 98.47% of the total oil (Table 4.11). The essential oil mainly comprises of oxygenated monoterpenes (24.78%), oxygenated non-terpenes (23.28%), monoterpene hydrocarbons (16.8%) and non-terpene hydrocarbons (15.07%).

Oxygenated monoterpene formed the most abundant group in this oil (24.78%) with the presence of myrtenol (14.13%), L-pinocarveol (3.39%) and isopinocamphone (3.03%). The other oxygenated monoterpenes consist of isocyclocitral (1.29%), borneol (1.11%), linalool (0.79%), pinocarvone (0.54%), terpinen-4-ol (0.39%) and decahydronaphtho(2,3-b)oxirene (0.11%).

Hydrocarbons made up 24.89% of the total oil of this species. Monoterpene hydrocarbons (16.8%) comprise of eight compounds such as L-β-pinene (12.09%), (E)-ocimene (1.42%), (R)-α-pinene (1.10%) and D-limonene (0.70 %). Meanwhile, there are fifteen sesquiterpene hydrocarbons (8.09%), among other include longipinane, (E)- (1.67%) and allo-aromadendrene (1.02%).

The rest of the oils detected in this oil were oxygenated non-terpenes (23.28%), non-terpene hydrocarbons (15.07%), oxygenated diterpenes (2.67%), oxygenated sesquiterpenes (1.89%), and miscellaneous (5.89%).

Table 4.11: Chemical components of the leaf oil of *Boesenbergia prainiana*

No	Compounds	Group	KI	Composition (%)	RI	Method of ID
1	(R)-α-Pinene	MH	937	1.10	10.032	KI,MS
2	**L-β-Pinene**	**MH**	**981**	**12.09**	**11.860**	**KI,MS**
3	D-Limonene	MH	1033	0.70	14.061	KI,MS
4	Terpinolene	MH	1087	0.40	16.593	KI,MS
5	Linalool	OM	1101	0.79	17.273	KI,MS
6	5-Methyl hexanal	ONT	1106	0.44	17.514	KI,MS
7	L-Pinocarveol	OM	1146	3.39	19.245	KI,MS
8	Pinocarvone	OM	1167	0.54	20.229	KI,MS
9	Borneol	OM	1177	1.11	20.693	KI,MS
10	Isopinocamphone	OM	1181	3.03	20.898	KI,MS
11	Terpinen-4-ol	OM	1184	0.39	21.060	KI,MS
12	**Myrtenol**	**OM**	**1199**	**14.13**	**21.827**	**KI,MS**
13	Decahydronaphtho(2,3-b)oxirene	OM	1205	0.11	22.060	KI,MS
14	1,2,3,4-Tetrahydro-1,5,8-trimethylnaphthalene	NTH	1222	0.15	22.798	MS
15	**Tetracyclo[3.3.1.1(1,8).0(2,4)]decane**	**NTH**	**1298**	**10.93**	**26.261**	**MS**
16	Myrtenyl acetate	ONT	1326	4.46	27.431	KI,MS
17	1,5,5-Trimethyl-6-methylene-cyclohexene	MH	1331	0.42	27.654	KI,MS
18	α-Terpinene	SH	1334	0.29	27.758	KI,MS
19	5,8-Dimethylquinoline	OT	1337	0.10	27.899	KI,MS
20	β-Damascenone	SH	1380	0.15	29.800	KI,MS
21	2-oxa-1,3-disilacyclohexane,1,1,3,3-tetramethyl-	OT	1388	4.88	30.137	MS
22	21-Cycloheptane,1,4-dimethyl-3-(2-methyl-1-propane-1-yl)-4-vinyl-	SH	1390	1.17	30.243	MS
23	Prehnitene	MH	1406	0.38	30.942	KI,MS
24	β-Caryophyllene	SH	1410	0.67	31.089	KI,MS
25	α-Ionone	ONT	1421	0.57	31.520	KI,MS
26	1,3-Dimethyl-5-tert-butylbenzene	NTH	1423	0.60	31.607	KI,MS
27	(E)-Ocimene	MH	1429	1.42	31.848	KI,MS
28	α-Yalangene	SH	1434	0.15	32.038	KI,MS
29	*cis*-Muurola-3,5-diene	SH	1443	0.30	32.397	KI,MS
30	(Z)-β-Farnesene	SH	1451	0.16	32.728	KI,MS
31	10-epi-γ-Eudesmol	OS	1462	0.24	33.165	KI,MS
32	β-Ionone	ONT	1474	0.17	33.700	KI,MS
33	4,5-di-epi-Aristolochene	SH	1477	0.31	33.824	KI,MS
34	α-Selinene	SH	1480	0.45	33.924	KI,MS
35	Allo aromadendrene	SH	1486	1.02	34.182	KI,MS
36	α-Cubebene	SH	1490	0.36	34.378	KI,MS
37	β-Bisabolene	SH	1503	0.63	34.925	KI,MS
38	γ-Selinene	SH	1508	0.31	35.091	KI,MS

Table 4.11, Continued,

No	Compounds	Group	KI	Composition (%)	RT	Method of ID
39	2-Butenamide,N-phenyl-	OT	1525	0.13	35.73	KI,MS
40	(E)-Nerolidol	OS	1562	1.65	37.19	KI,MS
41	Patchoulane	SH	1578	0.24	37.82	KI,MS
42	4-(1,3,3-Trimethyl-bicyclo[4.1.0]hept-2-yl)-but-3-en-2-one	ONT	1606	0.13	38.90	MS
43	2,6,10,10-Tetramethylbicyclo[7.2.0]undeca-2,6-diene	SH	1611	0.43	39.09	MS
44	But-3-enal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexenyl)-	ONT	1635	1.21	39.95	MS
45	1,3,5,7-Tetramethyl-adamantane	NTH	1640	0.48	40.15	KI,MS
46	Cyclohexanol 2-methylene-3-(1-methylethenyl)-,acetate, cis-	ONT	1657	0.30	40.78	KI,MS
47	2,2-Dimethyl-3-(3,7,16,20-tetramethyl-heneicosa-3,7,11,15,19-pentaenyl)-oxirane	ONT	1661	0.47	40.92	MS
48	Selina-3,7(11)-diene	SH	1667	0.22	41.15	KI,MS
49	Longipinane, (E)-	SH	1678	1.67	41.56	KI,MS
50	Oxalic acid,allyl dodecyl ester	OT	1723	0.16	43.20	KI,MS
51	Isocyclocitral	OM	1746	1.29	44.00	KI,MS
52	3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)-	NTH	1801	2.91	45.98	MS
53	Isophytol	OD	1945	0.91	50.74	KI,MS
54	Butyl cyclohexyl phthalate	OT	1953	0.15	50.98	KI,MS
55	**Hexadecanoic acid**	**OTH**	**1971**	**12.37**	**51.58**	**KI,MS**
56	3-Iodomethyl-3,6,6-trimethyl-cyclohexene	OT	2076	0.47	54.86	MS
57	Phytol	OD	2107	1.76	55.79	KI,MS
58	Linoleic acid	ONT	2131	1.69	56.51	KI,MS
59	9,17-Octadecadienal	ONT	2137	1.32	56.69	KI,MS
			Total	**98.47**		

Legend:

MH	: Monoterpene hydrocarbons
OM	: Oxygenated monoterpenes
SH	: Sesquiterpene hydrocarbons
OS	: Oxygenated sesquiterpenes
OD	: Oxygenated diterpenes
NTH	: Non-terpene hydrocarbons
ONT	: Oxygenated non-terpenes
OT	: Other
Composition (%)	: Obtained by using CBP-5 capillary column
MS	: Mass fragmentation
KI	: Kovats retention indices
RT	**:** Retention time (minutes)
Components (%)	: Major (≥20) (Bakkali *et al.,* 2008)
	: Main (≥5)
	: Trace (≤0.1) (Nampoothiri *et al*., 2012)

The group classifications of the chemical constituents for the rhizome and leaf oils of five wild *Boesenbergia* species are summarized in Table 4.12 and Table 4.13, respectively.

Table 4.12: Chemical composition of the rhizome oils of five wild *Boesenbergia* species

Compounds	**Formula molecule**	**Composition (%) (Rhizome)**				
		B.a	***B.s***	***B.* sp. *nova***	***B.pli***	***B. pra***
Monoterpene hydrocarbons						
α-Pinene	$C_{10}H_{16}$	-	-	-	-	1.64
(R)-α-Pinene	$C_{10}H_{16}$	0.47	1.16	0.48	-	-
β-Pinene	$C_{10}H_{16}$	2.69	2.64	0.59	-	-
L-β-Pinene	$C_{10}H_{16}$	-	-	-	0.78	9.51
β-Ocimene	$C_{10}H_{16}$	0.61	-	-	-	0.99
(E)-Ocimene	$C_{10}H_{16}$	-	-	0.53	-	-
cis-β-Ocimene	$C_{10}H_{16}$	-	0.71	-	-	-
Myrcene	$C_{10}H_{16}$	-	0.25	-	-	-
Limonene	$C_{10}H_{16}$	-	0.2	-	-	-
Isolimonene	$C_{10}H_{16}$	-	-	-	-	10.09
D-Limonene	$C_{10}H_{16}$	-	-	-	-	1.00
Camphene	$C_{10}H_{16}$	-	0.57	0.46	-	-
α-Terpinene	$C_{10}H_{16}$	-	0.39	-	-	-
1,5,5-Trimethyl-6-methylene-cyclohexene	$C_{10}H_{16}$	-	-	-	-	0.51
Endo-tricyclo[5.2.1.0(2.6)]decane	$C_{10}H_{16}$	-	-	-	-	0.54
Tetracyclo[3.3.1.1(1,8).0(2,4)]decane	$C_{10}H_{14}$	-	-	-	-	2.38
Tricyclo[3.1.0.0(2,4)]hexane, 3,3,6,6-tetramethyl-, (1.alpha.,2.beta.,4.beta., 5.alpha.)-	$C_{10}H_{16}$	-	-	-	-	0.52
1,2-Dimethylcyclooctene	$C_{10}H_{18}$	-	-	-	-	0.22
Isocamphene	$C_{10}H_{18}$	-	-	-	28.07	-
Total		**3.77**	**5.92**	**2.06**	**28.85**	**27.40**

Table 4.12, Continued,

Compounds	**Formula molecule**	**Composition (%) (Rhizome)**				
		B.a	***B.s***	***B.* sp. *nova***	***B.pli***	***B. pra***
Oxygenated monoterpenes						
Pinocarvone	$C_{10}H_{14}O$	-	-	-	-	1.29
(E)-Pinocamphone	$C_{10}H_{16}O$	-	-	-	-	1.67
Linalool	$C_{10}H_{18}O$	19.83	0.36	-	-	-
Borneol	$C_{10}H_{18}O$	0.30	2.44	2.63	-	7.52
α-Terpineol	$C_{10}H_{18}O$	0.28	-	-	-	-
1,8-Cineole	$C_{10}H_{18}O$	0.14	-	-	-	-
Terpinen-4-ol	$C_{10}H_{18}O$	-	-	-	-	0.89
Methyl cinnamate	$C_{10}H_{10}O_2$	-	55.42	-	-	-
Fenchol	$C_{10}H_{18}O$	-	-	-	-	0.57
p-Menth-2-en-9-ol, trans-	$C_{10}H_{18}O$	-	-	-	-	0.39
Dihydrocarveol	$C_{10}H_{18}O$	-	-	-	-	0.20
Total		**20.55**	**58.22**	**2.63**	**-**	**12.53**
Sesquiterpene hydrocarbons						
(-)-Aristolene	$C_{15}H_{24}$	0.63	-	-	-	-
β-Elemene	$C_{15}H_{24}$	1.53	-	0.33	-	5.00
δ-Elemene	$C_{15}H_{24}$	0.91	9.25	-	-	0.55
γ-Elemene	$C_{15}H_{24}$	-	1.57	-	-	1.41
Caryophyllene	$C_{15}H_{24}$	-	-	-	-	1.86
β-Caryophyllene	$C_{15}H_{24}$	0.29	6.86	2.11	-	-
α-Guaiene	$C_{15}H_{24}$	0.24	-	-	-	-
α-Cubebene	$C_{15}H_{24}$	1.64	-	7.27	-	-
β-Cubebene	$C_{15}H_{24}$	-	-	0.45	-	-
Isosativene	$C_{15}H_{24}$	1.34	0.71	-	-	-
α-Farnesene	$C_{15}H_{24}$	-	-	-	-	1.59

Table 4.12, Continued,

Compounds	Formula molecule	Composition (%) (Rhizome)				
		B.a	***B.s***	***B.* sp. *nova***	***B.pli***	***B. pra***
(Z)-α-Farnesene	$C_{15}H_{24}$	-	-	-	0.31	-
(E)-β-Farnesene	$C_{15}H_{24}$	-	-	2.02	-	0.69
(Z)-β-Farnesene	$C_{15}H_{24}$	1.88	0.31	-	-	-
4,5-di-epi-Aristolochene	$C_{15}H_{24}$	-	-	-	-	2.02
2-Isopropyl-5-methyl-9-methylenebicyclo[4.4.0] dec-1-ene	$C_{15}H_{24}$	0.99	-	-	-	-
α-Santalene	$C_{15}H_{24}$	5.78	3.49	0.28	-	-
β-Selinene	$C_{15}H_{24}$	0.94	-	4.08	-	1.44
α-Selinene	$C_{15}H_{24}$	0.69	-	-	-	2.64
γ-Selinene	$C_{15}H_{24}$	-	-	-	0.40	-
4,11-Selinadiene	$C_{15}H_{24}$	-	-	10.18	-	0.56
epi-β-Santalen	$C_{15}H_{24}$	-	-	-	-	0.57
α-Gurjunene	$C_{15}H_{24}$	14.27	-	-	-	-
γ-Gurjunene	$C_{15}H_{24}$	1.86	-	-	-	-
(+)-Aromadendrene	$C_{15}H_{24}$	1.40	-	1.86	-	-
Allo-aromadendrene	$C_{15}H_{24}$	-	0.34	1.85	1.11	-
Aromadendrene	$C_{15}H_{24}$	-	0.69	-	-	-
α-Bergamotene	$C_{15}H_{24}$	-	0.80	-	-	-
trans-α-Bergamotene	$C_{15}H_{24}$	-	-	-	-	0.76
δ-Cadinene	$C_{15}H_{24}$	-	1.02	2.62	-	-
α-Cadinene	$C_{15}H_{24}$	-	0.47	-	-	-
(-)-β-Cadinene	$C_{15}H_{24}$	-	-	-	-	0.33
α-Guaiene	$C_{15}H_{24}$	-	-	1.55	-	-
Epizonarene	$C_{15}H_{24}$	-	0.32	-	-	-
β-Bisabolene	$C_{15}H_{24}$	5.40	0.53	3.62	-	1.26
α-Copaene	$C_{15}H_{24}$	-	1.51	-	-	-
α-Longipinene	$C_{15}H_{24}$	-	-	-	0.64	-
β-Sesquiphellandrene	$C_{15}H_{24}$	-	-	-	-	0.29

Table 4.12, Continued,

Compounds	**Formula molecule**	**Composition (%) (Rhizome)**				
		B.a	***B.s***	***B.* sp. *nova***	***B.pli***	***B. pra***
γ-Maaliene	$C_{15}H_{24}$	-	-	18.43	-	-
α-Muurolene	$C_{15}H_{24}$	-	-	0.39	-	
γ-Muurolene	$C_{15}H_{24}$	-	-	2.91	-	1.09
ε-Muurolene	$C_{15}H_{24}$	-	-	-	-	0.24
Germacrene D	$C_{15}H_{24}$	-	3.68	-	-	0.42
Patchoulene	$C_{15}H_{24}$	-	-	-	-	1.16
β-Patchoulene	$C_{15}H_{24}$	-	-	-	-	1.11
Thujopsene	$C_{15}H_{24}$	-	-	-	-	1.04
1,4,7-Cycloundecatriene 1 5 9 9-tetramethyl- z z z-	$C_{15}H_{24}$	1.97	2.47	1.47	-	-
α-Panansinene	$C_{15}H_{24}$	-	-	8.90	-	-
Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)-(.+/-.)-	$C_{15}H_{24}$	-	-	6.67	-	-
6,10-Dimethyl-3-(1-methylethylidene)-1-cyclodecene	$C_{15}H_{26}$	-	-	-	0.30	-
Thujopsene	$C_{15}H_{24}$	-	-	-	1.81	-
Valencene	$C_{15}H_{24}$	-	-	0.78	-	0.63
Total		**41.76**	**34.02**	**77.77**	**4.57**	**26.66**
Oxygenated sesquiterpenes						
Nootkatone	$C_{15}H_{22}O$	-	-	0.54	-	-
p-Heptylacetophenone	$C_{15}H_{22}O$	-	-	-	0.22	-
cis-α-Santalol	$C_{15}H_{24}O$	0.83	-	-	-	-
Spathulenol	$C_{15}H_{24}O$	-	-	1.24	-	-
7R,8R-8-Hydroxy-4-isopropylidene-7-methylbicyclo[5.3.1]undec-1-ene	$C_{15}H_{24}O$	0.30	-	0.26	-	-

Table 4.12, Continued,

Compounds	**Formula molecule**	**Composition (%) (Rhizome)**				
		B.a	***B.s***	***B.* sp. *nova***	***B.pli***	***B. pra***
Ledene oxide	$C_{15}H_{24}O$	-	1.06	-	-	-
Ledene oxide (I)	$C_{15}H_{24}O$	-	-	1.62	-	-
Humulene epoxide (II)	$C_{15}H_{24}O$	0.21	-	-	-	-
diepi-α-Cedrene epoxide	$C_{15}H_{24}O$	-	-	0.16	-	-
Longifolenaldehyde	$C_{15}H_{24}O$	-	-	0.71	-	-
7-Hydroxyfarnesen	$C_{15}H_{24}O$	-	-	-	-	0.71
Cyclopentanone, 3-[3,5-decadienyl]-,(Z,Z)-	$C_{15}H_{24}O$	-	-	-	-	0.89
Caryophyllene oxide	$C_{15}H_{24}O$	-	-	-	-	0.19
Nerolidol	$C_{15}H_{26}O$	22.77	-	-	-	-
Nerolidol 2	$C_{15}H_{26}O$	-	-	-	-	2.71
Longiborneol	$C_{15}H_{26}O$	-	-	-	6.90	-
γ-Eudesmol	$C_{15}H_{26}O$	1.82	-	-	-	-
β-Eudesmol	$C_{15}H_{26}O$	1.55	-	-	-	-
α-Eudesmol	$C_{15}H_{26}O$	2.27	-	-	-	-
Palustrol	$C_{15}H_{26}O$	-	-	4.21	-	-
Ledol	$C_{15}H_{26}O$	-	-	-	-	0.65
Viridiflorol	$C_{15}H_{26}O$	-	-	-	-	1.56
(7a-Isopropenyl-4,5-dimethyloctahydroinden-4-yl)methanol	$C_{15}H_{26}O$	-	-	-	-	0.60
2-Pentadecanone	$C_{15}H_{30}O$	-	-	-	-	0.30
n-Pentadecanal	$C_{15}H_{30}O$	-	-	-	-	1.51
1-Pentadecanal	$C_{15}H_{30}O$	-	-	-	1.77	-
Total		**29.75**	**1.06**	**9.9**	**8.89**	**9.12**
Diterpene hydrocarbons						
Kaurene	$C_{20}H_{32}$	-	0.29	-	-	-
Total		-	**0.29**	-	-	-

Table 4.12, Continued,

Compounds	**Formula molecule**	**Composition (%) (Rhizome)**				
		B.a	***B.s***	***B.* sp. *nova***	***B.pli***	***B. pra***
Oxygenated diterpenes						
Retinal	$C_{20}H_{28}O$	-	-	-	1.06	-
Total		-	-	-	**1.06**	-
Non-terpene hydrocarbons						
Methylcyclohexane	C_7H_{14}	0.20	0.12	0.16	-	-
Bicyclo[4.1.0]heptane, 7-methylene-	C_8H_{12}	-	-	-	0.69	-
Cyclohexane, 1,2,4-tris(methylene)-	C_9H_{12}	-	-	-	0.19	-
1,7-Octadiene, 3-methylene-	C_9H_{16}	-	-	-	-	0.31
2,2,7-Trimethyl-3-octyne	$C_{11}H_{20}$	-	-	-	0.90	-
Tricyclo[3.1.0.0(2,4)]hexane, 3,6-diethyl-3,6-dimethyl-, trans-	$C_{12}H_{20}$	-	-	-	0.74	-
1-Ethyldecalin, trans	$C_{12}H_{22}$	-	-	-	2.57	-
1,1,6-Trimethyl-1,2-dihydronaphthalene	$C_{13}H_{16}$	-	-	0.57	-	-
Naphthalene, 1,2-dihydro-1,1,6-trimethyl-	$C_{13}H_{16}$	-	-	-	-	0.48
1H-Indene	$C_{13}H_{18}$	-	-	-	0.57	-
(+)-2-Carene, 4-.alpha.-isopropenyl-	$C_{13}H_{20}$	-	-	-	1.21	-
Benzene, 1,2-bis(1-buten-3-yl)-	$C_{14}H_{18}$	-	-	-	0.96	-
3,5-Octadiene, 2,2,4,5,7,7-Hexamethyl-,(E,Z)-	$C_{14}H_{26}$	-	-	-	-	5.05
Total		**0.2**	**0.12**	**0.73**	**7.83**	**5.84**
Oxygenated non-terpenes						
2-Methyl-3-butyn-2-ol	C_5H_8O	0.53	-	-	-	-
2-Nonanone	$C_9H_{18}O$	-	-	-	-	0.78

Table 4.12, Continued,

Compounds	**Formula molecule**	**Composition (%) (Rhizome)**				
		B.a	***B.s***	***B.* sp. *nova***	***B.pli***	***B. pra***
2-Nonanol	$C_9H_{20}O$	-	-	-	-	0.83
5,6,7,8-Tetrahydro-2-methyl-1,4-naphthoquinone	$C_{11}H_{12}O_2$	-	-	0.34	-	-
11-Oxatetracyclo[5.3.2.0(2,7).0(2,8)]dodecan-9-one	$C_{11}H_{14}O_2$	-	-	1.93	-	-
2-Undecanol	$C_{11}H_{24}O$	-	-	-	-	0.19
Myrtenyl acetate	$C_{12}H_{18}O_2$	-	-	-	-	0.42
Sabinyl acetate	$C_{12}H_{18}O_2$	-	-	-	-	4.58
Bornyl acetate	$C_{12}H_{20}O_2$	-	-	-	-	1.04
n-Butyl cinnamate	$C_{13}H_{16}O_2$	0.37	-	-	-	-
1-{2-[2-Methyl-2-(5-methyl-2-furyl)propyl]cyclopropyl}ethanone	$C_{14}H_{20}O_2$	-	-	0.71	-	-
Pentadecanoic acid	$C_{15}H_{30}O_2$	-	-	-	0.80	-
Hexadecanoic acid	$C_{16}H_{32}O_2$	-	-	-	0.36	2.63
2H-Phenanthro[9,10-b]pyran	$C_{17}H_{12}O$	-	-	-	1.77	-
Podocarpan-12-ol	$C_{17}H_{30}O$	-	-	-	1.37	-
9,17-Octadecadienal	$C_{18}H_{32}O$	-	-	-	2.58	-
Linoleic acid	$C_{18}H_{32}O_2$	-	-	-	5.99	-
Octadecanoic acid	$C_{18}H_{36}O_2$	-	-	-	0.22	-
Total		**0.9**	**-**	**2.98**	**13.09**	**10.47**
Other						
Chloro(ethyl)diisopropylsilane	-	-	-	0.49	-	-
2(1H)-Quinolinone, 1-methyl-	-	-	-	0.45	-	-
1,3,5-Triphenyl-4,5-dihydro-1H-pyrazole	-	-	-	0.72	-	-
4-Hydroxy-3a,7a-dimethly-4,5-dihydro-3H-2-benzofuran-1-one	-	-	-	-	0.38	-

Table 4.12, Continued,

Compounds	Formula molecule	Composition (%) (Rhizome)				
		B.a	***B.s***	***B.* sp. *nova***	***B.pli***	***B. pra***
Neoisolongifolene, 8-bromo-	-	-	-	-	0.33	0.63
Butyl 2-ethylhexylphthalated	-	-	-	-	0.34	-
Tricyclo[3.2.1.0(2,4)] octane-3-carboxamide, N-(4-ethoxyphenyl)-	-	-	-	-	1.01	-
Epirizole	-	-	-	-	0.44	-
Trichloroacetic acid, 2-methyloct-5-yn-4-yl ester	-	-	-	-	4.46	-
Trichloroacetic acid, 2-methyloct-5-yn-4-yl ester	-	-	-	-	-	1.03
Butyl cyclohexyl phthalate	-	-	-	-	-	0.28
2(1H)-Benzo cyclooctene,decahydro-10a-methyl-,trans-	-	-	-	-	-	0.42
1,4-Dimethyl-8-isopropylidene tricyclo[5.3.0.0(410)]decane	-	-	-	-	-	0.45
3,9-Dimethyl bicyclo[4.2.1.1(2,5)]decan-9-ol	-	-	-	-	-	0.34
Difluoro-(2-hydroxynaphthalen-1-yl sulfonyl)-acetic acid methyl ester	-	-	-	-	-	0.39
1-Isopropyl12-oxatetracyclo[5.2.1.1(2,6).1(9,11)]dodecane	-	-	-	-	-	2.17
Total		-	-	**1.66**	**6.96**	**5.71**
Grand total		**96.93**	**99.63**	**97.73**	**71.25**	**97.73**

Legend:

The rhizome of five wild *Boesenbergia* species:
B.a : *Boesenbergia armeniaca*
B.s : *Boesenbergia stenophylla*
B. sp. *nova* : *Boesenbergia sp. nova*
B.pli : *Boesenbergia plicata*
B.pra : *Boesenbergia prainiana*
Composition (%) : Obtained by using CBP-5 capillary column

Table 4.13: Chemical composition of the leaf oils of five wild *Boesenbergia* species

Compounds	**Formula molecule**	**Composition (%) (Leaf)**				
		B.a	***B.s***	***B.* sp. *nova***	***B. pli***	***B. pra***
Monoterpene hydrocarbons						
Prehnitene	$C_{10}H_{14}$	-	-	-	-	0.38
(R)-α-Pinene	$C_{10}H_{16}$	0.84	1.22	-	-	1.10
(-)-α-Pinene	$C_{10}H_{16}$	-	-	0.93	-	-
β-Pinene	$C_{10}H_{16}$	4.83	4.84	2.81	-	-
L-β-Pinene	$C_{10}H_{16}$	-	-	-	-	12.09
(-)-β-Pinene	$C_{10}H_{16}$	-	-	-	21.33	-
β-Ocimene	$C_{10}H_{16}$	0.32	0.27	-	-	-
(E)-Ocimene	$C_{10}H_{16}$	-		-	2.53	1.42
Limonene	$C_{10}H_{16}$	-	0.11	-	-	-
D-Limonene	$C_{10}H_{16}$	-		-	1.94	0.70
Camphene	$C_{10}H_{16}$	-	0.23	0.13	-	-
β-Phellandrene	$C_{10}H_{16}$	-	0.11	-	-	-
Terpinolene	$C_{10}H_{16}$	-	-	-	0.26	-
Isodurene	$C_{10}H_{14}$	-	-	-	0.59	-
α-Terpinene	$C_{10}H_{16}$	-	-	-	-	0.29
Terpinolene	$C_{10}H_{16}$	-	-	-	-	0.40
1,5,5-Trimethyl-6-methylene-cyclohexene	$C_{10}H_{16}$	-	-	-	-	0.42
Total		**5.99**	**6.78**	**3.87**	**26.65**	**16.8**
Oxygenated monoterpenes						
Pinocarvone	$C_{10}H_{14}O$	-	-	0.26	-	0.54
Hotrienol	$C_{10}H_{16}O$	0.53	-	-	-	-
L-Pinocarveol	$C_{10}H_{16}O$	-	-	-	-	3.39
α-Cyclocitral	$C_{10}H_{16}O$	-	-	0.64	-	-
Isocyclocitral	$C_{10}H_{16}O$	-	-	-	-	1.29
L-*trans*-Pinocarveol	$C_{10}H_{16}O$	-	-	0.28	-	-
Myrtenol	$C_{10}H_{16}O$	-	-	0.79	-	14.13

Table 4.13, Continued,

Compounds	**Formula molecule**	**Composition (%) (Leaf)**				
		B.a	***B.s***	***B.* sp. *nova***	***B.pli***	***B. pra***
(-)-Myrtenol	$C_{10}H_{16}O$	-	-	-	3.84	-
1,3,4-Trimethyl-3-cyclohexen-1-carboxaldehyde	$C_{10}H_{16}O$	-	-	-	0.37	-
Isopinocamphone	$C_{10}H_{16}O$	-	-	-	-	3.03
Decahydronaphtho(2,3-b)oxirene	$C_{10}H_{16}O$	-		-	-	0.11
Methyl cinnamate	$C_{10}H_{10}O_2$	-	83.17	-	-	-
Linalool	$C_{10}H_{18}O$	11.63	1.18	-	3.21	0.79
Borneol	$C_{10}H_{18}O$	0.22	0.55	1.05	-	1.11
L-Borneol	$C_{10}H_{18}O$	-	-	-	1.05	-
α-Terpineol	$C_{10}H_{18}O$	0.91	0.22	-	-	-
1,8-Cineole	$C_{10}H_{18}O$	0.78	-	-	-	-
L-4-Terpineol	$C_{10}H_{18}O$	0.42	-	-	-	-
Terpinen-4-ol	$C_{10}H_{18}O$		-	-	-	0.39
cis-4-Decenal	$C_{10}H_{18}O$	-	-	-	0.29	-
Total		**14.49**	**85.12**	**3.02**	**8.76**	**24.78**
Sesquiterpene hydrocarbons						
α-Elemene	$C_{15}H_{24}$	0.32	-	-	-	-
β-Elemene	$C_{15}H_{24}$	0.21	-	0.64	0.79	-
δ-Elemene	$C_{15}H_{24}$	-	0.31	-	-	-
γ-Elemene	$C_{15}H_{24}$	-	0.21	-	-	-
α-Cubebene	$C_{15}H_{24}$	-	-	8.98	-	0.36
β-Cubebene	$C_{15}H_{24}$	-	-	0.57	-	-
(Z)-β-Farnesene	$C_{15}H_{24}$	-	-	-	-	0.16
Selinene	$C_{15}H_{24}$	-	-	-	3.41	-
β-Selinene	$C_{15}H_{24}$	-	-	6.31	-	-
α-Selinene	$C_{15}H_{24}$	-	-	-	-	0.45
γ-Selinene	$C_{15}H_{24}$	-	-	6.28	-	0.31

Table 4.13, Continued,

Compounds	**Formula molecule**	**Composition (%) (Leaf)**				
		B.a	***B.s***	***B.* sp. *nova***	***B.pli***	***B. pra***
Selina-3,7(11)-diene	$C_{15}H_{24}$	-	-	-	-	0.22
4,11-Selinadiene	$C_{15}H_{24}$	-	-	-	1.56	-
4,5-di-epi-Aristolochene	$C_{15}H_{24}$	-	-	-	-	0.31
α-Guaiene	$C_{15}H_{24}$	-	-	1.09	-	-
Allo-aromadendrene	$C_{15}H_{24}$	-	-	0.29	-	1.02
α-Caryophyllene	$C_{15}H_{24}$	-	-	0.62	-	-
β-Caryophyllene	$C_{15}H_{24}$	6.25	0.82	2.97	9.85	0.67
Isocaryophyllene	$C_{15}H_{24}$	0.32	-	-	-	-
epi-β-Santalen	$C_{15}H_{24}$	0.48	-	-	-	-
α-Gurjunene	$C_{15}H_{24}$	3.13	-	-	-	-
β-Gurjunene	$C_{15}H_{24}$	0.40	-	-	-	-
β-Bisabolene	$C_{15}H_{24}$	3.96	-	1.83	0.80	0.63
α-Copaene	$C_{15}H_{24}$	0.95	0.21	-	-	-
Bergamotene	$C_{15}H_{24}$	-	-	-	1.19	-
α-Bergamotene	$C_{15}H_{24}$	0.66	-	0.14	-	-
α-Calacorene	$C_{15}H_{20}$	-	-	0.45	-	-
β-Sesquiphellandrene	$C_{15}H_{24}$	1.46	-	-	0.53	-
β-Bourbonene	$C_{15}H_{24}$	-	-	2.29	-	-
Germacrene D	$C_{15}H_{24}$	0.51	0.58	-	-	-
δ-Cadinene	$C_{15}H_{24}$	0.46	0.22	1.96	-	-
cis-Muurola-3,5-diene	$C_{15}H_{24}$	-	-	-	-	0.30
Elixene	$C_{15}H_{24}$	-	0.50	-	-	-
1,4,7-Cycloundecatriene 1 5 9 9-tetramethyl- z z z-	$C_{15}H_{24}$	1.58	3.59	-	-	-
γ-Maaliene	$C_{15}H_{24}$	-	-	22.82	-	-
α-Panansinene	$C_{15}H_{24}$	-	-	9.12	-	-
Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene	$C_{15}H_{24}$	-	-	0.66	-	-
1R,3Z,9S-2,6,10,10 Tetramethylbicyclo[7.2.0]undeca-2,6-diene	$C_{15}H_{24}$	-	-	0.58	-	-

Table 4.13, Continued,

Compounds	**Formula molecule**	**Composition (%) (Leaf)**				
		B.a	***B.s***	***B.* sp. *nova***	***B.pli***	***B. pra***
6,10-Dimethyl-3-(1-methylethylidene)-1-cyclodecene	$C_{15}H_{26}$	-	-	-	0.95	-
2,6,10,10-Tetramethylbicyclo[7.2.0]undeca-2,6-diene	$C_{15}H_{24}$	-	-	-	-	0.43
α-Yalangene	$C_{15}H_{24}$	-	-	-	-	0.15
Longipinane, (E)-	$C_{15}H_{2}$	-	-	-	-	1.67
Viridiflorene	$C_{15}H_{24}$	-	-	0.82	-	
Patchoulene	$C_{15}H_{24}$	-	-	5.83	-	0.24
21-Cycloheptane,1,4-dimethyl-3-(2-methyl-1-propane-1-yl)-4-vinyl-	$C_{15}H_{24}$	-	-	-	-	1.17
Total		**20.69**	**6.44**	**74.25**	**19.08**	**8.09**
Oxygenated sesquiterpenes						
Nootkatone	$C_{15}H_{22}O$	-	-	0.53	-	-
Humulene epoxide II	$C_{15}H_{24}O$	-	0.32	-	-	-
Ledene oxide-(II)	$C_{15}H_{24}O$	-	-	1.29	-	-
Caryophyllene oxide	$C_{15}H_{24}O$	-	-	1.90	-	-
Isoaromadendrene epoxide	$C_{15}H_{24}O$	-	-	1.30	-	-
cis-α-Santalol	$C_{15}H_{24}O$	0.54	-	-	-	-
Nerolidol	$C_{15}H_{26}O$	42.55	0.19	-	2.65	-
(E)-Nerolidol	$C_{15}H_{26}O$	-	-	-	-	1.65
γ-Eudesmol	$C_{15}H_{26}O$	0.70	-	-	-	-
β-Eudesmol	$C_{15}H_{26}O$	0.64	-	-	-	-
α-Eudesmol	$C_{15}H_{26}O$	0.85	-	-	-	-
10-epi-γ-Eudesmol	$C_{15}H_{26}O$	-	-	-	-	0.24
Elemol	$C_{15}H_{26}O$	2.67	-	-	-	-
n-Pentadecanal	$C_{15}H_{30}O$	-	-	-	5.45	-
Total		**47.95**	**0.51**	**5.02**	**8.10**	**1.89**

Table 4.13, Continued,

Compounds	**Formula molecule**	**Composition (%) (Leaf)**				
		B.a	***B.s***	***B.* sp. *nova***	***B.pli***	***B. pra***
Diterpene hydrocarbons						
Kaurene	$C_{20}H_{32}$	0.56	0.40	-	-	-
Total		**0.56**	**0.40**	-	-	-
Oxygenated diterpenes						
Isophytol	$C_{20}H_{40}O$	1.03	-	-	-	0.91
Phytol	$C_{20}H_{40}O$	3.15	-	0.64	14.52	1.76
3,7,11,15-Tetramethylhexadec-1-en-3-ol	$C_{20}H_{40}O$	-	-	-	0.77	-
Total		**4.18**	**-**	**0.64**	**15.29**	**2.67**
Non-terpene hydrocarbons						
Methylcyclohexane	C_7H_{14}	0.22	0.14	0.17	-	-
4-Methyl-1,5-heptadiene	C_8H_{14}	1.54	-	-	-	-
3-Undecen-5-yne, (E)-	$C_{11}H_{18}$	-	-	-	0.59	-
1,3-Dimethyl-5-tert-butylbenzene	$C_{12}H_{18}$	-	-	-	-	0.60
1,7,7-Trimethyl-2-vinylbicyclo[2.2.1]hept-2-ene	$C_{12}H_{18}$	-	0.61	-	-	-
6-Dodecyne	$C_{12}H_{22}$	-	-	-	0.34	-
1,1,6-Trimethyl-1,2-dihydronaphthalene	$C_{13}H_{16}$	-	-	0.57	-	-
1,2,3,4-Tetrahydro-1,5,8-trimethylnaphthalene	$C_{13}H_{18}$	-	-	-	-	0.15
Tetracyclo[3.3.1.1(1,8).0(2,4)]decane	$C_{13}H_{18}$	-	-	-	-	10.93
1,3,5,7-Tetramethyl-adamantane	$C_{14}H_{24}$	-	-	-	-	0.48
3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)-	$C_{14}H_{26}$	-	-	-	-	2.91
8-Heptadecene	$C_{17}H_{34}$	-	-	-	0.93	-
Total		**1.76**	**0.75**	**0.74**	**1.86**	**15.07**

Table 4.13, Continued,

Compounds	Formula molecule	Composition (%) (Leaf)				
		B.a	***B.s***	***B.* sp. *nova***	***B.pli***	***B. pra***
Oxygenated non-terpenes						
5-Methyl hexanal	$C_7H_{14}O$	-	-	-	-	0.44
5-Methylene-1,3a,4,5,6,6a-hexahydropentalen-1-ol	$C_9H_{12}O$	-	-	-	0.73	-
(E)-2-Butenoic acid, 2-(methylenecyclopropyl) prop-2-yl ester	$C_{11}H_{16}O_2$	1.15	-	-	-	-
Bicyclo[3.1.1]hept-3-ene, 2-formylmethyl-4,6,6-trimethyl-	$C_{12}H_{18}O$	-	-	-	0.40	-
Myrtenyl acetate	$C_{12}H_{18}O_2$	-	-	-	0.36	4.46
Cyclohexanol 2-methylene-3-(1-methylethenyl)-,acetate, cis-	$C_{12}H_{18}O_2$	-	-	-	-	0.30
Isobutyl cinnamate	$C_{13}H_{16}O_2$	0.85	-	-	-	-
β-Damascenone	$C_{13}H_{18}O$	-	-	-	-	0.15
α-Ionone	$C_{13}H_{20}O$	-	-	-	0.71	0.57
β-Ionone	$C_{13}H_{20}O$	-	-	-	0.84	0.17
6,8-Nonadien-2-one, 6-methyl-5-(1-methylethylidene)-	$C_{13}H_{20}O$	-	-	-	0.30	-
2-Butyl-2-ethyl-5-methyl-3,4-hexadienal	$C_{13}H_{22}O$	-	-	-	1.55	-
Mayurone	$C_{14}H_{20}O$	-	-	1.34	-	-
Ethanone,1-(1,3a,4,5,6,7-hexahydro-4-hydroxy-3,8-dimethyl-5-azulenyl)-	$C_{14}H_{20}O_2$	-	-	2.52	-	-
4-(1,3,3-Trimethyl-bicyclo[4.1.0]hept-2-yl)-but-3-en-2-one	$C_{14}H_{22}O$	-	-	-	-	0.13
But-3-enal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexenyl)-	$C_{14}H_{22}O$	-	-	-	-	1.21
Hexadecanal	$C_{16}H_{32}O$	1.07	-	-	-	-
Hexadecanoic acid	$C_{16}H_{32}O_2$	-	-	-	7.71	12.37

Table 4.13, Continued,

Compounds	**Formula molecule**	**Composition (%) (Leaf)**				
		B.a	***B.s***	***B.* sp. *nova***	***B.pli***	***B. pra***
Linoleic acid	$C_{18}H_{32}O_2$	-	-	-	-	1.69
9,17-Octadecadienal	$C_{18}H_{32}O$	-	-	-	-	1.32
Hexahydrofarnesyl acetone	$C_{18}H_{36}O$	-	-	-	1.48	-
1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane	$C_{25}H_{26}O$	-	-	-	0.61	-
2,2-Dimethyl-3-(3,7,16,20-tetramethyl-heneicosa-3,7,11,15,19-pentaenyl)-oxirane	$C_{29}H_{48}O$	-	-	-	-	0.47
1,6,10,14,18,22-Tetracosahexaen-3-ol,2,6,10,15,19,23-hexamethyl-,(all-E)-	$C_{30}H_{50}O$	0.55	-	-	-	-
Total		**3.62**	**-**	**3.86**	**14.69**	**23.28**
Others						
2,4-Dimethylquinoline	-	-	-	2.04	-	-
Pentafluoropropionic acid, 1-adamantyl methyl ester	-	-	-	1.79	-	-
Phenacetic acid, 2-carbmethoxy-	-	-	-	0.93	-	-
6,6-Dimethylspiro[2,3-diazabicyclo[3.1.0]hex-2-ene-4,1'-cyclopropane]	-	-	-	-	0.57	-
6.beta.Bicyclo[4.3.0]nonane, 5.beta.-iodomethyl-1.beta.-isopropenyl-4.alpha.,5.alpha.-dimethyl-,	-	-	-	-	1.72	-
Dibutyl phthalate	-	-	-	-	0.41	-
2-oxa-1,3-disilacyclohexane,1,1,3,3-tetramethyl-	-	-	-	-	2.84	-

Table 4.13, Continued,

Compounds	**Formula molecule**	**Composition (%) (Leaf)**				
		B.a	***B.s***	***B.* sp. *nova***	***B.pli***	***B. pra***
2-Butenamide,N-phenyl-	-	-	-	-	-	0.13
3-Iodomethyl-3,6,6-trimethyl-cyclohexene	-	-	-	-	-	0.47
5,8-Dimethylquinoline	-	-	-	-	-	0.10
Oxalic acid,allyl dodecyl ester	-	-	-	-	-	0.16
Butyl cyclohexyl phthalate	-	-	-	-	-	0.15
2-oxa-1,3-disilacyclohexane,1,1,3,3-tetramethyl-	-	-	-	-	-	4.88
Total		**-**	**-**	**4.76**	**5.54**	**5.89**
Grand total		**99.24**	**100.0**	**96.16**	**99.97**	**98.47**

Legend:

The leaves of five wild *Boesenbergia* species:
B.a : *Boesenbergia armeniaca*
B.s : *Boesenbergia stenophylla*
B. sp. *nova* : *Boesenbergia sp. nova*
B.pli : *Boesenbergia plicata*
B.pra : *Boesenbergia prainiana*
Composition (%) : Obtained by using CBP-5 capillary column

Classification group of volatile compounds for the rhizome and leaf oils of five wild *Boesenbergia* species namely *Boesenbergia armeniaca*, *Boesenbergia stenophylla*, *Boesenbergia* sp. *nova*, *Boesenbergia plicata* and *Boesenbergia prainiana* are summarized in Table 4.14 and Table 4.15.

Table 4.14: Classification of chemical constituents of the rhizome oils of five wild *Boesenbergia* species according to their classification.

Classification	**Composition (%)**				
	B.a	*B.s*	*B.* sp. *nova*	*B. pli*	*B.pra*
Monoterpene hydrocarbons	3.77	5.92	2.06	**28.85**	**27.40**
Oxygenated monoterpenes	20.55	**58.22**	2.63	-	12.53
Sesquiterpene hydrocarbons	**41.76**	34.02	**77.77**	4.57	26.66
Oxygenated sesquiterpenes	29.75	1.06	9.90	8.89	9.12
Diterpene hydrocarbons	-	0.29	-	-	-
Oxygenated diterpenes	-	-	-	1.06	-
Non-terpene hydrocarbons	0.20	0.12	0.73	7.83	5.84
Oxygenated non-terpenes	0.90	-	2.98	13.09	10.47
Other	-	-	1.66	6.96	5.71
Total	**96.93**	**99.63**	**97.73**	**71.25**	**97.73**

Table 4.15: Classification of chemical constituents of the leaf oils of five wild *Boesenbergia* species according to their classification.

Classification	**Composition (%)**				
	B.a	*B.s*	*B.* sp. *nova*	*B. pli*	*B.pra*
Monoterpene hydrocarbons	5.99	6.78	3.87	**26.65**	16.8
Oxygenated monoterpenes	14.49	**85.12**	3.02	8.76	**24.78**
Sesquiterpene hydrocarbons	20.69	6.44	**74.25**	19.08	8.09
Oxygenated sesquiterpenes	**47.95**	0.51	5.02	8.10	1.89
Diterpene hydrocarbons	0.56	0.40	-	-	-
Oxygenated diterpenes	4.18	-	0.64	15.29	2.67
Non-terpene hydrocarbons	1.76	0.75	0.74	1.86	15.07
Oxygenated non-terpenes	3.62	-	3.86	14.69	23.28
Other	-	-	4.76	5.54	5.89
Total	**99.24**	**100.00**	**96.16**	**99.97**	**98.47**

In general, the group classification for the essential oils of five *Boesenbergia* species mainly consisted of monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, diterpene hydrocarbons and non-terpene hydrocarbons.

Overall, the rhizome and leaf oils of *Boesenbergia stenophylla* revealed the same major compound group classification that is oxygenated monoterpenes (Table 4.14 and Table 4.15). Similarly, the rhizome and leaf oils of *Boesenbergia* sp. *nova* and *Boesenbergia plicata* showed same major compound group classification that is sesquiterpene hydrocarbons and monoterpene hydrocarbons, respectively (Table 4.14 and Table 4.15).

4.3 Antibacterial properties of *Boesenbergia* species

The rhizome and leaf oils of *Boesenbergia armeniaca, Boesenbergia stenophylla, Boesenbergia* sp. *nova, Boesenbergia plicata* and *Boesenbergia prainiana* were investigated for their antibacterial activity against four food-borne pathogens namely *Staphylococcus aureus, Bacillus cereus, Salmonella enteritidis* and *Escherichia coli* by using disc-diffusion methods. The results are indicated in Table 4.16.

Table 4.16: *In vitro* antibacterial activity of the rhizome and leaf oils of *Boesenbergia* species against four foodborne pathogens.

Species	Plant parts	Zone of inhibition (mm) (10 mg/mL) (mean±S.D.)			
		Staphylococcus aureus	*Bacillus cereus*	*Escherichia coli*	*Salmonella enteritidis*
Boesenbergia armeniaca	Rhizome	17.1±0.3	16.2±0.3	11.3±1.1	11.8±0.3
	Leaves	13±0.2	12.6±0.4	8.4±1.0	11.1±0.3
Boesenbergia stenophylla	Rhizome	14.4±0.6	14.1±0.2	11.2±1.1	9.5±0.7
	Leaves	15±0.2	13±0.7	11.7±0.9	10±0.3
Boesenbergia sp. *nova*	Rhizomes	12.3±0.7	15.3±0.6	10.3±1.4	11±0.2
	Leaves	12±0.9	19.8±0.3	7.6±0.6	8.6±0.6
Boesenbergia plicata	Rhizomes	12.8±0.3	12±1.0	9.4±0.4	5.5±0.6
	Leaves	17.2±0.5	26.5±0.5	10.3±0.4	9.1±0.4
Boesenbergia prainiana	Rhizomes	17.9±0.3	12.2±0.6	10.6±1.1	10.3±0.3
	Leaves	15±0.2	23.3±0.6	8.3±0.9	11.9±0.4
Kanamycin (50µg/ml)		48.5±1.5	44.2±2.1	41.0±2.6	42.4±2.0

*≥20 mm zone of inhibition = extremely sensitive
*15-19 mm zone of inhibition = very sensitive
*9-14 mm = sensitive
*<8 mm = not sensitive

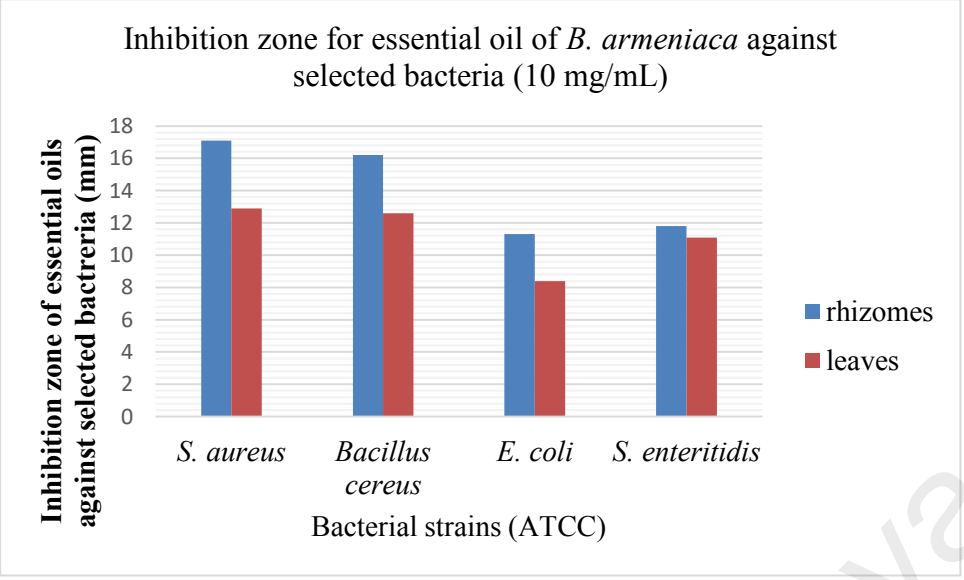


Figure 4.2: Inhibition zone of rhizome and leaf oils of *Boesenbergia armeniaca* against selected bacterial strains

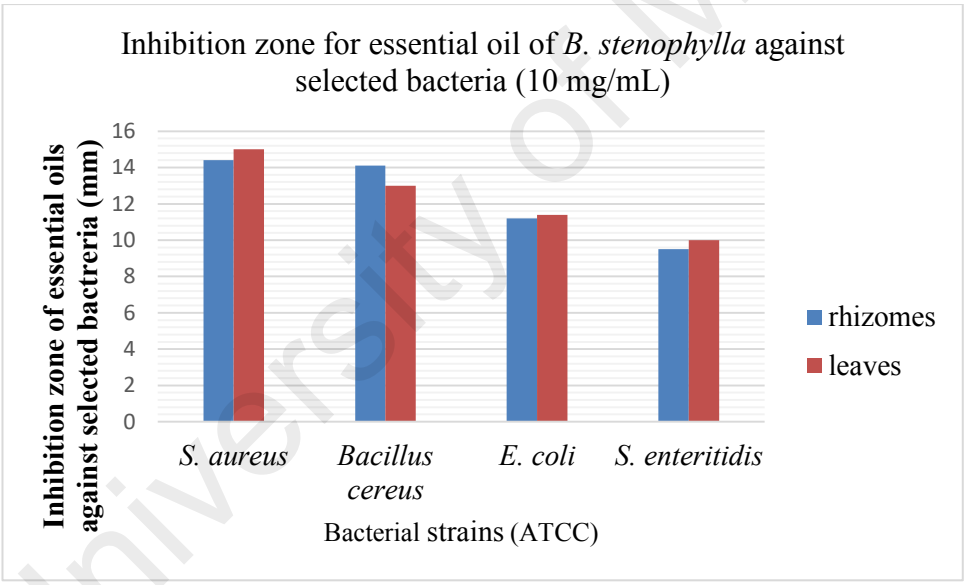


Figure 4.3: Inhibition zone of rhizome and leaf oils of *Boesenbergia stenophylla* against selected bacterial strains

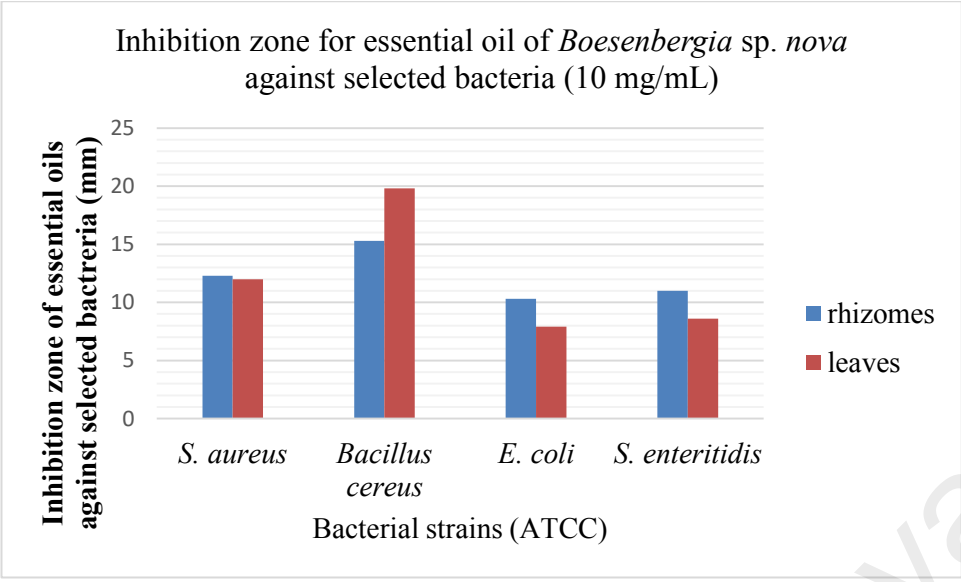


Figure 4.4: Inhibition zone of rhizome and leaf oils of *Boesenbergia* sp. *nova* against selected bacterial strains

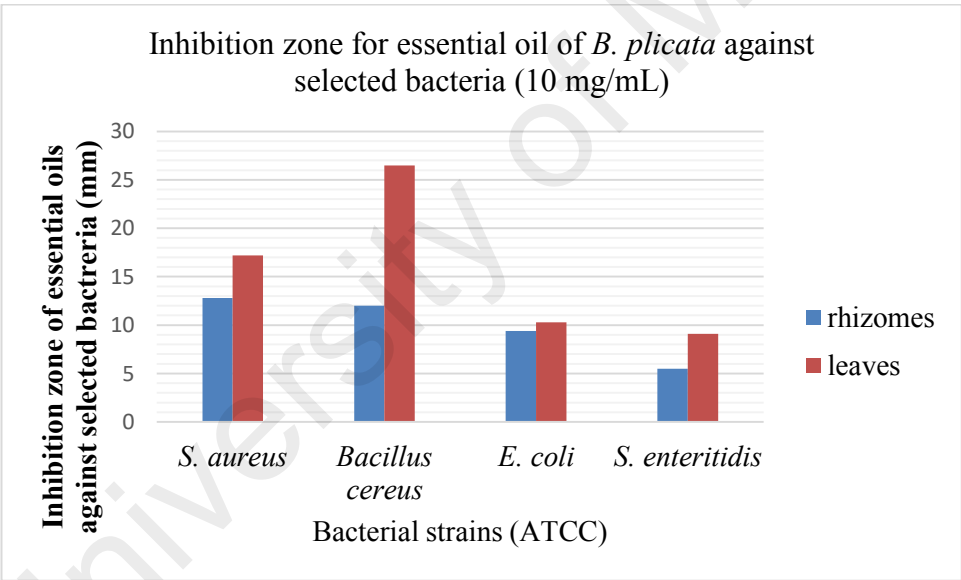


Figure 4.5: Inhibition zone of rhizome and leaf oils of *Boesenbergia plicata* against selected bacterial strains

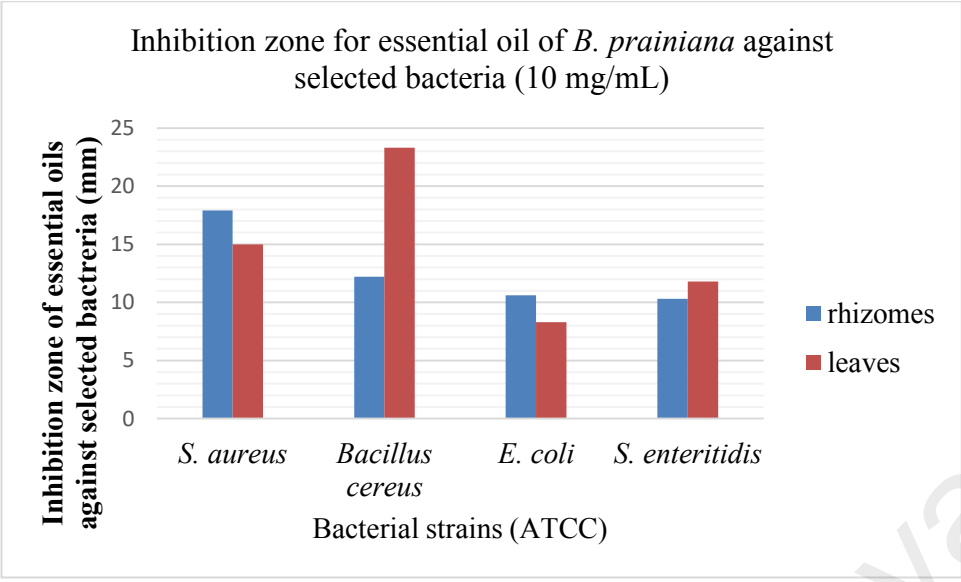


Figure 4.6: Inhibition zone of rhizome and leaf oils of *Boesenbergia prainiana* against selected bacterial strains

The antibacterial activity of the rhizome and the leaf oils from five *Boesenbergia* species exhibited a wide range activity against four food-borne pathogens namely*: Staphylococcus aureus, Bacillus cereus, Salmonella enterica* and *Escherichia coli.* Overall, Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*), showed higher inhibition compared to Gram-negative bacteria (*Salmonella enterica* and *Escherichia coli*) in all species tested (Figure 4.2, 4.3, 4.4, 4.5, 4.6).

Based on the Table 4.17, the inhibition zone from leaf oils of *Boesenbergia plicata* and *Boesenbergia prainiana* against *Bacillus cereus* showed an extremely sensitive inhibition with 26.5 mm and 23.3 mm, respectively while the other rhizome and leaf oils showed varies inhibition against Gram-positive bacteria and Gram-negative bacteria with a very sensitive inhibition (15-19 mm), sensitive inhibition (9-14 mm) and no inhibition (<8 mm). The inhibition zone of the rhizome and leaf oils of *B. armeniaca* and *B. plicata* against *Bacillus cereus* are shown in Appendix L and Appendix M, respectively.

4.3.1 Minimum inhibitory concentration (MIC)

Table 4.17: The minimum inhibitory concentration (MIC) of essential oils of *Boesenbergia* species (mg/mL) against four bacterial strains

Bacterial strains (ATCC) / **Species**	**Minimum Inhibitory Concentration (MIC) (mg/mL)**			
	Staphylococcus aureus	*Bacillus cereus*	*E. coli*	*Salmonella enteritidis*
Boesenbergia armeniaca				
• Rhizome	2.5	2.5	5	5
• Leaf	2.5	2.5	5	5
Boesenbergia stenophylla				
• Rhizome	2.5	2.5	5	5
• Leaf	2.5	2.5	5	5
Boesenbergia sp. *nova*				
• Rhizome	5	2.5	5	5
• Leaf	2.5	2.5	5	5
Boesenbergia plicata				
• Rhizome	2.5	2.5	5	5
• Leaf	2.5	1.25	5	5
Boesenbergia prainiana				
• Rhizome	2.5	2.5	5	5
• Leaf	2.5	2.5	5	5

MIC (mg/mL)	**Activity status**
≥ 5	Weak
< 5 – 2.5	Moderate
< 2.5	Strong

The minimum inhibitory concentration (MIC) values for the bacteria were determined by the disc-diffusion assay in serial two-fold dilution method. Minimum inhibitory concentration (MIC) value for the rhizomes and leaf oils were also determined against *Staphylococcus aureus, Bacillus cereus, Salmonella enterica* and *Escherichia coli*. The MIC results for the rhizome and leaf oils of selected *Boesenbergia* species are presented in Table 4.18. Based on the results, the MIC values demonstrate a wide range of activity (MIC of 1.25-5 mg/mL) against four bacterial strains.

The leaf oil of *Boesenbergia plicata* had the highest efficiency against *Bacillus cereus* with a minimum concentration of 1.25 mg/mL. *Bacillus cereus* proved to be the most sensitive of the tested bacteria in this present study since it is susceptible to all essential oils investigated. Results revealed that the two Gram-negative bacteria were resistant to all essential oil tested with MIC values of 5 mg/mL.

CHAPTER 5: DISCUSSION

Essential oils of rhizomes and leaves obtained by hydrodistillation from *Boesenbergia armeniaca, Boesenbergia stenophylla, Boesenbergia* sp. *nova*, *Boesenbergia plicata* and *Boesenbergia prainiana* revealed different percentages of yield and colours of the essential oils. The colour of the leaf and rhizome oils range from lighter yellow to intense yellow for instance golden yellow in the rhizome oil of *Boesenbergia armeniaca* while the percentage yield varies from 0.01% (the leaf oil of *Boesenbergia armeniaca*) to 0.14% (the rhizome oil of *Boesenbergia* sp. *nova*). The total percentage of the essential oil constituents detected ranged from 71.25% (the rhizome oil of *Boesenbergia plicata*) to 100% (the leaf oil of *Boesenbergia stenophylla*).

Of the five *Boesenbergia* species studied, the rhizome oils of *Boesenbergia stenophylla*, *Boesenbergia* sp. *nova* and *Boesenbergia prainiana* revealed higher total number of compounds compared to the leaf oils. On the contrary, the leaf oils of *Boesenbergia armeniaca* and *Boesenbergia plicata* showed higher total number of compounds compared to the rhizome oils. In this study, out of the five species investigated *Boesenbergia prainiana* showed the highest total number of compounds with fifty-nine compounds constituting 98.47% of the leaf oil and sixty-six compounds constituting 97.73% of the rhizome oil. The monoterpene hydrocarbons dominated the volatile profile of the rhizome oil of *Boesenbergia prainiana* showing a composition of 27.40% from the total oil and mainly characterized by isolimonene (10.09%) and L-β-pinene (9.51%), while the leaf oil of *Boesenbergia prainiana* is dominated by oxygenated monoterpene representing 24.78% of the total oil comprising of myrtenol (14.13%), L-pinocarveol (3.39%) and isopinocamphone (3.03%).

Among the essential oils analysed, five major compounds were identified. These are methyl cinnamate (55.42% - 83.17%), nerolidol (22.77% - 42-55%) present in the leaf and rhizome oils of *Boesenbergia stenophylla* and *Boesenbergia armeniaca*, respectively. Whereas γ-maaliene (22.82%) is found in the leaf oil of *Boesenbergia* sp. *nova*, and isocamphene (28.07%) and (-)-β-pinene (21.33%) are detected in the rhizome and leaf oil of *Boesenbergia plicata*, respectively. Meanwhile, no major compounds were detected in the rhizome and leaf oils of *Boesenbergia prainiana*. From previous studies, a constituent is considered as major if its percentage composition in the oil is 20% and above (Bakkali *et al.,* 2008).

The rhizome and leaf oils of *B. stenophylla* can be exploited as a useful natural source for methyl cinnamate since it is a phenylpropanoid derivative that is abundant in the essential oil. It gives a pleasant and strong aromatic constituent of fruits and culinary spices that are used in the flavor industry. Methyl cinnamate also acts as a fragrant ingredient that can be found in decorative cosmetics, fine fragrances, shampoos, as well as in non-cosmetic products such as household cleaners and detergents (Sharma & Kanwar, 2012). In other studies, a similarly high percentage of methyl cinnamate was reported to be present in several other Zingiberaceae species, such as the leaf oil of *Alpinia malaccensis* var. *nobilis* (63.0%) from Pahang; the leaf oil of *Alpinia malaccensis* var. *nobilis* (88.0%) from Terengganu and the rhizome oil of *Alpinia nieuwenhuizii* (67.8%) from Sabah (Azah *et al*., 2005; Mashitah *et al*., 2011; Vejayan *et al*., 2017).

Meanwhile, nerolidol found in *Boesenbergia armenica* is known to exhibit anti-inflammatory, antinociceptive, anti-schistomotal and antiulcer activities (Klopell *et al*., 2007; Silva *et al*., 2014; Fonseca *et al*., 2015). Nerolidol also gives a mild delicate sweet and floral odour. A similarly high percentage of nerolidol was reported in several other Zingiberaceae species such as the rhizome oil of *Hedychium coccineum* Buch.-

Ham. Ex Sm. (44.40%) from Mauritius; the seed oils of *Aframomum dalzielii* Hutch. (91.20%), *Aframomum letestuanum* Gagnep (88.0%) and *Aframomum pruinosum* Gagnep. (95.10%) from Cameroon (Gurib-Fakim & Maudarbaccus, 2002; Nguikwie *et al.,* 2013).

In general, the rhizome and leaf oils of the five *Boesenbergia* species investigated in this project consisted mainly of two to four of the main compounds except for *Boesenbergia* sp. *nova.* The main constituents of the rhizome oil of *Boesenbergia* sp. *nova* comprised of five compounds namely, γ-maaliene (18.43%), 4,11-selinadiene (10.18%), α-panansinene (8.90%), α-cubebene (7.27%), and β-cadinene (6.67%). Meanwhile, the six main compounds of the leaf oil of *Boesenbergia* sp. *nova* are γ-maaliene (22.82%), α-panansinene (9.12%), α-cubebene (8.98%), β-selinene (6.31%), γ-selinene (6.28%) and patchoulene (5.83%). A compound is considered as "main" if its percentage composition is from ≥5% to <20%.

Linalool found in the rhizome (19.83%) and leaf oils (11.63%) of *Boesenbergia armeniaca* is reported to exhibit anti-inflammatory, anti-tumor and anti-spasmodic activity (Peana *et al*., 2002; Chang & Shen, 2014; Rekha *et al.,* 2014). Linalool possesses a mild and light floral odour with slight citrus impression. Therefore, it is regarded as one of the most popular perfume ingredients that were found in 90% of all fragrances. For instance, it is used in large quantities of soap and perfume products (Bickers *et al*., 2003).

Phytol is an aromatic ingredient used in many fragrant products and also can be found in cosmetic and non-cosmetic products (McGinty *et al.,* 2010). Several studies have been reported regarding the effectiveness of phytol in reducing cholesterol levels in blood and also in biological activities such as anti-schistosomal, antioxidant and antinociceptive activities (Santos *et al*., 2013; De Moraes *et al.,* 2014). The average

worldwide use of phytol ranged from 0.1 to 1.0 metric tons per year while the average maximum that go into fine fragrances has been reported to be 0.20% (McGinty *et al.,* 2010). Since phytol is present in appreciable amount in the leaf oil of *Boesenbergia plicata* (14.52%), it could be potentially exploited as one of the natural source for phytol.

From previous studies, *Boesenbergia longiflora* from Thailand, *Boesenbergia stenophylla* from Sarawak and *Boesenbergia plicata* from Kedah showed that the volatile consituents were dominated by sesquiterpenoid groups such as longipinocarvone (81.69%), (E)-methyl cinnamate (53.4%), β-pinene (11.4%), while *Boesenbergia rotunda* from Pahang was reported to be highly rich in monoterpenoids with abundance of nerol (39.56%) and L-camphor (36.01%) (Ahmad & Jantan, 2003; Kar *et al*., 2014; Baharudin *et al.,* 2015; Omar *et al.*, 2015). In this study results showed that *Boesenbergia armeniaca* and *Boesenbergia* sp. *nova* were dominated by sesquiterpenoids, whereas *Boesenbergia stenopylla, Boesenbergia plicata* and *Boesenbergia prainiana* were dominated by monoterpenoids (Table 4.14 and Table 4.15).

Monoterpenes can be considered as broad-spectrum molecules with antimicrobial activity, especially antibacterial and antifungal effects (Bakkali *et al*, 2008). According to Dhanik *et al*., (2017), the monoterpene hydrocarbons are believed to be the most important contributors to the aroma of ginger (*Zingiber officinale*). Sesquiterpenes are known for their anti-inflammatory and insect repellent activities. For instance, β-caryophyllene and α-caryophyllene showed significant activities against the human pathogenic fungi such as *Candida glabrata* and *Candida albicans* (Sabulal *et al*., 2006). Dhanik *et al.,* (2017), also reported that oxygenated sesquiterpenes were discovered to be the significant contributors to the flavour properties in *Zingiber officinale*.

Interestingly, in this study, it was found that none of the identified compounds are common in the rhizome oils of five *Boesenbergia* species investigated. As for the leaf oils, only one compound, that is β-caryophyllene is found to be common in the five *Boesenbergia* species. These results implied that the five *Boesenbergia* species selected for this study are not closely related. The composition of β-caryophyllene varies in the order of: *Boesenbergia plicata* (9.85%) > *Boesenbergia armeniaca* (6.25%) > *Boesenbergia* sp. *nova* (2.97%) > *Boesenbergia stenophylla* (0.82%) > *Boesenbergia prainiana* (0.67%). Studies by other researchers showed that β-caryophyllene is responsible for the anti-bacterial, anti-inflammatory and anti-arthritic activity (Bakir *et al.,* 2008; Vijayalaxmi *et al*., 2015; Alencar Filho *et al.,* 2017). In addition, β-caryophyllene is commonly used as a fragrance chemical due to its pleasant odour (Sköld *et al*., 2006).

Overall, *Boesenbergia armeniaca, Boesenbergia stenophylla* and *Boesenbergia sp. nova* collected from the highlands of Sabah showed higher percentage of terpenoid compounds compared to *Boesenbergia plicata* and *Boesenbergia prainiana* which are lowland species from Peninsular Malaysia. Said *et al.,* (2011) reported that terpenoid content of the leaf extract of *Pistacia lentiscus* vary with altitude, revealing increasing compounds of monoterpene hydrocarbons with higher elevation. Terpenes have been reported to exhibit cytotoxicity against tumor cells and anti-inflammatory effect (Preedy, 2014).

The antibacterial activity and the minimum inhibitory concentration (MIC) of the rhizome and leaf oils of five wild *Boesenbergia* species namely *Boesenbergia armeniaca, Boesenbergia stenophylla, Boesenbergia* sp. *nova*, *Boesenbergia plicata* and *Boesenbergia prainiana* are summarized in Tabel 4.16 and Table 4.17, respectively. All the essential oils tested using disc-diffusion method at the concentration of 10mg/ml

exhibited a wide range activity against four food-borne pathogens namely*: Staphylococcus aureus, Bacillus cereus, Salmonella enterica* and *Escherichia coli.*

The negative control, methanol (MeOH), showed no inhibiting effect whereas the positive control, kanamycin (50 µg/ml), showed different zones of inhibition against *Staphylococcus aureus* (48.5 mm) > *Bacillus cereus* (44.2 mm) > *Salmonella enteritidis* (42.4 mm) > *E. coli* (41.0 mm) (Tabel 4.16). The level of inhibition of essential oils depends on the growth of inhibition diameter (mm) which can be classified as: extremely sensitive inhibitory (≥20 mm), very sensitive inhibitory (15-19 mm), sensitively inhibitory (9-14 mm) and no inhibitory (<8 mm) (Ponce *et al*., 2003). Based on this classification, the leaf oils of *Boesenbergia plicata* (26.5 mm) and *Boesenbergia prainiana* (23.3 mm) appear to exhibit the largest inhibition zone suggesting an extremely sensitive inhibitory activity (Table 4.16). However, on the overall, the rhizome oils of the five *Boesenbergia* species studied, showed larger zones of inhibition against the bacterial strains compared to the leaf oils. These results may be due to the presence of linalool (19.83%), δ-elemene (9.25%) and β-elemene (5.00%) which, in previous studies were reported to exhibit antibacterial activity (Beier *et al*., 2014; Oyedeji & Afolayan, 2005; Zhu *et al,* 2013). A study by Kasture *et al*., (2015) showed that the rhizome oil of *Acorus calamus* showed higher antibacterial activity than the leaf oil against selected bacterial strains and fungus.

Of the essential oils tested, the leaf oils of *Boesenbergia prainiana* (23.3 mm inhibition zone) with MIC value of 2.5 mg/mL and *Boesenbergia plicata* (26.5 mm inhibition zone) with MIC value of 1.25 mg/mL exhibited strong activity against *Bacillus cereus*. The β-caryophyllene (0.67%-9.85%), linalool (0.79%-3.21%) and phytol (1.76%-14.52%) present in the leaf oils may be responsible for the good antibacterial activity exhibited by these oils.

Almost all of the rhizome and leaf oils displayed relatively moderate level of MIC values (2.5 mg/mL) against *Staphylococcus aureus* and *Bacillus cereus* except for the rhizome oil of *Boesenbergia* sp. *nova* which showed weak activity with MIC value of 5 mg/mL against *Staphylococcus aureus* and the leaf oil of *Boesenbergia plicata* against *Bacillus cereus* which showed strong activity with MIC value of 1.25 mg/mL (Table 4.17).

Overall, all the essential oils of the five *Boesenbergia* species studied exhibited low antibacterial activity against the Gram-negative bacteria (*Salmonella enterica* and *E. coli*) with MIC value of 5 mg/mL. Of these, the rhizome oil of *Boesenbergia plicata* against *Salmonella enteritidis* (5.5 mm inhibition zone) exhibited the lowest antibacterial activity with MIC value of 5 mg/mL. Based on the report by Joy *et al*., (2007), the oxygenated monoterpene of 1,8-cineole (eucalyptol) was discovered as one of the important compound against the Gram-negative bacteria. Thus, the weak inhibitory antibacterial activity of the oils against the Gram-negative bacteria in the present study were not suprising since the compound 1,8-cineole is not present in any of the essential oils that were analysed. The Gram-negative bacteria are less susceptible since they possess an outer membrane surrounding the cell wall, which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering (Vaara, 1992).

Interestingly, the leaf oils of five *Boesenbergia* species in the present study showed potent activity against Gram-positive bacteria of *Bacillus cereus*. The order of activity is as follows: *Boesenbergia plicata* (26.5 mm inhibition zone) with MIC value of 1.25mg/mL > *Boesenbergia prainiana* (23.3 mm inhibition zone) with MIC value of 2.5mg/mL > *Boesenbergia* sp. *nova* (19.8 mm inhibition zone) with MIC of 2.5mg/mL > *Boesenbergia stenophylla* (13.0 mm inhibition zone) with MIC value of 2.5mg/mL > *Boesenbergia armeniaca* (12.6 mm inhibition zone) with MIC value of 2.5mg/mL.

Volatile constituents such as phytol (14.52%), β-caryophyllene (9.85%) and linalool (3.21%) that were present in the leaf oils may be responsible for the activity since they were reported to display antibacterial activity against *Bacillus cereus* (Ghaneian *et al.*, 2015; Silva *et al.,* 2015).

Other studies on the antibacterial activity of *Boesenbergia rotunda* showed a wide spectrum on inhibitory activity against microorganisms (Wannisorn *et al*., 2009; Rahman *et al*., 2016). It is reported that the essential oils from the rhizome of *Boesenbergia rotunda* showed antibacterial activity against bacteria, fungi and yeast (Jitvaropas *et al*., 2012; Jantapan *et al*., 2017; Taechowisan *et al*., 2017). Natta *et al.,* (2008) reported the antibacterial activity of the rhizome oil of *Boesenbergia rotunda* against *Staphylococcus aureus,* (MIC value of 12.5 µg/mL) *Bacillus cereus* (MIC value of 12.5µg/mL) and *E. coli* which showed the diameter of the inhibiton zone of 15.0 mm, 16.0 mm and 9.0 mm, respectively.

On the overall, the different performances on anti-bacterial activity tested on the rhizome and leaf oils in this research can be linked to their chemical compositions such as the content of monoterpenes, sesquiterpenes and others (Celikel & Kavas, 2008).

CHAPTER 6: CONCLUSION

The data on profiling of volatile constituents from essential oils of *Boesenbergia armeniaca, Boesenbergia stenophylla, Boesenbergia* sp. *nova* (from Sabah), *Boesenbergia plicata* and *Boesenbergia prainiana* from Peninsular Malaysia are reported for the first time and results revealed that the most abundant compounds were dominated by monoterpenoids (*Boesenbergia stenophylla, Boesenbergia plicata* and *Boesenbergia prainiana*) and sesquiterpenoids (*Boesenbergia armeniaca* and *Boesenbergia* sp. *nova*).

The results showed that only β-caryophyllene (sesquiterpene hydrocarbon) is common in the leaf oils of the *Boesenbergia* species but present in different percentage of composition: *Boesenbergia plicata* (9.85%) > *Boesenbergia armeniaca* (6.25%) > *Boesenbergia* sp. *nova* (2.97%) > *Boesenbergia stenophylla* (0.82%) > *Boesenbergia prainiana* (0.67%). Since β-caryophyllene has been reported to have anti-bacterial, anti-inflammatory and also as an effective anti-arthritic agent (Bakir *et al.,* 2008; Vijayalaxmi *et al*., 2015; Alencar Filho *et al.,* 2017), it can be suggested that the leaf oils of the five *Boesenbergia* species might be potentially used as the agent of biological activity as well as for other purposes.

Of the essential oils analysed, methyl cinnamate (55.42%-83.17%) and nerolidol (22.77-42.55%) were found to be the most abundant major compounds that were present in the rhizome and leaf oils of *Boesenbergia stenophylla* and *Boesenbergia armeniaca,* respectively. Methyl cinnamate and nerolidol are the fragrance ingredients that have been used in many fragrance products (Bhatia *et al.,* 2007: Lapczynski *et al*., 2008). Hence, the essential oils of these species have great potential to be used in detergents, beauty soaps, perfume, cosmetics and lotions.

The essential oils of the five *Boesenbergia* species exhibited a wide spectrum of antibacterial activity against four foodborne pathogens namely *Staphylococcus aureus, Bacillus cereus, Salmonella enteritidis* and *Escherichia coli.* The leaf oil of *Boesenbergia plicata* showed the highest efficiency against Gram-positive bacteria, *Bacillus cereus* with strong inhibitory activity (26.5 mm inhibition zone) with MIC of value of 1.25 mg/mL. Therefore, it is suggested that this species has potential to be developed as a food preservative agent to control foodborne pathogens especially *Bacillus cereus* since the oil possess good antibacterial activity.

This study has provided the essential oil profiling data on five *Boesenbergia* species revealing several major compounds in significant amounts. These could provide useful resources for further future studies on selected biological activities.

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LIST OF PUBLICATION

Publication

Nor, N. A. M., & Ibrahim, H. (2018). Chemical constituents of essential oils of *Boesenbergia armeniaca* and *Boesenbergia stenophylla* growing wild in Borneo. *Pakistan Journal of Botany, 50(5),* 1917-1922.