Faculty of Resource Science and Technology

CHEMICAL COMPOSITION OF *ARTOCARPUS ODORATISSIMUS*, *CANARIUM ODONTOPHYLLUM* AND *LITSEA GARCIAE* FRUITS FROM SARAWAK

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(Analytical Chemistry)
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DECLARATION

I declare that this report entitled "Chemical Composition of *Artocarpus odoratissimus*, *Canarium odontophyllum* and *Litsea garciae* Fruits from Sarawak" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not currently submitted in candidature of any other degree.

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Specially dedicated to my father, Poli bin Dahlan, my mother, Rohah binti Menol, my dear siblings and also my friends.

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ABSTRACT

Canarium odontophyllum, *Litsea garciae* and *Artocarpus odoratissimus* are among of the indigenous edible fruits that can be found in Sarawak and these highly seasonal fruits are very popular among local people. The fruits collected from different Divisions in Sarawak were analysed for their nutritional properties. The properties analysed include proximate analysis such as moisture content, ash content, crude fat, crude fiber and vitamin C. The results show that both pulps and kernels of *C. odontophyllum*, *L. garciae* and *A. odoratissimus* proved to be important sources of necessary nutrients for human diet. Mineral content in the fruit pulps analysed using atomic absorption spectrophotometer (AAS) shows that potassium (K) is the major element detected in all fruit samples with the highest abundance was detected in the pulps of *C. odontophyllum* (6116.47–10814.84 mg/kg). The samples are also rich in calcium (Ca), magnesium (Mg) and sodium (Na). Both the fruit pulps and kernels were extracted using Soxhlet extractor and the fatty acids in the oils were esterified prior to gas chromatography-mass spectrometry (GC-MS). The major components occurred in all pulp oils are palmitic acid (C16:0), linoleic acid (C18:2n6c) and oleic acid (C18:1n9c). The highest abundance of oleic acid was detected in the pulp oils of L. garciae (45.25-51.36%) among the three species, while pulp oils of C. odontophyllum contain the highest amount of palmitic acid (40.93-55.75%). Significant amount of linoleic acid in pulp oils of A. odoratissimus was detected in the range between 38.02 to 45.60%. The major compound in kernel oils of each fruit sample differs from each other where lauric acid (C12:0) and linoleic acid (C18:2n6c) are the dominant compounds in kernel oil of *L.garciae* and *A. odoratissimus*, respectively. Oleic acid and palmitic acid are the compounds with the highest and almost equal abundance in kernel oil of *C. odontophyllum*. Total phenolic content measured in the methanol extracts

of the fruit pulps using Folin-Ciocalteu reagent assay shows the highest abundance detected in *C. odontophyllum* extracts (19.33-37.09 mg GAE/g dw). Antioxidant activity determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay also shows the extracts of *C. odontophyllum* possessed the highest antioxidant activity compare to the other extracts with IC_{50} of between 87.58 to 119.50 µg/mL.

KOMPOSISI KIMIA DI DALAM BUAH *Canarium odontophyllum*, *Litsea garciae* DAN *Artocarpus odoratissimus* DARI SARAWAK

ABSTRAK

Canarium odontophyllum, *Litsea garciae* dan *Artocarpus odoratissimus* adalah antara buah berasal dari Sarawak yang boleh dimakan. Buah-buahan bermusim ini sangat digemari dalam kalangan penduduk tempatan. Buah yang dikumpulkan dari beberapa Bahagian di Sarawak telah dianalisis untuk sifat-sifat nutrisi. Sifat-sifat yang dianalisis adalah analisis proksimat seperti kandugan lembapan, kandungan abu, lemak mentah, serat kasar dan vitamin C. Hasil analisis menunjukkan isi dan biji *C. odontophyllum*, *L. garciae* dan *A. odoratissimus* adalah sumber yang penting untuk nutrien yang diperlukan untuk diet manusia. Kandungan mineral di dalam isi buah dianalisis menggunakan spektrofotometer serapan atom mununjukkan bahawa kalium (K) adalah unsur utama dikesan dalam semua sampel buah dan kelimpahan kalium yang paling tinggi dikesan dalam isi buah *C. odontophyllum* (6116.47–10814.84 mg/kg). Sampel-sampel tersebut juga kaya dengan kalsium (Ca), magnesium (Mg) dan natrium (Na). Isi dan biji buah telah diekstrak dengan menggunakan pengekstrak Soxhlet dan asid lemak dalam minyak telah diesterifikasi sebelum dianalisis menggunakan kromatografi gas-spektrometri jisim. Komponen utama dikenalpasti dalam semua minyak isi buah adalah asid palmitik (C16:0), asid linoleik (C18:2n6c) dan asid oleik (C18:1n9c). Di antara tiga spesies tersebut, kelimpahan asid oleik yang tertinggi telah dikesan dalam minyak isi buah *L. garciae* (45.25-51.36%), manakala minyak isi buah *C. odontophyllum* mengandungi kelimpahan asid palmitik yang tertinggi (40.93-55.75%). Kandungan asid linoleik dalam minyak isi buah *A. odoratissimus* dikesan dalam julat di antara 38.02 sehingga 45.60%.

Komponen utama di dalam minyak biji adalah berlainan daripada buah yang berbeza. Asid laurik (C12: 0) dan asid linoleik (C18:2n6c) ialah komponen dominan dalam minyak biji buah *L. garciae* dan *A. odoratissimus*, masing-masing nya. Asid oleik dan asid palmitik adalah komponen dengan kelimpahan paling tinggi dalam minyak biji *C. odontophyllum* dan kelimpahannya adalah hampir sama. Jumlah kandungan fenolik dalam ekstrak metanol isi buah yang ditentukan menggunakan assei bahan uji Folin-Ciocalteu menunjukkan bahawa kelimpahan tertinggi dikesan dalam ekstrak *C. odontophyllum* dari Mukah (37.09 ± 0.20 mg GAE/g dw). Aktiviti antioksida ditentukan dengan menggunakan assei radikal-memerangkap 1,1-difenil-2-picrihidrazil juga menunjukkan bahawa ekstrak dari *C. odontophyllum* mempunyai aktiviti antioksida yang tertinggi jika dibandingkan dengan ekstrak-ekstrak yang lain dengan nilai IC_{50} dari 87.58 sehingga 119.50 µg/mL.

TABLE OF CONTENTS

CHAPTER 2: LITERATURE REVIEW

CHAPTER 3: EXPERIMENTAL

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AAS	-	Atomic absorption spectrophotometer
AOAC	-	Official Methods of Analysis
BHT	-	Butylated hydroxytoluene
DPPH	-	1,1-diphenyl-2-picrylhydrazyl
EPA	-	Environmental Protection Agency
FAMEs	-	Fatty acid methyl esters
FAO	-	Food and Agriculture Organisation
GC	-	Gas chromatography
GC-MS	-	Gas chromatography-mass spectrometry
HPLC	-	High-performance liquid chromatography
IC_{50}	-	Concentration of extract at 50% inhibition
ICP-AES	-	Inductively coupled plasma-atomic emission spectroscopy
ISE	-	Ion-selective electrodes
LLE	-	Liquid-liquid extraction
MS	-	Mass spectrometry
MUFA	-	Monounsaturated fatty acid
PUFA	-	Polyunsaturated fatty acid
SFA		Saturated fatty acid
UV	-	Ultraviolet
UV-Vis	-	Ultraviolet-visible
WHO	-	World Health Organization

LIST OF UNITS

%	-	percentage
µg/mL	-	microgram per milliliter
µL	-	microliter
cm	-	centimeter
g	-	gram
g/100 g		gram per hundred gram
kg	-	kilogram
M	-	molarity
m	-	meter
mg	-	milligram
mg GAE/g dw	-	milligram gallic acid equivalent in gram dry weight
mg/100 g	-	milligram per hundred gram
mg/L	-	milligram per liter
mg/mL	-	milligram per milliliter
mL	-	milliliter
nm	-	nanometer
ºC	-	degree Celsius
rpm	-	rotations per minute
v/v	-	volume over volume
w/v	-	weight per volume
µL	-	microliter

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Fruits are low in energy but their benefits to health surpass their caloric contribution due to the presence of vitamins and provitamins such as ascorbic acid, tocopherols, carotenoids and others, minerals, antioxidants and various phyto-nutrients which provides plenty of soluble dietary fibre. Furthermore, they are also rich in a wide variety of phenolic substances and important source of essential elements (Brug *et al.*, 2006; Loliger, 1991). Several evidences have been reported on the role of fruit in the prevention of chronic diseases such as cardiovascular diseases (He *et al*., 2007; Dauchet *et al*., 2006), hypertension (Dauchet *et al*., 2007), stroke (He *et al*., 2006; Dauchet *et al*., 2005) and probable evidence for the protection against some cancers of the gastrointestinal and respiratory tracts (Brug *et al.*, 2006; FAO/WHO, 2004) if consumed in recommended amounts. Furthermore, there is evidence that high fruit intake is associated with a reduced risk of osteoporosis and some eye diseases such as cataracts and age-related nuclear lens opacities (Christen *et al*., 2005; Bell and Whiting, 2004; Lin *et al*., 2003). Fruit consumption may also play an important role in weight management as it is low in calories and fat, thus prevent overweight and obesity (Bes-Rastrollo *et al.*, 2006; Rolls *et al*., 2004).

Increased risk of health diseases for instance obesity, diabetes and cancer, as a result of the excessive consumption of energy-dense, nutrient poor food in combination with sedentary lifestyle has led to 1.4 billion adults and school children who are overweight and

half a billion who suffer from obesity, worldwide (WHO, 2012). On the contrary, about one billion people are hungry (FAO, 2010) and two billion suffer from micronutrient deficiencies (WHO, 2007) despite that global agricultural production is theoretically adequate to feed the world's population. Supplementary efforts are required to address all forms of malnutrition (undernourishment, micronutrient deficiency and over nutrition) by escalating the availability and affordability through production of a wide range of diverse foods that are needed for a healthy diet. Such foods include fruits, vegetables and some animal source foods (Charrondiere *et al*., 2013; Wolf and Elmadfa, 2010).

The consumption of tropical fruits is increasing in recent years and intensive investigation on them has also increased (Konczak & Roulle, 2011; Ikram *et al*., 2009). However, the information on their nutritive value is scarce, especially for exotic fruits that can only be found in certain places. The potential of indigenous, neglected or underutilized edible fruits to improve dietary diversity remains largely unknown and underexploited.

Borneo Island is the third largest island in the world with a total area of 287,000 square miles. The island is divided into four parts, Malaysian states of Sarawak and Sabah, Kalimantan (province of Indonesia) and Brunei Darussalam. Situated along the equator, the island receives abundance of rainfall, and has one of the most diverse floras in the world. Borneo has nearly 11,000 species of plants where about a third of which are indigenous (Julaini, 2009).

Sarawak is not exempted from sharing this speciality. Sarawak is the largest state in Malaysia, situated on the north-west of Borneo Island and is known as 'Bumi Kenyalang' (Land of Hornbill). Sarawak has a vast tropical rainforest which mainly contributes to the unique and bizarre plant diversity of Borneo. Not only rich with diverse language, culture and lifestyle, the land is also rich with numerous indigenous fruits.

Canarium odontophyllum, *Litsea garciae* and *Artocarpus odoratissimus* are among of the indigenous edible fruits that can be found in Sarawak. These highly seasonal fruits are very well-liked by the people in Sarawak. *C. odontophyllum* which is known as dabai by the local people is a fruit with oblong shape and dark purple/black skin when it is ripe. *L. garciae* on the other hand is called as engkala by the local people. This fruit comes with round shape with pink skin and green petal when it is ripe. *A. odoratissimus* (terap) is native to Borneo and it is related to breadfruit and jackfruit. This fruit has a strong scent and is considered superior in flavour to both Jackfruit and Chempedak. The aim of this study is to emphasize the nutritional value (proximate composition, minerals, phenolic content and antioxidant activity) of selected widely consumed fruits in Sarawak and to determine the fatty acids in their pulp and kernel oils.

1.2 Problem Statement

Chronic diseases, for instance diabetes, cancer and obesity are not new problems encountered by human kind nowadays since the modern lifestyle has taken over. Excessive consumption of carbohydrate, fatty and processed foods with lack of physical work out explains the health predicaments that occur not only in developed countries but also in under-developed and developing countries (WHO, 2000). Nevertheless, malnutrition and under-nutrition have also becoming a concern. Consequently, in order to alleviate such issues, continuous efforts are needed in searching for food sources with health enhancing properties. Fruit is one of the alternatives as it is a vital source of essential elements (Tahvonen, 1993). Increased fruit consumption can help replace foods with high content of saturated fats, salt and sugar, therefore improve the intake of most dietary fibre and micronutrients. This fact has

lead to increasing attention toward edible indigenous tropical fruits for the region and its people (Konczak & Roulle, 2011; Ikram *et al*., 2009; Leterme *et al*., 2006). In Sarawak, several fruits including *C. odontophyllum*, *L. garciae* and *A. odoratissimus* remain underutilized and only known and consumed by the local people. The local community in Sarawak consumes large amount of these fruits during the ripening season without knowing much about them. Studies on their nutritive values are vital to enrich food composition databases as they may be rich in essential elements to improve dietary diversity.

1.3 Objectives

The objectives of this study were:

(a) To determine proximate composition such as moisture content, ash content, crude fat, crude fiber and vitamin C in *C. odontophyllum*, *L. garciae* and *A. odoratissimus* fruits.

(b) To analyse fatty acids in the pulp and kernel oils of the fruit samples using gas chromatography-mass spectrometry (GC-MS).

(c) To determine mineral contents in the fruit pulp using atomic absorption spectroscopy (AAS).

(d) To determine total phenolic content and antioxidant activity in the methanol extracts of the fruit pulp using ultraviolet-visible (UV-Vis) spectroscopy.

1.4 Scope of Study

This research focuses in the analysis of three selected edible fruits (*C. odontophyllum*, *L. garciae*, and *A. odoratissimus*) from different divisions in Sarawak (Kuching, Kota Samarahan, Sibu, Mukah, Kapit and Miri). Proximate analysis including moisture content, ash content, crude fat, crude fiber and vitamin C were examined according to respective method. The kernel and pulp of the fruit samples were extracted using Soxhlet extraction method and the FAMEs composition in the esterified oils were analysed using GC-MS. Mineral content in the fruit pulp was investigated using AAS. The fruit pulp was also extracted with methanol and the extracts were subjected to antioxidant activity and phenolic content analysis. Folin-Ciocalteu reagent method was used to determine phenolic content while 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was used for the antioxidant activity. Both assays were analysed using ultraviolet-visible (UV-Vis) spectroscopy.

1.5 Significance of Study

This study introduces three indigenous edible fruits that are well known in Sarawak but remain underexploited. The information on proximate analysis, fatty acids, minerals, antioxidant activity and phenolic content of the fruit samples were expected to be valuable for future reference on the chemical composition of underutilized edible fruits from Sarawak. In addition, the fruits could be introduced and exported to Peninsula Malaysia or to other countries.

CHAPTER 2

LITERATURE REVIEW

2.1 *Canarium odontophyllum* Miq.

Canarium is a large genus in the family of *Burseraceae*, consisting of 75 species circulated throughout tropical Africa, Asia and the Pacific islands (Evans, 1992). The *Burseraceae* are best known for producing fragrant resins of economic, medicinal and cultural value such as frankincense, myrrh and copal (Langenheim, 2003). Comprises of 18 genera (ca. 700 spp.), the family is divided into three tribes namely *Canarieae*, *Protieae* and *Bursereae*, each of which is disseminated pan tropically across a broad range of low-elevation, frost-free habitats including rainforest, dry deciduous forest and desert (McGregor, 1991). The *Burseraceae* is also known as the Torchwood family, the frankincense and myrrh family or simply as the incense tree family (Weeks *et al*., 2005). More than 55 species are native to the tropics of Southeast Asia and they are valued for their fragrant resins, lumber, edible oil-rich kernels and the fruit pulp of some species are edible (Sui *et al*., 1997; McGregor, 1991). Table 2.1 listed several species from the genus *Canarium* of tribe *Canarieae* and their common names, uses, origin and geographical distribution.

Table 2.1: Common name, uses, origin and geographical distribution of several species from the genus *Canarium.*

	Common name	Uses	Origin and geographical distribution	References
Canarium album L.	Chinese olive, gan lan or qing guo (China)	Processed fruit consumed as beverage, candy and confections. Dried fruit as traditional folk medicine, anti-bacterium, anti-virus, anti-inflammation and detoxification.	Native to Southeast area of China. Introduced to other Asian tropical and subtropical regions.	He & Xia (2007)
Canarium harveyi Seem.	*Canarium* nut(English), ‘ai (Tonga), ai (Niue), kaunigai (Fiji), nangae (Vanuatu), Santa Cruz ngali nut (Solomon Island)	Nuts edible. Timber for canoe manufacture, food bowls, firewood etc. Used as traditional medicine. Oil as cooking fuel.	Solomon Island, Vanuatu, Fiji, Tonga and Niue.	Thomson & Evans (2006)
Canarium indicum L.	*Canarium* nut(English), galip nut (Papua New Guinea), nangai (Vanuatu), ngali (Solomon Island)	Nuts edible. Timber for canoe manufacture, light construction and other wood articles. Bark as traditional medicine for the treatment of chest pains. Burning resin used in traditional ceremonies and churches. Oil as substituted for coconut oil.	Native to Eastern Indonesia, Papua New Guinea, Solomon Island and Vanuatu. Introduced to Fiji.	Thomson & Evans (2006)
Canarium odontophyllum Miq.	Dabai, Sibu Olive (Sarawak)	Fruits edible.	Sumatra, Borneo (Sarawak, Sabah and East Kalimantan) and Philippines.	Khoo *et al.* (2012)
Canarium pimela L.	Black Chinese olive, Wu lan (China)	Processed fruits edible. Chinese traditional medicine: arresting bleeding and resolving phlegm, reducing swelling and easing pain. Oil used in food industry.	Native to South China. Introduced to tropical and subtropical regions of eastern Asia.	Zhen-Cheng *et a*l. (2010)

In Latin, *C. odontophyllum* means "toothed leaf". The tree of *C. odontophyllum* can be found in Sumatra, Borneo (Sarawak, Sabah and East Kalimantan) and the Philippines. It has also been introduced and cultivated in Queensland, Australia (Khoo *et al.*, 2012). The fruit of *C. odontophyllum* is known as dabai among the local community in Sarawak and it is similar in appearance to Mediterranean olive (*Olea europaea* L.). The green colour skin of an immature *C. odontophyllum* fruit turns to dark purple or black colour when the fruit is fully ripe (see Figure 2.1). The fruits are ovoid drupes, weighing 10.0 – 18.0 g and measuring 3.5-4.5 cm in length and 2.0 – 3.0 cm in diameter. The skin and the pale yellow fleshy pulp of *C. odontophyllum* fruit are edible, while the single three-angled seed is discarded.

Figure 2.1: Ripe *C. odontophyllum* fruit.

C. odontophyllum fruit is considered as one of the 100 high-value fruit species in Sarawak and has been classified as underutilized fruit. This fruit is also listed as a plant genetic resource for food and agriculture in Sabah (Wong *et al*., 2008; Jamadon *et al*., 2007). This highly seasonal fruit is very much appreciated by the local community during the fruit ripening season which is only between October to December. The whole ripe fruit is soaked in warm water to soften the pulp and then is consumed raw or seasoned with sugar or salt for better taste. Some people prefer to eat the fruit with salty soy sauce and chillies. Alternatively, the fruit can be processed into pickle product and the pickle can be fried with rice as 'Nasi goreng dabai' (Dabai fried rice) which can only be found in certain restaurants in Sarawak.

In recent years, *C. odontophyllum* fruit has caught considerable attention from local researchers seeing that the number of publications on the analysis of the fruit has increased since 2010. Most of the studies were focused on the strong antioxidant properties in the fruit and its effectiveness towards preventing diseases. A comparison of fatty acids, vitamin E and physicochemical properties between *C. odontophyllum*, olive and palm oils was documented by Azlan *et al*. (2010). In this paper, palmitic acid, myristic acid, oleic acid and linoleic acid were detected in *C. odontophyllum* pulp oil (36.1, 5.8, 41.5 and 11.8%) and kernel oil (46.4, 9.3, 35.1 and 2.8%) suggested that these oils have a high potential to be developed into healthy cooking oils.

Studies by Shakirin *et al*. (2010) and Prasad *et al*. (2011) showed that the antioxidant capacities in the peel of *C. odontophyllum* are higher than in the pulp and the kernel. Their finding also showed a high correlation between total phenolic content and antioxidant activity. Chew *et al*. (2011) and Ali Hassan *et al*. (2013b) have found a correlation between antioxidant activity, flavonoid, anthocyanin and carotenoid contents in *C. odontophyllum* fruits. Quantification of phenolic compounds in *C. odontophyllum* using high-performance

liquid chromatography (HPLC) showed the abundance of two phenolic acids, five flavonoids, ethyl gallate, three anthocyanidins and four anthocyanins (Chew *et al*., 2012). The fruit is also rich in calcium (28.47±1.56 – 43.72±22.72 mg/100 g fresh weight), potassium (5.02±0.13 – 6.93±1.71 mg/100 g fresh weight) and sodium (8.77±0.34 – 12.05±3.45 mg/100 g fresh weight) (Chew *et al*., 2011). *C. odontophyllum* fruit, especially the defatted pulp has the potential of cholesterol lowering ability (Shakirin *et al.,* 2012) and may serve as a great alternative treatment in the prevention of atherosclerosis (Nurulhuda *et al.,* 2013). Furthermore, Azlan *et al*. (2013) suggested the defatted fruit pulp as a future active pharmaceutical ingredient due to its strong *in vivo* antioxidant capacities.

2.2 *Litsea garciae* Vidal.

L. garciae is from the genus *Litsea*, a part of the *Lauraceae* family. The *Lauraceae* family, generally known for spicy leaves is a large family comprised of about ~55 genera and about 2500-3500 species. Nowadays, the family is centred in tropical America, South-East Asia and Australia, growing mainly in warm, tropical, lowland to mountain rainforests, although some genera extend to humid subtropical and temperate regions in both hemispheres (Li *et al*., 2008; Mabberley, 2008).

The evergreen genus *Litsea* is the second largest genus under *Lauraceae* family with an estimated of over 200-400 species. It is distributed abundantly in various countries throughout tropical and subtropical areas particularly in Asia, with a few species in the islands of the Pacific, Australia and from North America to South America. This genus is represented with 72 species in China, predominantly growing in the south and south-western parts of the country on the mountains of 1500 m above sea level or higher (Ng, 2005). While

in Borneo, there are about 22 *Litsea* species identified in which 47 species are reduced to synonymy including *L. firma*, *L. grandis*, *L. monopetala*, *L. resinosa*, *L. tomentosa*, *L. ochracea* and *L. garciae* (Yan *et al*., 2000). Some *Litsea* species are used as traditional medicines, to treat sprained or swollen ankle or knees, scabies and gastric. *Litsea* plants also exhibit a variety of biological activities including antimicrobial, antibacterial, antifungal, antioxidant, hypothermic and antitumor (Jiang *et al*., 2009; Menon *et al*., 1970).

L. garciae is native to Borneo Island. In Sarawak, the tree can be found in inland riparian forest, secondary forest and rarely in mixed dipterocarp forest. The tree is a medium-sized tree with long brittle spreading branches and the leaves lanceolate or obovate, often with unequal side, 25 cm long or more and non-hairy (Kueh *et al.*, 2000). The English name of *L. garciae* is bagnolo/ wuru lilin and the common name differs according to the local language: engkala (Malay Sarawak), Madang enkala/ pedar (Iban), Ta'ang (Bidayuh) and pengolaban (Sabah) (Johnny *et al*., 2011; Kueh *et al.*, 2000). The fruit of *L. garciae* is round in shape with a unique green stem cap, about 4.0- 6.0 cm in length (with the stem cap) and 3.0- 6.0 cm in diameter (see Figure 2.2). The thin skin turns to pinkish, bright pink or greenish white when it is ripe, depending on the variety. The edible white creamy flesh is very soft in texture and the taste is somewhat similar to avocado (*Persea americana*) (Ali Hassan *et al.*, 2013a). *L. garciae* is a highly seasonal fruit which is available only from September to December and it is very well-liked by the local community in Sarawak. The fruit is typically eaten raw after soaking it in warm water for 5 to 10 minutes or some people steams the fruit with rice, while the seed is discarded. A pinch of salt usually added to enhance the flavour.

(a)

(b)

(c)

Figure 2.2: The (a) diameter and (b) length of a *L. garciae* fruit; (c) the interior of a ripe *L. garciae* fruit with the kernel.

A study by Ali Hassan *et al.* (2013a) on the antioxidant activity and phytochemistry of *L. garciae* from Sabah reported that the stem cap of *L. garciae* displayed the highest antioxidant activity compare to the pulp and kernel. Total phenolic content and total flavonoid content were also found highest in the stem cap with the value of 8.29±0.70 mg gallic acid equivalents/g and 6.90±0.61 mg rutin equivalents/g, respectively. On the other hand, the fruit pulp had the highest abundance of total anthocyanin content (4.12±0.10 mg cyanidin-3-glucoside equivalents/100 g).

2.3 *Artocarpus odoratissimus* Blanco.

A. odoratissimus is from the genus of *Artocarpus* (Moraceae). The family of Moraceae consists of 60 genera which comprises of 1,400 species disseminated in the tropical and subtropical regions of Asia with a few species in the temperate regions (Jagtap and Bapat, 2010). As a group among tree plants, *Artocarpus* species are known to occupy a variety of ecological niches across different habitats and are being diverse in various forest ecosystems. A number of *Artocarpus* species are used as food and for traditional folk medicines in South-East Asia, Indonesia, Western part of Java and India. *Artocarpus* plants offer advantages as a lucrative multipurpose crop for producing fruits and timber. The fruits and fruit products hold potential in the diet as they possess not only pleasant taste but also natural source of instant energy (Kanzaki *et al.*, 1997; Verheij and Coronel, 1992).

A. altilis (breadfruit) and *A. heterophyllus* (jackfruit) are the main species in this genus. Breadfruit is native to Papua New Guinea and neighbouring Pacific Islands and has long been consumed as an important staple food by the Polynesians. As early as the 1700s, the European colonialists had recognized the potential of this crop to provide an abundant

source of food for the rest of the tropics (Jones *et al*., 2011). Traders and explorers introduced the breadfruit to South and Central America in 18th century and it is currently cultivated extensively in the humid tropics (Verheij and Coronel, 1992). Historical reports suggest that jackfruit is supposed to have originated in the rain forests of the Western Ghats in the South-western part of India. The tree produces the largest tree-borne fruits in the world and a fully grown tree can yield between 10-200 fruits (Azad *et al*., 2007; Reddy *et al*., 2004). Jackfruit is the national fruit of Bangladesh and is considered to be an extremely important tree by the natives (Bose, 1985). Other species in this genus also have good potential for commercial use as fruit crops. Table 2.2 shows the common name, uses, origin and geographical distribution of several species from the genus *Artocarpus.*

A. odoratissimus is less popular compared to jackfruit and breadfruit as the species is native to Borneo and Philippines only (Jagtap and Batap, 2010). The synonyms of *A. odoratissimus* are *Artocarpus tarap* Becc. and *Artocarpus mutabillis* Becc.. The common name for this species varies from different place. Table 2.3 shows the various common names of *A. odoratissimus* in different languages.

Table 2.2: Common names, uses, origin and geographical distribution of several species from the genus *Artocarpus* (Jagtap and Bapat, 2010).

Species	Synonyms	Common names	Uses	Origin and geographical distribution
A. altilis (Parkinson) Fosberg	*A. camansi* Blanco, *A. communis* J.R. & G. Forst, *A. incise*, *A. incisus* (Thunb) L.f.	Breadfruit	Fruit pulp tonic for liver, leaves to treat liver cirrhosis, hypertension and diabetes.	Native to Pacific and Tropical Asia, Indonesia, Papua New Guinea
A. chama Buch.-Ham.	*A. chaplasha* Roxb. *A. melinoxylus* Gagnep	Chaplasha	-	India, Burma
A. chempeden Spreng.	-	Chempedak	Seeds in diarrhea, roots in malaria fever.	South-East Asia, Indonesia
A. elasticus Reinw. Ex Blume	-	-	Bark in inflammation and female contraception, latex in dysentery, leaves to treat tuberculosis.	South-East Asia, West Malaysia
A. gomezianus Wall. Ex Trecul.	*A. pomiformis* Teijsm & Binn	Tampang Burung	-	Western part of Indonesia
A. heterophyllus Lam.	*A. brasiliensis* Gomez, *A. integra*, *A. integrifolia* auct., *A. jaca* Lam., *A. maxima* Blanco, *A. philippensis* Lamk	Jackfruit	Fruits edible, roots in diarrhea and fever, leaves as antisyphilic and vermifuge, ulcers and wound healing, leaves and stem barks used to treat anemia, asthma, dermatitis, diarrhoea, cough.	Native to Western Ghats India. Introduced in South-East Asian region

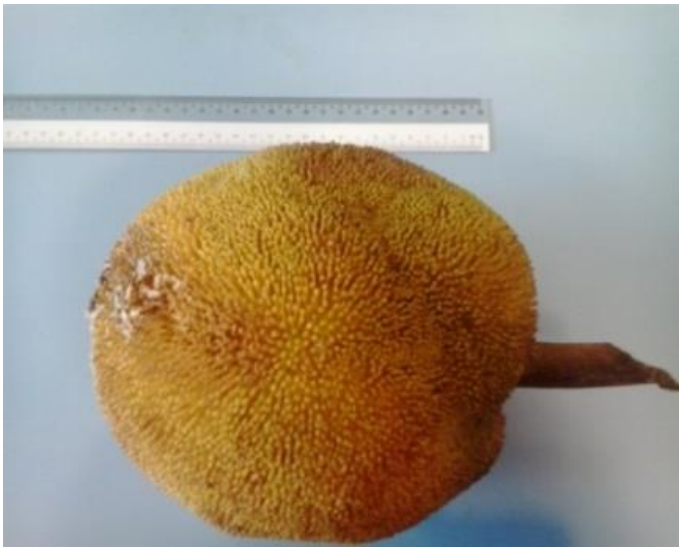
A. hirsutus Lam.	*A. hirsute* Lam.	-	-	South India
A. integer (Thunb.) Merr.	*A. champeden* (Lour.) Stokes, *A. integrifolius* L.f., *A. polyphema* Persoon, *Polyphema champeden* Lour., *Radermachia integra* Thunb.	-	Fruits edible.	Burma, Peninsular Thailand, Peninsular Malaysia, Sumatra, Borneo, Sulawesi lingga, Archipelago
A. lacucha Buch.-Ham.	*A. lakoocha* Roxb.	Monkeyjack, Lakoocha	Bark chewed like betel nut used to treat skin ailments.	Native to humid sub. Himalayan Regions of India, South China, South-East Asia
A. lowii King	-	Miku	Sap used as an ointment and as cooking oil.	Rare species in Malaysia
A. nobilis Thw.	-	-	Seeds and young fruits edible.	Endemic to Sri Lanka
A. odoratissimus Blanco.	*A. tarap* Becc., *A. mutabillis* Becc.	Marang, Terap	Fruits edible, leaves (ash) used as antidote for centipede bites and scorpion stings.	Borneo, Philippines
A. rotunda (Hout) Panzer	-	-	-	South-East Asia, Indonesia

Table 2.3: Common names of *A. odoratissimus* in different languages (Subhadrabandhu, 2001).

Languages	Common name
Iban	Pingan
Bidayuh	Pi-ien
Kelabit	Keiran
Malay	Terap
Sulu	Marang
Lanao	Madang
Tagalog	Loloi
Thailand	Khanun sampalor
Brunei	Tarap

A. odoratissimus tree is evergreen and can grow up to 25 m tall. The fruit is large, average from 16 to 24 cm in length, 12 to 17 cm in diameter and weigh about 1 kg. The fruits are spheroid in shape and thickly studded with short, brittle, greenish yellow spines (see Figure 2.3). The flesh is juicy and aromatic. Galang (1955) described the flesh as clinging to the central core and each segment of the flesh contains a seed with size of 8 × 15 mm. Ripe arils of *A. odoratissimus* are very sweet and consumed raw, while the young fruits are usually eaten as vegetables, cooked with spices and coconut milk or stewed with meat. The seeds are boiled or roasted and are eaten as a snack as they have nutty taste. Some local people use the sweet flesh to make terap fritters, ice-cream flavour, or organic vinegar. In traditional medication, ash obtained from the leaves is used as antidote for centipede bites and scorpion stings. Some people believe that malevolent spirits can be dispelled by hanging the leaves on the door of the house (Chai, 2006).

(a)



(b)

(c)

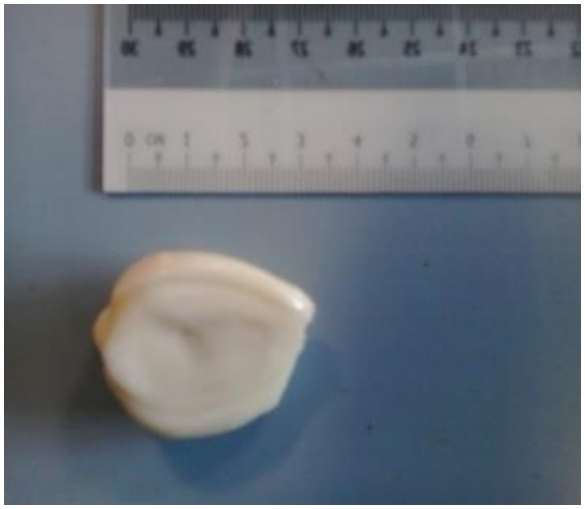


Figure 2.3: The (a) length of an *A. odoratissimus* fruit; (b) interior of a ripe *A. odoratissimus* fruit; and (c) the edible fruit pulp.

Several studies on the fruit of *A. odoratissimus* have been documented. Tang *et al*. (2013) have reported data on the proximate analysis of *A. odoratissimus* in Brunei Darussalam and suggested that this fruit is comparable in term of nutrients with *A. heterophyllus*, *A. altilis* and *A. integer*. The findings showed that *A. odoratissimus* is rich in potassium for both pulp (176–298 mg/100 g) and kernel (352–443 mg/100 g). Fructose is the dominant sugar in the fruit pulp with the value of 5.8–13.7 g/100 g (Tang *et al*., 2013). A study conducted by Abu Bakar *et al*. (2009) on the phytochemicals and antioxidant activity of different parts of *A. odoratissimus* from Sabah showed the kernel was higher in total phenolic, flavonoid content and antioxidant activities compared to the fruit pulp of this species. However, subsequent investigation has verified no cytotoxicity activity of crude extract from *A. odoratissimus* against selected cancer cell lines including ovarian, liver and colon cancer (Abu Bakar *et al*., 2010).

2.4 Fatty Acids

2.4.1 Fatty acids in fruit

Fatty acid is a carboxylic acid with a long aliphatic tail chain, which is either saturated or unsaturated. Saturated fatty acids have no double bond between the carbon atoms, while unsaturated fatty acids have one or more double bonds (Daubert, 1949). The terms lipids, fats and oils are often used interchangeably. Lipids are fats and oils that regularly referred to the broad, total collection of food molecules that are by definition, soluble in organic solvents and insoluble in water. Fats generally referred to lipids that are in solid form at room temperature while oils commonly refer to lipids that are in liquid form at room temperature. Triglyceride

is the main component in fats and oils and it consists of glycerine and three fatty acids. The existence of different kinds of fatty acids makes the triglyceride molecular species complex given that loads of different combination among the three fatty acids is formed (Min and Ellefson, 2010; Daubert, 1949).

Fruits contain minor amount of lipid components. As they are considered as the precursor for diverse volatile odorous principles of fruits, they are alleged to contribute to the development of characteristic aromas and flavours during ripening (Gholap and Bandyopadhyay, 1980). Moreover, Supran (1978) reported that lipids contribute to the nutritional and industrial value. Fatty acids profile is the major determinant of the oil quality in coriander fruit, mainly with the percentage of linoleic and oleic acids. Oils with different fatty acid composition are required depending on their use in the industry or for human consumption (Gholap and Bandyopadhyay, 1980; Supran, 1978).

High oleic acid proportion in oils is more stable than others because oleic acid is a lot more resistant to autoxidation, thus it is believed to reduce human's cardiovascular diseases (Msaada *et al*., 2009). Linoleic, linolenic acids and their isomers are essential fatty acids, required by the human body but cannot be synthesised from other substrates and consequently must be obtained from food. It is reported that essential fatty acids are necessary for growth and protection of skin, hair and nails (Cakir, 2004). Essential fatty acids balance the skin's metabolism by controlling the flow of oils and nourish collagen which is the supporting structure underneath the skin. Lack of this fatty acids leads to cutaneous problems such as alopecia, peeling of epidermis, eczema, psoriasis, dandruff and hair loss. Nowadays, plants that are rich in linoleic acid have been extensively cultivated and their oils are widely consumed (Cunnane & Anderson, 1997; Cakir, 2004).

2.4.2 Fatty acids in kernel oil

In recent years, there is growing interest in searching for newer sources of edible oils, for example plant seeds which offer oils with high nutritional, industrial and pharmaceutical importance (Shah *et al*., 2004). Kernel oils generally have broad range of utilization as foods, lubricants, fuel for paraffin lamps, additives for paint formulations, soup ingredient and also in medicinal applications. Some kernel oils have the potential to be developed for oleochemical industries (Ahmad *et al*., 2007). The depletion of world petroleum reserves as a result of increasing energy demands together with environmental concerns has prompted the efforts to discover various alternative sources of petroleum-based fuels. In this context, biodiesel has gained substantial position in public, over the world (Ahmad *et al*., 2007; Shah *et al*., 2004).

Biodiesel, which is made up of mono-alkyl esters of fatty acids derived from vegetable oils, animal fats or waste cooking oils is environmentally sound alternative to the conventional diesel fuels. Most of the commercial biodiesel production is performed through a transesterification of triglyceride with the mono-alkyl alcohol in the presence of either acid or alkali as catalysts. This leads to the production of fatty acid methyl esters along with glycerol. The reaction by using alkali catalysts is faster, though the triglyceride must contain low level of free fatty acids (Berrios *et al*., 2007; Ma and Hanna, 1999). The option of the fats or oils to be used in biodiesel production is a process involving both chemistry and economics. With respect to process chemistry, the greatest difference among the selections of fats and oils is the amount of free fatty acids, which is related to triglycerides (Van Gerpen, 2005). Typical vegetable oil feedstock for biodiesel production includes the commodity seed oils, such as soybean, sunflower, rapeseed and canola. These oils are readily converted to the

corresponding alkyl esters by base catalyst and offer acceptable fuel properties in modern diesel engines (Schwab *et al*., 1987; Van Gerpen, 2005). Nevertheless, the usage of conventionally grown edible oils leads to alleviate food versus fuel issue. Consequently, the process economics may be improved by exploration of newer and lower cost feedstock, not only due to the high oil costs but also due to their ever-increasing demand (Azam *et al*., 2005).

A number of tropical plants growing within the rainforest in Sarawak, some of which produce edible fruits that are widely consumed by the local community, but the kernels are largely discarded. Studies on these seeds are essential in order to discover new sources, whether for human consumption or for industrial purposes.

2.5 Minerals in Fruits

Minerals are one of the crucial regulators of physiological processes in humans. In the earlier part of this century, scientists could qualitatively distinguish small amounts of several mineral elements in living organisms (Anke *et al*., 1984). Experiments in cell culture and in intact organisms revealed the significance of trace elements in numerous metabolic processes and functions throughout the life cycle. More than one-third of all human proteins need metal ions in order to function, thus deficiency in these ions will affect our health significantly. Research on humans and animals revealed that optimal consumption of elements such as sodium, potassium, magnesium, calcium, manganese, copper, zinc, and iodine might diminish individual risk factors including those related cardiovascular disease (Anke *et al*., 1984; Mertz, 1982).

Minerals are important in the diet even though they comprise only 4-6% of the human body. Macro minerals include calcium, phosphorus, magnesium, sulphur, potassium, chlorine and sodium are those required in amounts greater than 100 mg per day by the adult and they represent 1% or less of bodyweight (Macrae *et al*., 1993). They serve as structural components of tissues and function in cellular and basal metabolism and water and acid-base balance. An additional of 10 minerals are required in much smaller amounts, less than 100 mg per day and make up less than 0.01% of bodyweight. They are referred as trace elements, and including zinc, iron, silica, manganese, copper, fluoride, iodine molybdenum, selenium and chromium. (Nielsen, 1984; Macrae *et al*., 1993). The major functions of several mineral elements in human body are presented in Table 2.4.

The human food chain is linked through fruit, vegetable and tuber consumption to the nature of the soil which provides the mineral ions. Several minerals that are vital to human nutrition are accumulated in different parts of plant as it accumulates essential minerals for growth from the environment (Mitchell and Burridge, 1979). In fruit, mineral concentrations have been related to ripening, internal disorders and disease severity. For example, calcium has been implicated in fruit quality because of its role in cell wall and membrane function (Hofman and Smith, 1994; Poovaiah *et al*., 1988). The final level of mineral elements in fruit are influenced by genetic factors, soil and weather conditions, the use of fertilizers and the state of the fruit's maturity at harvest. Mineral amounts in fruit must be monitored to ensure their presence in the diet is in the required level since some trace elements may be nonessential or profoundly have deleterious effects on health (Sanchez-Castillo *et al*., 1998; Kozlowski *et al*., 2009).

Table 2.4: Major functions of selected mineral elements in human body (Endres *et al.*, 2012).

Mineral element	Function
Calcium (Ca)	Strengthens bones and teeth, involved in muscle contraction and relaxation, blood clotting, water balance, nerve function. Required for chromosome segregation.
Copper (Cu)	Necessary for formation of haemoglobin. Part of energy metabolism enzymes.
Iron (Fe)	Functions in the haemoglobin in red blood cells, which transports oxygen from the lungs to the body's tissues, including the muscles and the brain. Used in energy utilization.
Magnesium (Mg)	Involved in protein synthesis, muscle contraction, nerve transmission, DNA synthesis and chromosome segregation
Manganese (Mn)	Essential for normal bone development. Activates enzymes.
Potassium (K)	Facilitates many reactions, especially protein synthesis, water balance, nerve transmission, muscle contraction.
Sodium (Na)	Promotes acid-base balance, water balance, nerve impulse transmission, muscle activity.
Zinc (Zn)	Part of insulin and enzymes, vitamin A transport, wound healing, fetus and sperm development, immunity, promotes enzyme activity and metabolism.

Mineral analysis is a valuable model for understanding the fundamental structure of analysis procedures to separate and measure. Separation of minerals from food matrix is often specific for example complexometric titrations or precipitation titrations, or it can be nonspecific which involves procedures such as ashing or acid extraction. These nonspecific separations require a specific measurement to be made as provided by colorimetry, ion-selective electrodes (ISE), atomic absorption spectroscopy (AAS) or inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (Ward and Carpenter, 2010).

2.6 Phenolic Content and Antioxidant Activity

2.6.1 Phenolic content in fruits

Phenolics or sometimes called phenols, are a class of chemical compounds consisting of a hydroxyl group (-OH) bonded directly to an aromatic hydrocarbon group. Phenolic compounds consist of a diverse group of phytochemicals that have one or more phenol group in their structure and these compounds may be present in plants as simple molecule to a complex high-molecular mass polymer (Balasundram *et al.*, 2006). Phenolic compounds are the most abundant secondary metabolites in plants and can be classified into water-soluble compounds (phenolic acids, phenylpropanoids, flavonoids and quinones) and water-insoluble compounds (condensed tannins, lignins and cell-wall bound hydroxycinammic acids). These compounds have a huge range of structures and functions such as to protect plants from UV light, infections or act as attractants for pollinators (Landete, 2012; Naczk and Shahidi, 2006; Rispail *et al*., 2005). Phenolics are synthesised during the normal development of a plant and have been considered the most significant, numerous and ubiquitous group of compounds in the plant kingdom (Naczk & Shahidi, 2004).

The structure of phenolic compounds is the key determinant of their radical scavenging and metal chelating activity. These compounds delay or inhibit the lipid autoxidation by acting as radical scavengers which an electron is donated to the free radical and convert it into an innocuous molecule, therefore protect against the propagation of the oxidative chain (Navarro *et al*., 2006). The antioxidant activity in phenolic compounds depends on the position and number of the hydroxyl groups bonded to the carboxyl functional group. Figure 2.4 shows an example of a structure of phenolic compound, gallic acid. The

antioxidant activity increases with the increasing of the degree of hydroxylation (Navarro *et al*., 2006; Naczk and Shahidi, 2006).

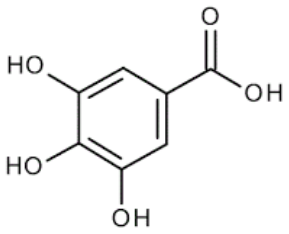


Figure 2.4: Structure of gallic acid.

Experimental studies on animal and human cell lines have demonstrated that these bioactive compounds can play role in preventing degenerative diseases, for example cancer and cardiovascular diseases when taken daily in adequate amounts (Wijngaard *et al*., 2009). In addition, polyphenolic compounds are proved to have a direct effect in the digestive system and serve as signalling molecules which involved in modulation of signal pathways, thus affecting cellular function and gene expression (Aaby *et al*., 2012).

Fruits are an important source of phenolics in the human diet and the compounds contribute to its colour, taste, aroma and flavour. Complex mixtures of phenols in fruits are not evenly distributed throughout the peel, pulp and kernel and they are strongly dependent on the degree of ripeness, variety, climate, soil composition, geographic location, light exposure and storage conditions, amongst other factors (Serrano *et al*., 2010; Manach *et al*., 2004). Phenolic compounds cannot be produced by the human body and therefore must be consumed essentially through the daily diet. It is important to analyse the composition of phenolic compounds in fruits given that the nutritional and therapeutic role of dietary phenolic antioxidants is vital for the improvement of health benefits. Numerous studies have been carried out on the phenolic acid compositions in various fruits and their related cultivars such

as apples and pears (Amiot *et al*., 1993), *Pyrus* (Challice and Williams, 1972), *Diospyros* (Ayaz *et al*., 1997), and carrots (Babic *et al*., 1993).

The major methodologies used to quantify the phenolic compounds in fruits that are widely described in the literature include; 1) the colorimetric method of Folin-Ciocalteu that estimates the total phenolics (Haminiuk *et al*., 2011), 2) the aluminium chloride colorimetric assay that quantifies the total flavonoids (Liu *et al*., 2008) and 3) the pH differential method that estimates the total anthocyanins (Granato *et al.*, 2010). The visible region of spectrum is generally used to quantify the total phenolics, flavonoids and tannins (Granato *et al*., 2010).

2.6.2 Folin-Ciocalteu reagent assay

Folin-Ciocalteu reagent assay is the most common procedure used to quantify the total phenolic content in foodstuffs and it was originated from the procedure developed by Otto Folin and Vintila Ciocalteu in 1927 (Haminiuk *et al*., 2012). This assay is based on the chemical reduction by a mixture of tungsten and molybdenum oxides where a blue colour is produced and absorbs light at 765 nm upon reaction with phenols. The intensity of the light absorption at that wavelength depends on the concentration of phenols (Everette *et al.*, 2010; Waterhouse, 2001). However, Folin-Ciocalteu reagent does not only measure total phenols but it also react with any reducing substance. Thus it should be noted that chemical compounds such as amino acids, proteins, sugars, organic acids and aromatic amines may also react with this reagent (Prior *et al*., 2005; Ikawa *et al*., 2003).

2.6.3 Antioxidant Activity

Antioxidants are substances that inhibits or delay oxidative damage of cellular components such as polyunsaturated fatty acids, proteins, carbohydrates and nucleic acids caused by reactive oxygen species as well as free radicals (Martinez-Cayuela, 1995). Reactive oxygen species for example superoxide radical, hydrogen peroxide, hydroxyl radical and singlet oxygen are of the greatest biological significance, while any chemical species that are capable of existing with one or more unpaired outer shell electrons may be classified as free radicals. In general, they are extremely reactive and highly unstable. These radicals can start chain reactions and when the chain reaction occurs in a cell, it can cause damage or death to the cell (Schoneich, 1999; Martinez-Cayuela, 1995).

Providentially within biological systems and chemical scavengers, dietary antioxidants (α-tocopherol, β-carotene, ascorbic acid, glutathione and uric acid), some hormones (estrogen and angiotensin) and endogenous enzymes (superoxide dismutase, glutathione peroxidase and catalase) are able to remove oxygen free radicals formed in cells and consequently protect against oxidative damage. Dietary antioxidants are often acted as reducing agents where they oxidized themselves by removing free radical intermediates and inhibit other oxidation reactions to terminate the chain reactions in a cell (Martinez-Cayuela, 1995; Halliwell and Gutteridge, 1990).

Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butyl hydroquinone (TBHQ) and propylgallate (PG) are food additives used to conserve against deterioration. However, due to their potential health risks and toxicity, their usage is increasingly controlled. Hence, there is an increase of interest nowadays in searching for new sources of natural antioxidants and to use them in foods and

pharmaceutical preparations to replace synthetic antioxidants (Moure *et al*., 2001). In fruits, the most eminent antioxidant constituents which may take part in the roles of prevention and protection are vitamin C and E, carotenoids, minerals (selenium and zinc), some peptides and phenolic compounds (Tomas-Barberan and Espin, 2001).

2.6.4 DPPH radical scavenging assay

DPPH assay was performed to evaluate the antioxidant activity of the methanol extracts from studied fruit samples. DPPH is a stable free radical due to the delocalisation of the spare electron over the molecule and the delocalisation gives rise to a deep violet colour. The violet colour will be decolourised when the solution of DPPH is mixed with a substance that can donate hydrogen atom, which the molecules of DPPH are reduced by molecules of the reductant (Molyneux, 2004). The reduction of DPPH radicals can be observed by a decrease in its absorbance at 517 nm. Figure 4.9 shows the reduction reaction of DPPH by antioxidant compound. R is antioxidant compound.

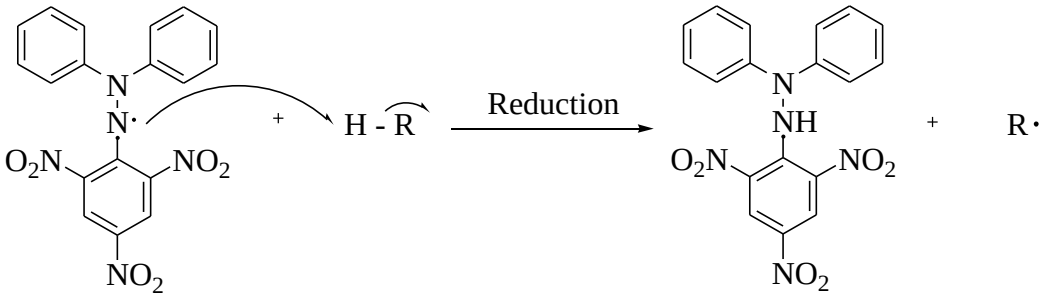


Figure 2.5: The reduction reaction of DPPH

CHAPTER 3

EXPERIMENTAL

3.1 Introduction

Three edible fruits in Sarawak namely *C. odontophyllum*, *L. garciae* and *A. odoratissimus* have been analysed for their potential health-enhancing properties. Proximate analysis such as moisture content, ash content, crude fat, crude fibre and vitamin C content were performed according to standard methods. The pulp and kernel of the fruits were extracted using Soxhlet extraction apparatus and the esterified fatty acids in the oil extracts were analysed using GC-MS. Mineral contents in the fruit pulp were analysed using AAS. The fruit pulp extracted with methanol was subjected to Folin-Ciocalteu reagent assay and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay for the determination of total phenolic content and antioxidant activity, respectively. The assays were analysed using UV-Vis spectroscopy.

3.2 Samples Collection

The fruit samples were collected from different divisions in Sarawak as presented in Table 3.1 from September to December 2012. Only ripe with no injuries fruits were chosen and brought back to the laboratory. The fruits were washed with water and the pulps were separated from their kernels and allowed to dry before they were measured for moisture and

ash contents. Remaining samples were freeze-dried, grounded, kept in air tight container and stored in the freezer prior to analysis.

Table 3.1: The locations of sample collected.

Sample	Locations			
C. odontophyllum	Sibu	Mukah	Kapit	Miri
L. garciae	Kuching	Kota Samarahan	Sibu	Mukah
A. odoratissimus	Kuching	Kota Samarahan	Sibu	Miri

3.3 Proximate Analysis

3.3.1 Chemicals and solvents

Petroleum ether was purchased from BDH chemicals (Pennsylvania, USA), sulphuric acid (H_2SO_4) was from J.T. Baker (Illinois, USA). Potassium iodide (KI), potassium iodate (KIO_3), sodium hydroxide (NaOH) pellets and ascorbic acid were purchased from Sigma-Aldrich (Missouri, USA). Hydrochloric acid (HCl) and boric acid were from HmbG Chemicals (Hamburg, Germany). All chemicals were used as received without further purification.

3.3.2 Moisture content

Moisture content was determined using the direct drying method according to AOAC method 945.32 (AOAC, 2000). Empty crucibles with lid were dried in the oven for 3 hours at 105˚C and transferred to desiccator to cool. Exactly 5 g of sample was weighed into the crucible and dried for 3 hours in an oven set at 105˚C until a constant weight of the sample was obtained. After drying, the crucible with the dry sample was cooled in the desiccator and then weighed. The difference in weight (initial-constant) is the moisture content.

$$\text{Moisture (\%)} = [(w1-w2)/w1] \times 100 \qquad \text{eq. 3.1}$$

w1 = weight (g) of fresh sample before drying

w2 = weight (g) of sample after drying

3.3.3 Ash content

Ash content was determined using the dry ashing method according to AOAC method 930.05 (AOAC, 2000). Cleaned crucible and lid were placed overnight in the furnace at the temperature of 550˚C to ensure the impurities on the surface of the crucible were burned off. The crucible then was cooled in the desiccator and weighed. Exactly 5 g of sample was weighed into the crucible and heated over low Bunsen flame with half covered lid until no more fumes produced. The crucible was then incinerated overnight in the furnace set at 550˚C until whitish/greyish ash was obtained. The crucible was cooled in desiccator and weighed.

Organic matter was burned off and the inorganic material remained was weighed (Tee et al., 1996).

Ash (%) = [weight of ash (g)/weight of fresh sample (g)] × 100 eq. 3.2

3.3.4 Crude fat

Crude fat was determined using Soxhlet extraction according to Tee *et al*. (1996). Approximate 5 g of dried grounded sample was weighed and placed into the extraction thimble. A total of 250 mL of petroleum ether was filled in a cleaned and dried round bottom flask and placed on the heating mantle. The sample was heated for 8 hours. After the extraction, the solvent left in the round bottom flask was evaporated using the rotary evaporator until it was completely dry. The flask was cooled in the desiccator and weighed. Percentage of crude fat was determined using equation 3.3:

Fat (%) = [weight of fat (g)/weight of dried sample (g)] × 100 eq. 3.3

3.3.5 Crude fiber

Determination of crude fiber was carried out according to AOAC method 978.10 (AOAC, 2000). Briefly, 2 g of defatted sample was placed in a round bottom flask and mixed with 200 mL of 1.25% H_2SO_4. The mixture was boiled for 30 minutes. The residue was then washed with four portions of 50 mL of distilled water and filtered with vacuum filter. The residue was collected and placed in a round bottom flask. Into the flask, 200 mL of 1.25%

NaOH was added and the mixture was boiled for another 30 minutes. The solid residue was washed and filtered into a crucible with minimum volume of aqueous solution, first with 30 mL of 1.25% H_2SO_4 and then with two portions of 30 mL of distilled water. The residue was collected and dried overnight in the oven at 105˚C. The crucible was cooled in the desiccator and weighed. The crucible was then placed in a furnace at 550˚C for 3 hours, re-cooled and weighed. The fiber content was estimated based on the difference between dry weight and ash content of the residue as the following equation:

$$\text{Crude fiber (\%)} = (w_i / w_{ds}) \times 100 \qquad \text{eq. 3.4}$$

w_i = loss in weight (g) on ignition

w_{ds} = weight (g) of dried defatted sample

3.3.6 Vitamin C

The determination of vitamin C content was performed according to the method by Suntornsuk *et al.* (2002). Approximately 100 g of fruit samples were blended with 100 mL of distilled water using food processor. The mixture was then filtered using cheesecloth and 50 mL of it was transferred into an Erlenmeyer flask. Exactly 0.5 g of KI, 5 mL of 1M HCl and 3 drops of 0.5% starch solution were added into the flask. The mixture was swirled thoroughly and then was titrated with 0.01M KIO_3. The end point of the titration was achieved when a dark blue colour formed. Ascorbic acid powder was used as the standard and the vitamin C content in the fruits was calculated and expressed as mg/100g.

3.4 Analysis of Fatty Acids

3.4.1 Chemicals and solvents

A mixture of FAMEs standard consisting 37 compounds was purchased from Supelco (Pennsylvania, USA). Petroleum ether was obtained from BDH chemicals (Pennsylvania, USA) and hexane was from Mallinckrodt Chemicals (Dublin, Ireland). Dichloromethane and methanol were from J.T. Baker (Illinois, USA), while sodium hydroxide (NaOH) pellets and butylated hydroxytoluene (BHT) were purchased from Sigma-Aldrich (Missouri, USA). All chemicals were used as received without further purification.

3.4.2 Sample extraction

Samples were extracted using Soxhlet extraction apparatus according to the procedure described by Tee *et al*. (1996). Approximately 5 g of dried grounded sample was weighed and placed into an extraction thimble with glass wool covered on top. A total of 250 mL of petroleum ether was filled in a cleaned and dry round bottom flask and 0.05% of BHT was added to prevent oxidation. The flask was placed on the heating mantle and then heated for 8 hours. The remaining solvent in the round bottom flask was then evaporated using the rotary evaporator until it was completely dry. The oil extract was diluted with dichloromethane in an air tight vial and kept in the freezer prior to GC-MS analysis. The pulps and kernels were extracted and analysed separately using the same extraction method.

3.4.3 Fatty acid methylation

In order to enable analysis of fatty acids in the oil extracts with GC-MS, the extracts need to be derivatized into FAMEs. Derivatization of fatty acids was carried out according to the procedure obtained by David *et al.* (2002). Exactly 200 µL of extracted oil was dissolved in 1 mL of hexane in an eppendorf tube. A total of 200 µL of 2M NaOH in methanol was added into the tube, capped and vortexed for 10 seconds. The role of NaOH in this reaction is to act as a catalyst in the esterification of the free fatty acids. The mixture was placed in a water bath at 50˚C for 20 seconds, cooled and vortexed again for 10 seconds. The mixture was then centrifuged at 6,000 rpm for 20 seconds to obtain a clear separation. These supernatant obtained was then analysed using GC-MS.

3.4.4 Gas chromatography analysis

GC analysis was performed on a Shimadzu gas chromatograph equipped with mass spectrometer model Shimadzu QP2010 PLUS Series. The GC separation was carried out using BPX-5 capillary column (29.5 m x 0.25 mm x 0.25 µm film thickness). The operating conditions for the gas chromatography were as follows: the column temperature was initially set at 90°C for 5 minutes and then was increased to 300°C at the rate of 3.5°C min^{-1} and held for 10 minutes. The injection temperature was kept at 260°C with a split ratio of 1:20. Helium gas was used as the carrier gas at a flow rate of 1.0 mL min^{-1}. Exactly 1 µL of 1,000 µg/mL mixture of FAMEs standard was injected. The identifications of fatty acids in the samples prepared at 1,000 µg/mL were made by comparison of the retention times with those of the

standard FAMEs mixture. The peak areas were recorded and the fatty acid compositions were expressed in percentage according to the following equation:

Composition of individual fatty acid (%) = $(p_i / p_t) \times 100$ eq. 3.5

p_i = peak area of individual fatty acid

p_t = total peak areas of all fatty acids

3.5 Mineral Analysis

3.5.1 Chemicals and solvents

Hydrochloric acid (HCl) was purchased from HmbG Chemicals (Hamburg, Germany) while nitric acid (HNO_3) from J.T. Baker (Illinois, USA). All chemicals were used as received without further purification.

3.5.2 Sample preparation for Atomic Absorption Spectroscopy analysis

Determination of mineral content was carried out according to the AOAC method 920.164 (AOAC, 2000). Accurately 4 g of dried grounded sample was placed in a dry crucible and kept in a furnace at 550ºC for 3 hours. The crucible was cooled after the ashing, added with 10 mL of 6M HCl and then transferred into a conical flask. The flask was heated on a hot plate until a clear solution was obtained. The residue was filtered into a 100 mL volumetric flask. It was then diluted with 0.1M HNO_3 solution, made up to mark and ready to be analysed with AAS.

3.5.3 Atomic Absorption Spectroscopy analysis

The AAS analyses were performed on a Perkin Elmer atomic absorption spectrophotometer model iCE 3000 Series equipped with flame and graphite furnace. Mineral contents in the samples were quantified against working standard solutions of known concentrations and the results were expressed as mg/kg of dry weight. The samples were determined for the presence of calcium (Ca), potassium (K), magnesium (Mg), lead (Pb), sodium (Na), zinc (Zn), copper (Cu), iron (Fe), cadmium (Cd), selenium (Se) and manganese (Mn). All determinations were done in triplicate.

3.6 Total Phenolic Content and Antioxidant Activity

3.6.1 Chemicals and solvents

Methanol was purchased from J.T. Baker (Illinois, USA), Folin-Ciocalteu reagent from Merck (Darmstadt, Germany) and gallic acid from Bio Basic (Ontario, Canada). Sodium carbonate (Na_2CO_3), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ascorbic acid were obtained from Sigma-Aldrich (Missouri, USA). All chemicals were used as received without further purification.

3.6.2 Sample extraction

The fruit pulp was extracted with methanol according to the method obtained by Ismail *et al*. (2004). Exactly 5 g of homogenized sample was weighed and transferred into a conical flask. A total of 25 mL of 70% (v/v) methanol: distilled water was added into the sample and then stirred at 200 rpm in an orbital shaker for 1 hour at room temperature. The extract was then separated from the residue by filtration. The remaining residue was re-extracted twice and the two extracts were combined. The residual solvent of methanol extract was evaporated under reduced pressure at 40˚C, transferred into vial and kept in the freezer prior to analysis. The methanol extracts were used for the determination of total phenolic content and total antioxidant activity.

3.6.3 Determination of total phenolic content

Total phenolic content was estimated according to procedure described by Velioglu *et al.* (1998). Briefly, 100 µL of extract was transferred into a test tube. Exactly 0.75 mL of Folin-Ciocalteu reagent which previously diluted 10-fold with deionised water was added into the test tube and mixed. The mixture was allowed to stand at room temperature for 5 minutes and 0.75 mL of 6% (w/v) Na_2CO_3 in distilled water was added to the mixture and then mixed gently. The absorbance of the mixture was measured by an automated UV–Vis spectrophotometer (Model: JASCO V-630) at the wavelength of 765 nm after standing at room temperature for 90 minutes. Gallic acid was used as reference by performing calibration on concentrations between 0 to 100 µg/mL for quantification purpose. The results were

expressed as mg gallic acid equivalent in g dry weight (mg GAE/g dw) basis and the determination was done in triplicate.

3.6.4 Antioxidant activity

Antioxidant activity in the fruit sample was performed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay according to procedure described by El Hajaji *et al*. (2011). In a test tube, 5 mL of different concentrations (10, 25, 50, 100, 500 µg/mL) of the methanol extract was added into 5 mL of methanol solution of 1.01×10^{-2} M DPPH. The mixture was shaken vigorously and allowed to stand in the dark for 30 minutes at room temperature. The absorbance of the mixture was measured at the wavelength of 517 nm using UV-Vis spectrophotometer (Model: JASCO V-630). A mixture of 5 mL methanol and 5 1.01×10^{-2} M mL DPPH solution was used as the control. The scavenging activity was expressed as inhibition percentage according to following equation:

$$\% \text{ Inhibition} = [(A_b - A_s)/A_b] \times 100 \qquad \text{eq. 3.5}$$

A_b = absorbance of the control reaction

A_s = absorbance of the test compound

Ascorbic acid was used as positive control. The testing was performed in triplicate. The concentration of extract at 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted against extract concentration.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Proximate Composition

Pulp and kernel of fruit samples collected from different locations were analysed for their proximate composition such as moisture content, ash content, crude fat and crude fiber. Vitamin C was also determined in the fruit pulp. The results show that both pulps and kernels of *C. odontophyllum*, *L. garciae* and *A. odoratissimus* proved to be important sources of necessary nutrients for human diet. The differences in the values between different districts might be due to the environmental factor and growth condition (Demir and Ozcan, 2001).

4.1.1 Moisture content

Table 4.1 shows the moisture content of the pulp and kernel for all of studied fruits. Generally, the moisture content in the fruit pulps is significantly higher than in the fruit kernel with the highest percentage was observed in fruit pulp of *A. odoratissimus* from Sibu (79.94±1.14%). Tang *et al*. (2013) reported 67.90-73.40% and 31.1-54.5% of moisture in fruit pulp and kernel of *A. odoratissimus*, respectively. Both pulp and kernel of *C. odontophyllum* fruit have the lowest moisture content ranged from 47.39-52.17% and 23.56-29.74%, respectively. This data is in agreement with Chew *et al.* (2011) who reported 50.44 to 51.91 g/100 g fresh weight of moisture content in *C. odontophyllum*. The moisture contents in the pulp and kernel of *L. garciae* were in the range of 61.48 to 66.72% and 38.08 to 43.38%,

respectively. Moisture content is important in determining the storage quality and as an indicator of the freshness of any food goods. Furthermore, it helps to determine the suitability in producing the foods into different value added product, whether for fresh consumption, dried or processed (Ali *et al*., 2011).

Table 4.1: Moisture content in pulp and kernel of studied fruit samples.

		Moisture content (%)			
		Sibu	Mukah	Kapit	Miri
C. odontophyllum	Pulp	50.02 ± 0.82	49.41 ± 2.12	47.39 ± 4.28	52.17 ± 0.39
	Kernel	23.56 ± 0.28	29.74 ± 0.81	29.50 ± 1.57	23.84 ± 0.37
		Kuching	K. Samarahan	Sibu	Mukah
L. garciae	Pulp	66.72 ± 0.77	61.48 ± 5.14	65.51 ± 0.28	63.38 ± 2.04
	Kernel	41.88 ± 0.37	38.08 ± 3.12	39.50 ± 0.99	43.38 ± 0.14
		Kuching	K. Samarahan	Sibu	Miri
A. odoratissimus	Pulp	72.41 ± 0.41	74.01 ± 1.03	79.94 ± 1.14	79.45 ± 0.56
	Kernel	49.32 ± 1.80	41.36 ± 0.89	45.99 ± 1.60	42.29 ± 0.16

4.1.3 Ash content

Ash content in the pulp and kernel of studied fruits is presented in Table 4.2. The ash content of the kernel is higher compared to that in the pulp for *C. odontophyllum* and *A. odoratissimus*. The lowest percentage of ash was observed in *A. odoratissimus* with 0.60-1.08% and 1.13–1.43% in pulp and kernel, respectively. These values are within the range of those reported by Tang *et al*. (2013) with 0.6-0.8 g and 1.0-1.5 g per 100 g fresh sample for pulp and kernel, respectively. The highest ash content was in the pulp (3.68±0.49%) and

kernel (3.98±0.74%) of *C. odontophyllum* from Mukah. Ash accounted for 2.02-2.75% and 1.32-1.87% in fruit pulp and kernel of *L. garciae*, respectively.

Table 4.2: Ash content in pulp and kernel of studied fruit sample.

		Ash content (%)			
		Sibu	Mukah	Kapit	Miri
C. odontophyllum	Pulp	2.26 ± 0.14	3.68 ± 0.49	2.85 ± 0.47	2.34 ± 0.24
	Kernel	2.95 ± 0.18	3.98 ± 0.74	3.42 ± 0.71	2.70 ± 0.14
		Kuching	K. Samarahan	Sibu	Mukah
L. garciae	Pulp	2.64 ± 0.02	2.75 ± 0.27	2.02 ± 0.05	2.58 ± 0.12
	Kernel	1.87 ± 0.03	1.40 ± 0.10	1.32 ± 0.19	1.75 ± 0.27
		Kuching	K. Samarahan	Sibu	Miri
A. odoratissimus	Pulp	0.72 ± 0.06	0.92 ± 0.02	1.08 ± 0.19	0.60 ± 0.08
	Kernel	1.43 ± 0.07	1. 13 ± 0.09	1.26 ± 0.05	1.28 ± 0.03

4.1.4 Crude fat

Table 4.3 shows a significant difference of crude fat in the pulp and kernel samples. Among the three species, pulp of *C. odontophyllum* had the highest amount of fat, followed by *L. garciae* and *A. odoratissimus*. The fat content in fruit pulp of *C. odontophyllum* ranged from 32.07 to 45.15% which is higher compared to the values reported by Chew *et al.* (2011), which ranged between 21.16 and 25.76%. Fat accounted for only 10.35-14.22% in the kernel of *C. odontophyllum*. In contrast to *C. odontophyllum*, the crude fat in the kernel of *L. garciae* and *A. odoratissimus* were higher than in the pulp samples.

Table 4.3: Crude fat in pulp and kernel of studied fruit samples.

		Crude fat (%)			
		Sibu	Mukah	Kapit	Miri
C. odontophyllum	Pulp	35.95 ± 0.64	37.37 ± 1.48	32.07 ± 1.42	45.15 ± 1.33
	Kernel	10.35 ± 0.54	12.02 ± 0.88	12.13 ± 0.27	14.22 ± 0.60
		Kuching	K. Samarahan	Sibu	Mukah
L. garciae	Pulp	21.29 ± 0.28	23.21 ± 0.70	23.95 ± 1.29	18.80 ± 0.96
	Kernel	34.29 ± 0.28	33.13 ± 1.46	37.31 ± 0.53	32.09 ± 0.55
		Kuching	K. Samarahan	Sibu	Miri
A. odoratissimus	Pulp	1.73 ± 0.20	1.80 ± 0.33	1.38 ± 0.21	1.62 ± 0.18
	Kernel	14.00 ± 0.50	14.76 ± 1.02	13.22 ± 0.46	13.55 ± 0.57

4.1.5 Crude fiber

Generally, the crude fiber present in the kernel is significantly higher than in the pulp of all three fruit samples (see Table 4.4), with a slight difference for fruits obtained from different district. These data show that the kernels are rich in fiber. The highest fiber content was observed in the kernel of *C. odontophyllum* (17.30-23.87%), followed by *L. garciae* (15.00-19.64 %) and *A. odoratissimus* (6.95-12.50%). *C. odontophyllum* and *L. garciae* are significantly high in fiber for both the pulp and kernel samples. Fruit pulp of *A. odoratissimus* from Kota Samarahan has the lowest content of fiber (0.85%). The consumption of these fruits may benefit our health given that the regular consumption of dietary fiber in the diet is related to a reduction risk of several pathologies (Michels *et al*., 2005).

Table 4.4: Crude fiber in pulp and kernel of studied fruit samples.

		Crude fiber (%)			
		Sibu	Mukah	Kapit	Miri
C. odontophyllum	Pulp	11.60 ± 1.02	10.65 ± 0.35	13.10 ± 0.05	10.30 ± 0.09
	Kernel	23.87 ± 2.86	20.70 ± 3.46	18.76 ± 2.03	23.10 ± 1.34
		Kuching	K. Samarahan	Sibu	Mukah
L. garciae	Pulp	8.05 ±0.03	6.20 ± 0.02	8.85 ± 0.21	10.40 ± 0.12
	Kernel	17.30 ± 0.10	19.64 ± 0.15	18.67 ± 0.04	15.00 ± 0.09
		Kuching	K. Samarahan	Sibu	Miri
A. odoratissimus	Pulp	1.75 ± 0.05	0.85 ± 0.03	1.04 ± 0.04	0.98 ± 0.16
	Kernel	6.95 ± 0.68	12.50 ± 1.55	11.05 ± 1.57	9.20 ± 0.62

4.1.6 Vitamin C

Table 4.5 shows that the pulp of *C. odontophyllum* contained the highest vitamin C compared to other fruit samples ranged between 10.37-16.30 mg/100 g. This value is comparable with the vitamin C concentration in watermelon and blackberries (Tarrago-Trani *et al*., 2012). The concentration of vitamin C in *A. odoratissimus* (1.11-4.07 mg/100 g) on the other hand is comparable with vitamin C content in macadamia nut, peach and tomato from Colombia (Contreras-Calderon *et al*., 2011). Vitamin C is an essential water-soluble vitamin that is naturally present in fruits and vegetables. It plays a crucial role in numerous physiological reactions in central biochemical processes of human body and also acts as a cellular antioxidant (Arrigoni and De Tullio, 2000). Daily consumption of vitamin C lowers the possibility of several chronic diseases such as diabetes, cancer and cardiovascular to occur (Ozyurek *et al*., 2007). Recommended daily allowance for vitamin C is 90 mg/day for adult

males, 75 mg/day for adult females and 25 mg/day for children age 4-8 years old (Monsen, 2000).

Table 4.5: Vitamin C content in pulp of studied fruit samples.

Fruit	Location	Vitamin C content (mg/100 g)
C. odontophyllum	Sibu	14.81 ± 0.21
	Mukah	16.30 ± 0.00
	Kapit	14.44 ± 0.00
	Miri	10.37 ± 0.26
L. garciae	Kuching	11.85 ± 0.00
	K.Samarahan	11.11 ± 0.21
	Sibu	12.59 ± 0.00
	Mukah	13.33 ± 0.37
A. odoratissimus	Kuching	1.11 ± 0.00
	K.Samarahan	2.59 ± 0.00
	Sibu	3.33 ± 0.26
	Miri	4.07 ± 0.00

4.2 Fatty Acids in Pulp and Kernel Oils

4.2.1 A mixture of FAMEs standard

Fatty acids need to be derivatized into FAMEs to make it volatile and stable enough for the analysis by gas chromatography (Rubinson and Neyer-Hilvert, 1997). Each FAME identified in GC analysis represents its underivatized form of fatty acid. For example, capric acid methyl ester signifies capric acid in the sample before derivatization. Standard solution of FAMEs mixture was injected into GC-MS to serve as reference for the identification of individual FAME. The gas chromatogram for the mixture of FAMEs standard is shown in Figure 4.1. A total of 31 individual FAMEs were eluted and identified in the chromatogram. The mean and standard deviation of retention times for the identified FAMEs standard are presented in Table 4.6 and these retention times were used as reference to determine the fatty acid components in oils from pulp and kernel samples.

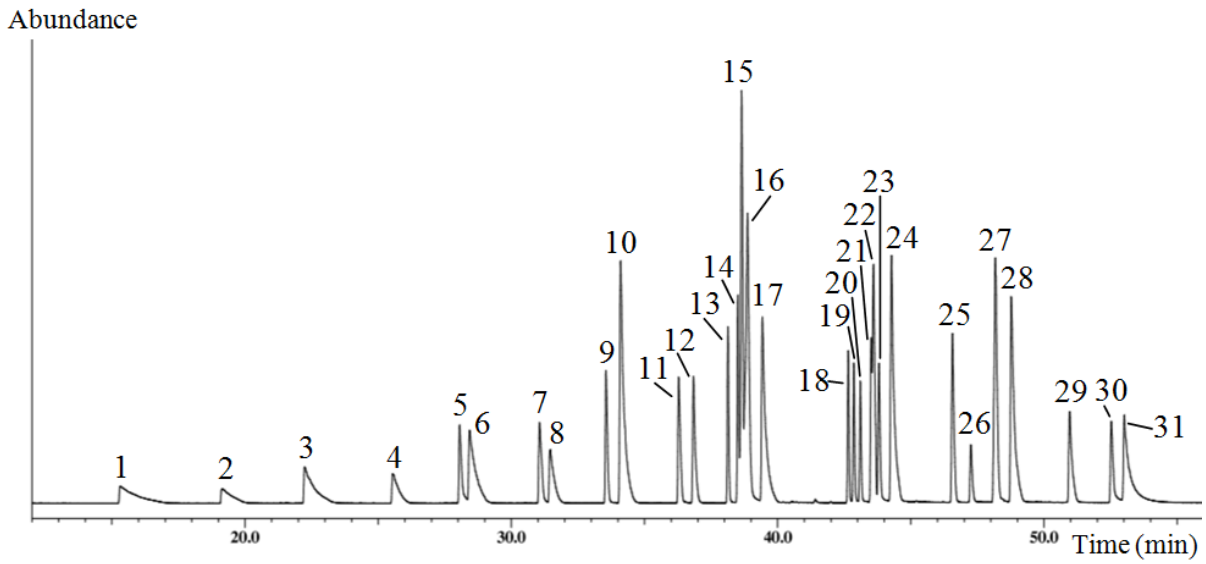


Figure 4.1: A GC-MS chromatogram for the mixture of FAMEs standard. Number of peak is referred to the individual FAME listed in Table 4.6.

Table 4.6: Mean of retention times for individual compound in a mixture of FAMEs standard, n=3.

	Compound	Lipid no.	Retention time (min)
1	Capric acid methyl ester	C10:0	15.48 ± 0.22
2	Undecanoic acid methyl ester	C11:0	19.28 ± 0.20
3	Lauric acid methyl ester	C12:0	22.34 ± 0.14
4	Tridecanoic acid methyl ester	C13:0	25.64 ± 0.13
5	Myristoleic acid methyl ester	C14:1	28.13 ± 0.11
6	Myristic acid methyl ester	C14:0	28.51 ± 0.11
7	Cis-10-pentadecenoic acid methyl ester	C15:1	31.13 ± 0.10
8	Pentadecanoic acid methyl ester	C15:0	31.53 ± 0.08
9	Palmitoleic acid methyl ester	C16:1	33.61 ± 0.08
10	Palmitic acid methyl ester	C16:0	34.15 ± 0.07
11	Cis-10- Heptadecanoic acid methyl ester	C17:1	36.34 ± 0.09
12	Heptadecanoic acid methyl ester	C17:0	36.90 ± 0.08
13	Linolenic acid methyl ester	C18:3n3	38.19 ± 0.08
14	Linoleic acid methyl ester	C18:2n6c	38.56 ± 0.08
15	Elaidic acid methyl ester	C18:1n9t	38.70 ± 0.07
16	Oleic acid methyl ester	C18:1n9c	38.93 ± 0.09
17	Stearic acid methyl ester	C18:0	39.49 ± 0.09
18	Cis-5,8,11,14,17-eicosapentaenoic acid methyl ester	C20:5n3	42.73 ± 0.12
19	Arachidonic acid methyl ester	C20:4n6	42.95 ± 0.12
20	Cis-8,11,14- eicosatrienoic acid methyl ester	C20:3n6	43.18 ± 0.11
21	Cis-11,14,17- eicosatrienoic acid methyl ester	C20:3n3	43.56 ± 0.07
22	Cis-11,14-eicosadienoic acid methyl ester	C20:2	43.67 ± 0.10
23	Cis-11-eicosenoic acid methyl ester	C20:1	43.89 ± 0.12
24	Arachidic acid methyl ester	C20:0	44.35 ± 0.11
25	Heneicosanoic acid methyl ester	C21:0	46.65 ± 0.12
26	Docosahexaenoic acid methyl ester	C22:6n3	47.37 ± 0.17
27	Erucic acid methyl ester	C22:1n9	48.26 ± 0.13
28	Behenic acid methyl ester	C22:0	48.86 ± 0.13
29	Tricosanoic acid methyl ester	C23:0	51.06 ± 0.13
30	Nervonic acid methyl ester	C24:1	52.63 ± 0.14
31	Lignoceric acid methyl ester	C24:0	53.11 ± 0.14

4.2.2 Fatty acids in pulp oils

Figures 4.2-4.4 show the GC-MS chromatograms for esterified fatty acids in pulp oils of *C. odontophyllum*, *L.garciae* and *A. odoratissimus* originated from different locations. It can be inferred from all the chromatograms that the major components exist in all pulp oil samples are palmitic acid (C16:0), linoleic acid (C18:2n6c) and oleic acid (C18:1n9c). The peak related to stearic acid (C18:0) was significantly detected in *C. odontophyllum* and *L. garciae*. However, this stearic acid peak was not detected in oil from *A. odoratissimus* pulp. Pulp oils of *C. odontophyllum* contained the highest number of compounds, followed by *L. garciae* and *A. odoratissimus*.

Total of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) in the pulp oils from all three fruits studied collected from different locations are presented in Table 4.7. The percentage of SFA which mainly contained palmitic acid is significantly high in *C. odontophyllum* (48.96-57.78%). This result is slightly higher compared to Azlan *et al.* (2010) which reported 44.43±0.07% of SFAs in *C. odontophyllum*. In contrast, *L. garciae* and *A. odoratissimus* are characterised by high amounts of unsaturated fatty acids compare to saturated analogues. MUFA is mostly represented by oleic acid in all pulp oil samples with the highest abundance observed in *L. garciae* (45.35-51.65%), followed by *C. odontophyllum* (35.80-40.92%) and *A. odoratissimus* (25.45-39.64%). These results are comparable with data reported by Edem (2002) who found 21.1%, 23.4% and 39.2% of MUFA in sunflower oil, soybean oil and palm oil, respectively. Table 4.7 also shows pulp oils of *A. odoratissimus* consist high percentage of PUFAs (38.02-46.07%) compared to pulp oils of *C. odontophyllum* (2.91-11.21%) and *L. garciae* (3.46-6.07%).

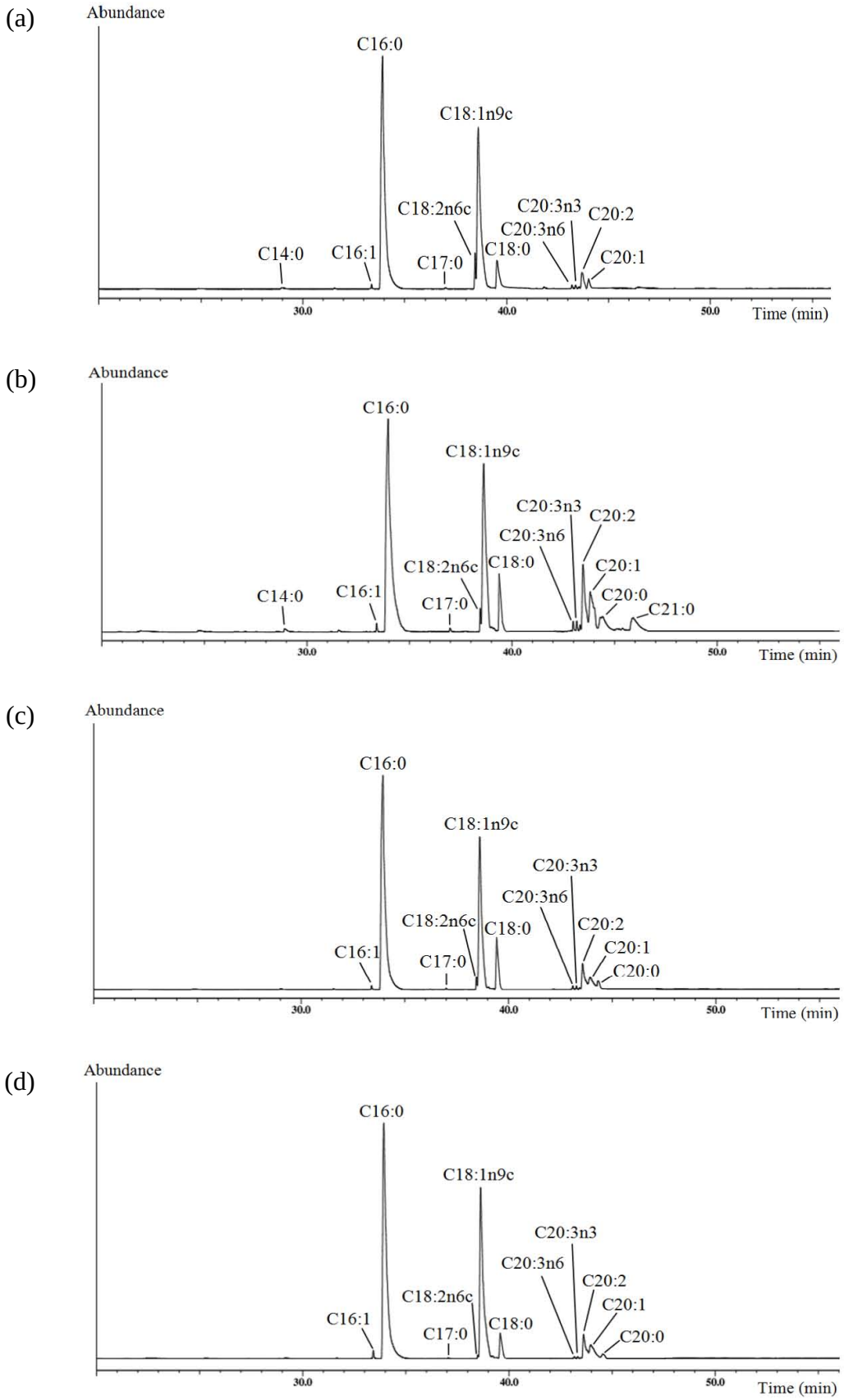


Figure 4.2: GC-MS chromatograms of the FAMEs in esterified pulp oils of *C. odontophyllum* collected from (a) Sibu, (b) Mukah, (c) Kapit and (d) Miri.

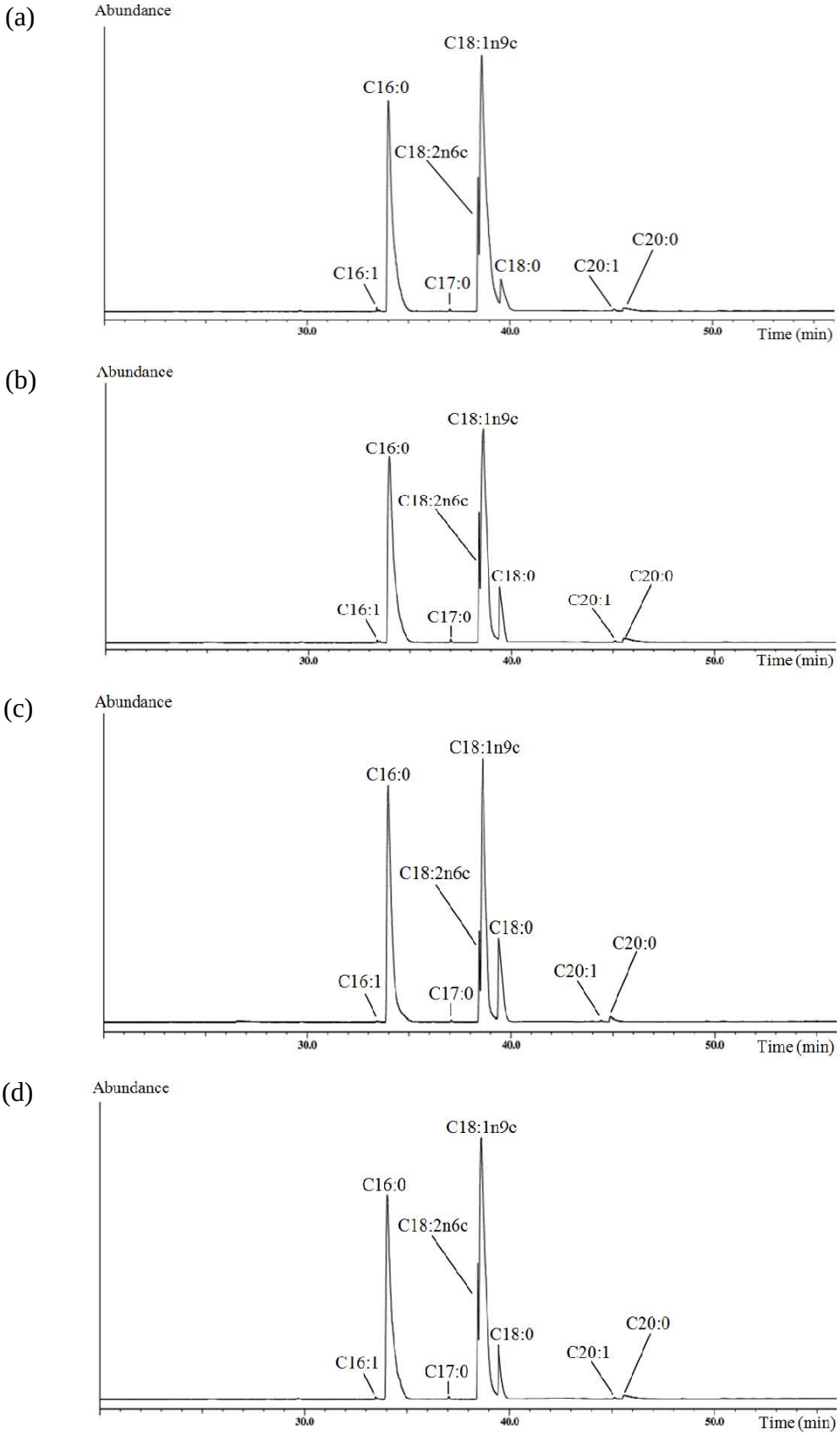


Figure 4.3: GC-MS chromatograms of the FAMEs in esterified pulp oils of *L. garciae* collected from (a) Kuching, (b) Kota Samarahan, (c) Sibu and (d) Mukah.

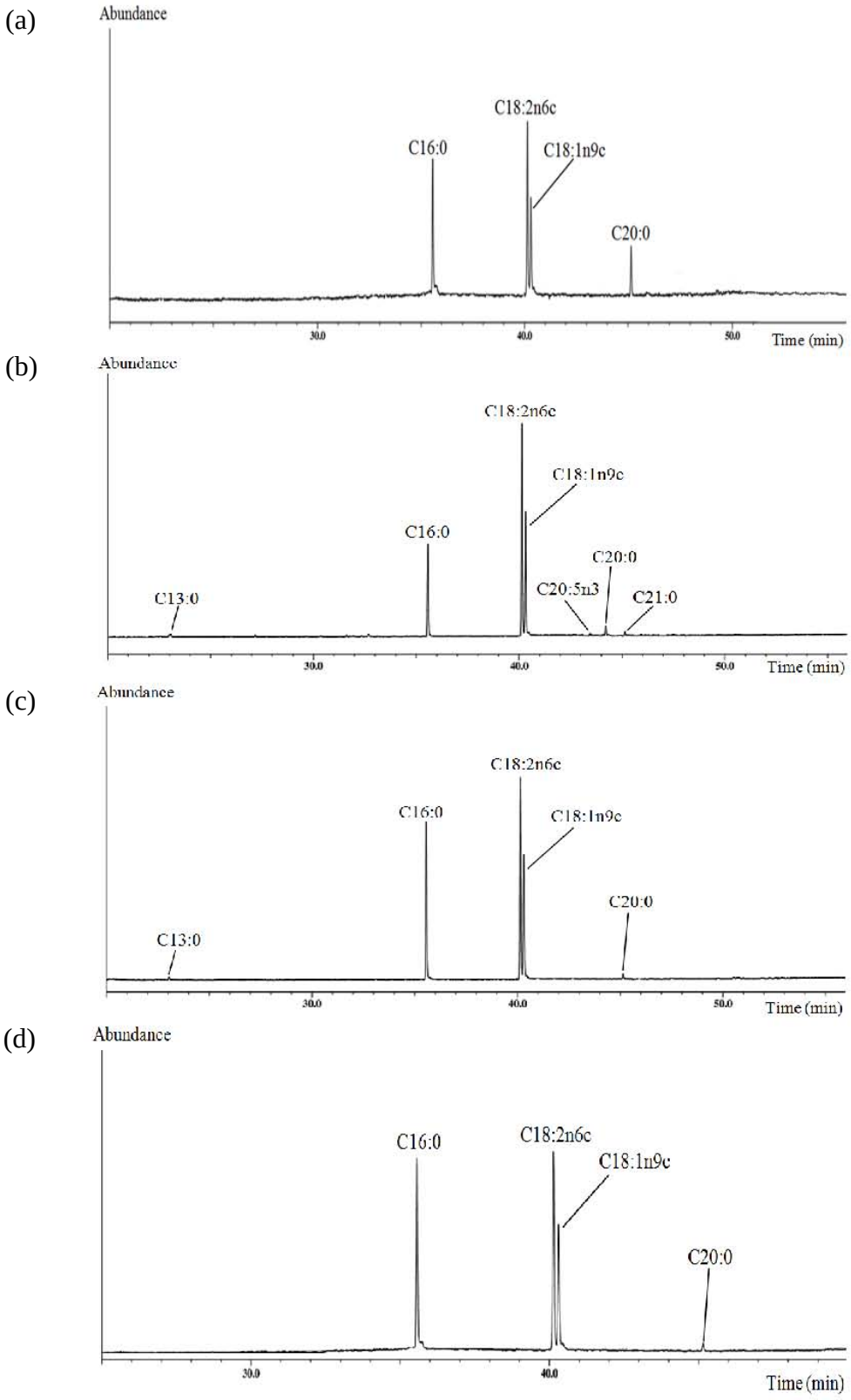


Figure 4.4: GC-MS chromatograms of the FAMEs in esterified pulp oils of *A. odoratissimus* collected from (a) Kuching, (b) Kota Samarahan, (c) Sibu and (d) Miri.

Table 4.7: The amount of SFA, MUFA and PUFA (%) in pulp oils of *C. odontophyllum*, *L. garciae*, and *A. odoratissimus*.

Fruit		Total SFA	Total MUFA	Total PUFA
C. odontophyllum	Sibu	57.63 ± 0.17	39.47 ± 0.08	2.91 ± 0.05
	Mukah	48.96 ± 0.03	39.81 ± 0.25	11.21 ± 0.03
	Kapit	57.78 ± 0.26	35.80 ± 0.08	6.20 ± 0.02
	Miri	55.14 ± 0.32	40.92 ± 0.10	3.96 ± 0.02
L. garciae	Kuching	42.92 ± 0.28	51.65 ± 1.85	5.43 ± 0.04
	K. Samarahan	48.24 ± 0.29	45.69 ± 1.48	6.07 ± 0.05
	Sibu	51.17 ± 0.43	45.35 ± 1.12	3.46 ± 0.01
	Mukah	45.66 ± 0.14	49.32 ± 0.02	5.02 ± 0.04
A. odoratissimus	Kuching	39.64 ± 0.69	22.35 ± 1.80	38.02 ± 1.21
	K. Samarahan	25.45 ± 0.56	28.47 ± 1.30	46.07 ± 0.13
	Sibu	33.83 ± 0.58	27.3 ± 0.96	38.88 ± 0.76
	Miri	36.77 ± 0.77	21.87 ± 3.67	41.36 ± 4.21

Several reports have shown the benefits of PUFA in reducing diseases such as cardiovascular, inflammatory, atherosclerosis, diabetes and heart diseases (Finley and Shahidi, 2001). A number of fatty acids also have a specific function in restoring the permeability barrier on the skin which reduces transepidermal water loss (Elias, 1983). The pulp oils from all studied fruits contained high amount of unsaturated fatty acids, primarily oleic and linoleic acid. Thus, the pulp oil of these fruits could be important for nutritional and industrial applications. Moreover, they are also rich in palmitic acid and stearic acid, which are the best natural sources of SFA as recommended by the National Cholesterol Education Program/American Heart Association (Hayes, 2002).

4.2.3 Composition of individual fatty acid in pulp oils

4.2.3.1 *Canarium odontophyllum*

Table 4.8 shows the composition of fatty acid in pulp oils of *C. odontophyllum* grouping into the total SFA, MUFA and PUFA. A total of 13 different fatty acids have been identified in *C. odontophyllum* pulp oils. However, not all of these fatty acids were detected in all samples collected from different district. Palmitic acid is the major fatty acid found in all samples with the highest percentage was detected in the sample from Sibu (55.75±0.64%), followed by Miri (51.91±1.18%), Kapit (49.13±0.80%) and Mukah (40.93±0.30%). Heneicosanoic acid was only detected in Mukah sample (0.31±0.02 %). The highest abundance of stearic acid was found in Kapit sample (8.35±0.2%), while the lowest abundance was in Sibu sample (1.71±0.02%). The oleic acid in pulp oil of *C. odontophyllum* (32.10-38.30%) is slightly lower compared to Azlan *et al.* (2010) which reported 41.54±0.06 % of oleic acid in *C. odontophyllum* pulp oil. Linoleic acid was also detected in the pulp oils in the range of 0.38-1.61%.

Table 4.8: Fatty acid compositions (%) in pulp oils of *C. odontopyllum*.

	Sibu	Mukah	Kapit	Miri
SFA				
C14:0	0.09 ± 0.00	0.28 ± 0.04	-	-
C16:0	55.75 ± 0.64	40.93 ± 0.30	49.13 ± 0.80	51.91 ± 1.18
C17:0	0.08 ± 0.00	0.13 ± 0.04	0.12 ± 0.01	0.04 ± 0.00
C18:0	1.71 ± 0.02	6.77 ± 0.05	8.35 ± 0.2	2.54 ± 0.07
C20:0	-	0.54 ± 0.01	0.18 ± 0.01	0.65 ± 0.02
C21:0	-	0.31 ± 0.02	-	-
Total SFA	**57.63 ± 0.17**	**48.96 ± 0.03**	**57.78 ± 0.26**	**55.14 ± 0.32**
MUFA				
C16:1	0.24 ± 0.03	0.29 ± 0.02	0.26 ± 0.01	0.48 ± 0.01
C18:1n9c	38.25 ± 0.12	33.15 ± 0.7	32.10 ± 0.21	38.30 ± 0.27
C20:1	0.98 ± 0.08	6.37 ± 0.04	3.44 ± 0.01	2.14 ± 0.01
Total MUFA	**39.47 ± 0.08**	**39.81 ± 0.25**	**35.80 ± 0.08**	**40.92 ± 0.10**
PUFA				
C18:2n6c	1.35 ± 0.07	1.61 ± 0.01	1.04 ± 0.03	0.38 ± 0.02
C20:2	1.10 ± 0.05	7.01 ± 0.08	4.72 ± 0.05	3.20 ± 0.04
C20:3n3	0.22 ± 0.03	1.28 ± 0.01	0.22 ± 0.00	0.07 ± 0.00
C20:3n6	0.24 ± 0.03	1.31 ± 0.02	0.22 ± 0.01	0.31± 0.02
Total PUFA	**2.91 ± 0.05**	**11.21 ± 0.03**	**6.20 ± 0.02**	**3.96 ± 0.02**

4.2.3.2 *Litsea garciae*

The predominant component in the pulp oils of *L. garciae* is oleic acid with the highest abundance was detected in Kuching sample (51.36±5.50%), followed by Mukah (49.20±0.07%), Kota Samarahan (45.51±4.43%) and Sibu (45.25±3.36%). However, Sibu sample has the highest percentage of palmitic acid (41.61±1.30%). Oleic acid has a significant role in the construction of nervous cell where it can be changed by organism into a set of compounds close to prostaglandins, which have an important function at the vessel level and

for blood coagulation, as well to prevent against cardiovascular diseases (Nehdi, 2011). It can also be inferred from Table 4.9 that stearic acid was identified in all samples in the range of 3.16-8.68%. Linoleic acid was the only PUFA detected in *L. garciae* with the highest abundance in Kota Samarahan sample (6.07±0.05%).

Table 4.9: Fatty acid compositions (%) in pulp oils of *L. garciae*.

	Kuching	K. Samarahan	Sibu	Mukah
SFA				
C16:0	39.27 ± 0.65	39.631 ± 0.73	41.61 ± 1.30	38.34 ± 0.14
C17:0	0.10 ± 0.00	0.12 ± 0.01	0.10 ± 0.01	0.10 ± 0.00
C18:0	3.16 ± 0.43	7.88 ± 0.36	8.68 ± 0.38	7.02 ± 0.37
C20:0	0.39 ± 0.03	0.61 ± 0.05	0.78 ± 0.03	0.20 ± 0.06
Total SFA	**42.92 ± 0.28**	**48.24 ± 0.29**	**51.17 ± 0.43**	**45.66 ± 0.14**
MUFA				
C16:1	0.15 ± 0.02	0.08 ± 0.01	0.05 ± 0.00	0.07 ± 0.00
C18:1n9c	51.36 ± 5.50	45.51 ± 4.43	45.25 ± 3.36	49.20 ± 0.07
C20:1	0.14 ± 0.04	0.10 ± 0.00	0.05 ± 0.01	0.05 ± 0.00
Total MUFA	**51.65 ± 1.85**	**45.69 ± 1.48**	**45.35 ± 1.12**	**49.32 ± 0.02**
PUFA				
C18:2n6c	5.43 ± 0.04	6.07 ± 0.05	3.46 ± 0.01	5.02 ± 0.04
Total PUFA	**5.43 ± 0.04**	**6.07 ± 0.05**	**3.46 ± 0.01**	**5.02 ± 0.04**

4.2.3.3 *Artocarpus odoratissimus*

As presented in Table 4.10, the total fatty acid detected in pulp oils of *A. odoratissimus* is lower compare to the other two fruit samples. Linoleic acid is the component with the highest percentage of abundance in this pulp oils, ranged from 38.02 to 45.60%. Linoleic acid is essential for the healthy growth of human skin where it can be transformed by organism into series of long fatty acid chains that act as the precursors of eicosanoids (Nasri *et al*., 2005). It was also noticed that oleic acid was the only MUFA detected with the highest abundance was identified in Kota Samarahan sample (28.47±1.30%) and the lowest abundance was in Miri sample (21.87±3.67%). The pulp oil from Miri has the highest amount of palmitic acid (34.58±1.44%), followed by Sibu (31.61±1.18%), Kuching (28.97±1.06%) and Kota Samarahan (21.77±2.06%).

Table 4.10: Fatty acid compositions (% of total fatty acids) of *A. odoratissimus* pulp oil.

	Kuching	K. Samarahan	Sibu	Miri
SFA				
C13:0	-	1.01 ± 0.01	0.79 ± 0.08	-
C16:0	28.97 ± 1.06	21.77 ± 2.06	31.61 ± 1.18	34.58 ± 1.44
C20:0	10.67 ± 0.32	1.46 ± 0.08	1.43 ± 0.47	2.19 ± 0.09
Total SFA	**39.64 ± 0.69**	**25.45 ± 0.56**	**33.83 ± 0.58**	**36.77 ± 0.77**
MUFA				
C18:1n9c	22.35 ± 1.80	28.47 ± 1.30	27.3 ± 0.96	21.87 ± 3.67
Total MUFA	**22.35 ± 1.80**	**28.47 ± 1.30**	**27.3 ± 0.96**	**21.87 ± 3.67**
PUFA				
C18:2n6c	38.02 ± 1.21	45.60 ± 1.24	38.88 ± 0.76	41.36 ± 4.21
C20:5n3	-	0.47 ± 0.02	-	-
Total PUFA	**38.02 ± 1.21**	**46.07 ± 0.13**	**38.88 ± 0.76**	**41.36 ± 4.21**

4.2.4 Fatty acids in kernel oils

Figures 4.5-4.7 show the GC-MS chromatograms for esterified fatty acids in the kernel oils of *C. odontophyllum, L. garciae* and *A. odoratissimus* obtained from different locations in Sarawak. Kernel oils of *A. odoratissimus* consists highest number of component of fatty acids compare to *C. odontophyllum* and *L. garciae*. The major component in kernel oils of each fruit sample differs from each other where lauric acid (C12:0) and linoleic acid (C18:2n6c) are the dominant compounds in *L. garciae* and *A. odoratissimus*, respectively. Oleic acid (C18:1n9c) and palmitic acid (C16:0) are the major compounds with almost similar percentage of abundance in kernel oils of *C. odontophyllum*. All chromatograms shows that stearic acid (C18:0) was detected in all kernel oil samples.

Table 4.11 shows total of SFA, MUFA and PUFA in the kernel oils of *C. odontophyllum, L. garciae*, and *A. odoratissimus*. The SFA in kernel oils of *C. odontophyllum* ranged between 49.69 to 57.02% is slightly lower compared to 60.84±0.04% of SFA reported by Azlan *et al*. (2010). The amount of SFA in kernel oils of *C. odontophyllum* is comparable with the percentage of SFA in palm oil (Azlan *et al*., 2010). The kernel oils of *A. odoratissimus* are rich in unsaturated fatty acids with the total of MUFA and PUFA accounted more than 60% of the total fatty acids. In contrast, *L. garciae* kernel oils are rich in SFA with the highest abundance detected in Kuching sample (79.05±0.53%), followed by Kota Samarahan (76.56±0.21%), Sibu (76.22±0.07%) and Mukah (75.50±0.25%). The amount of SFA in kernel oils of *L. garciae* is comparable with the SFA in palm kernel oil (82.1%) as reported by Edem (2002).

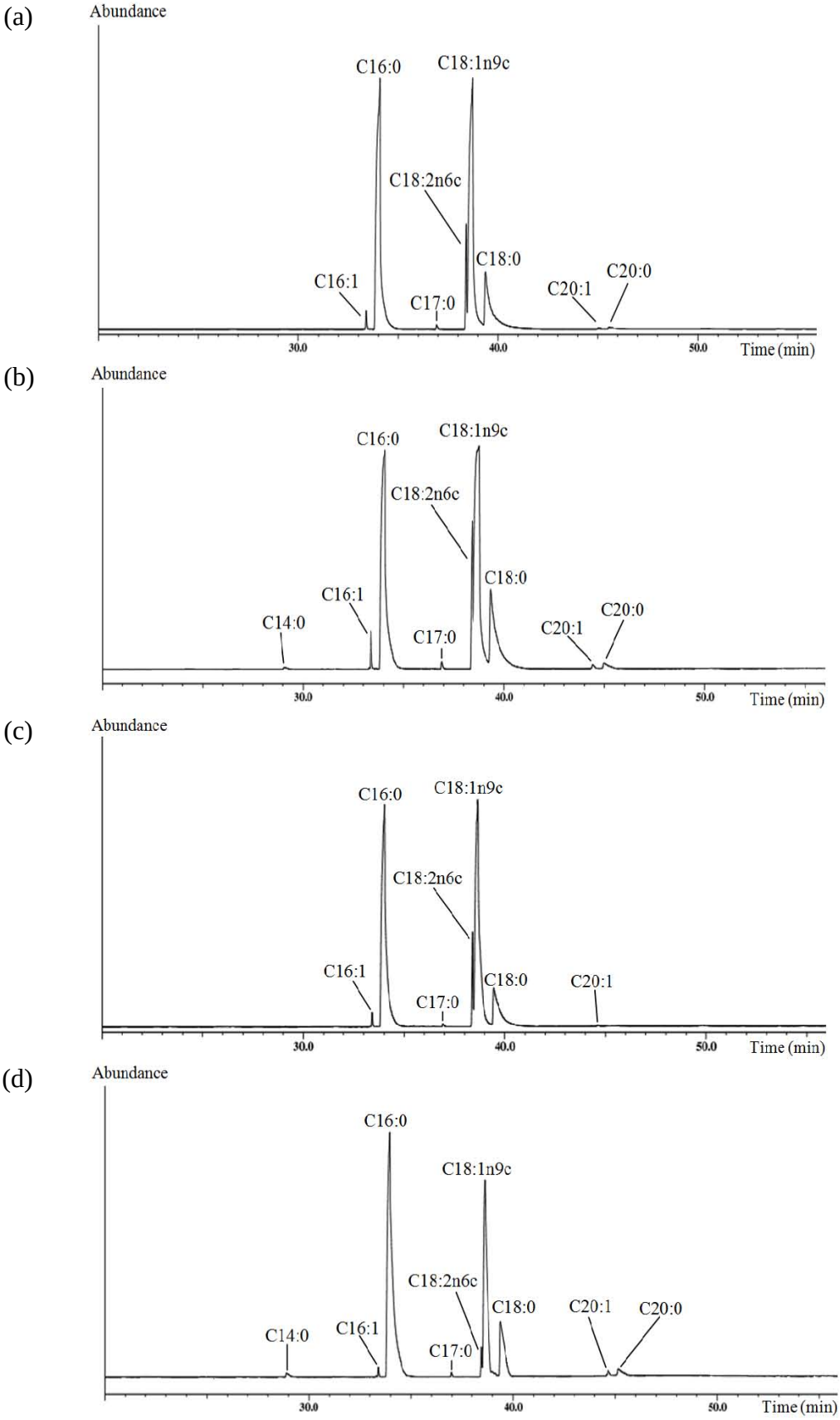


Figure 4.5: GC-MS chromatograms of the FAMEs in esterified kernel oils of *C. odontophyllum* collected from (a) Sibu, (b) Mukah, (c) Kapit and (d) Miri.

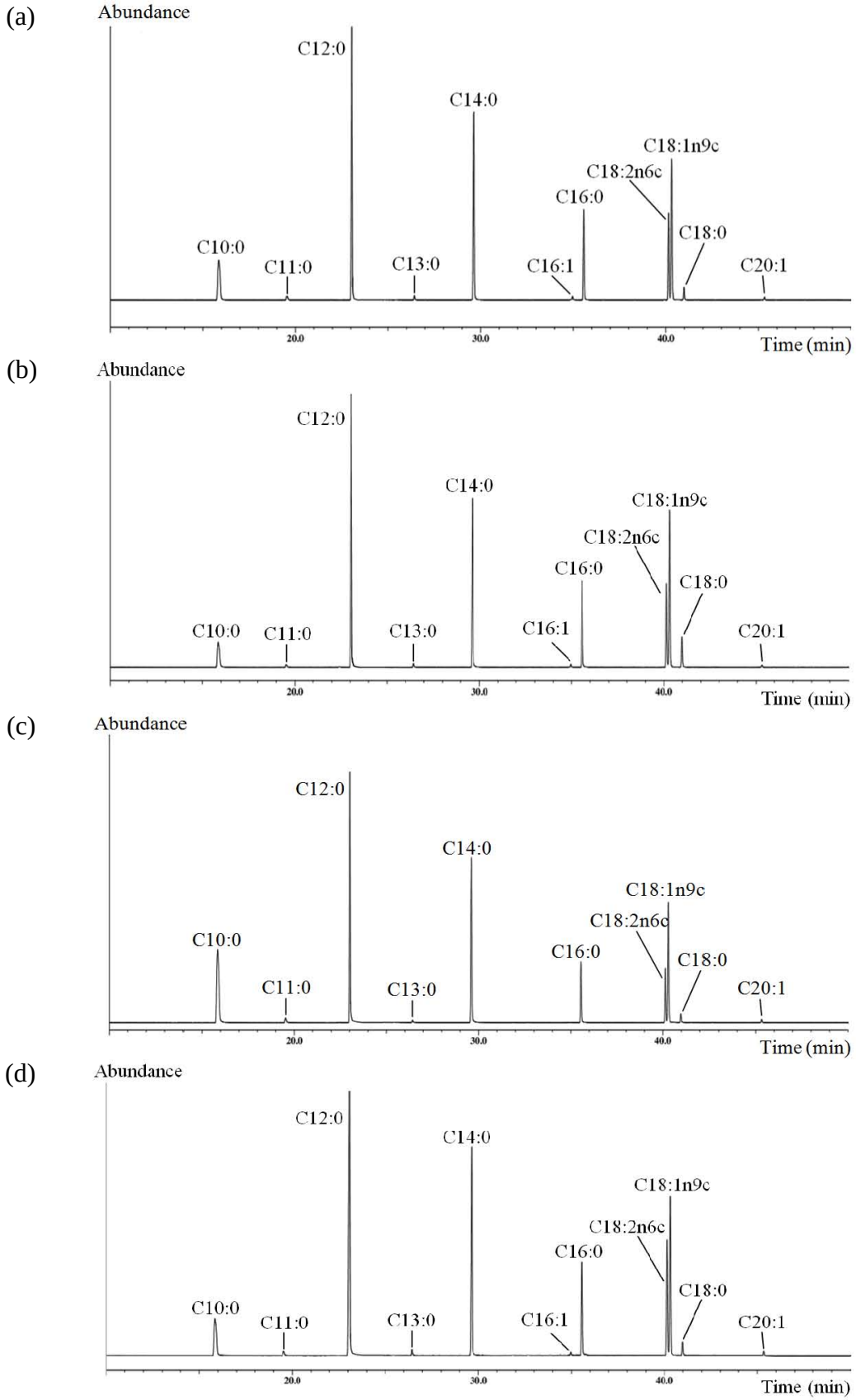


Figure 4.6: GC-MS chromatograms of the FAMEs in esterified kernel oils of *L. garciae* collected from (a) Kuching, (b) Kota Samarahan, (c) Sibu and (d) Mukah.

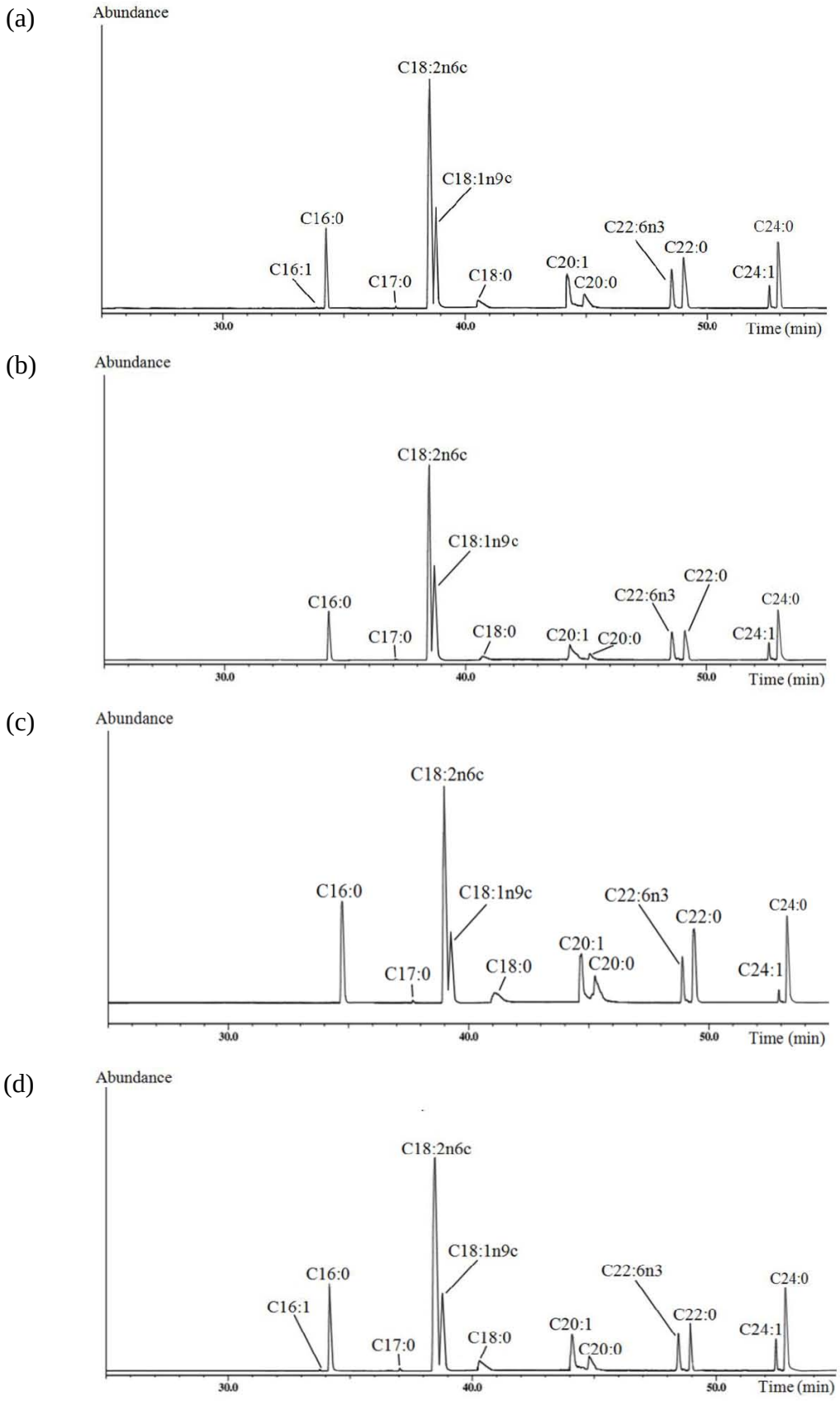


Figure 4.7: GC-MS chromatograms of the FAMEs in esterified kernel oils of *A. odoratissimus* collected from (a) Kuching, (b) Kota Samarahan, (c) Sibu and (d) Miri.

Table 4.11: The amount of SFA, MUFA and PUFA (%) in kernel oils of *C. odontophyllum*, *L. garciae*, and *A. odoratissimus*.

Fruit		Total SFA	Total MUFA	Total PUFA
C. odontophyllum	Sibu	49.69 ± 0.52	46.77 ± 3.15	3.53 ± 0.01
	Mukah	54.98 ± 0.32	38.90 ± 0.21	6.12 ± 0.06
	Kapit	52.67 ± 0.74	43.06 ± 0.73	4.28 ± 0.12
	Miri	57.02 ± 0.66	41.04 ± 2.75	2.01 ± 0.00
L. garciae	Kuching	79.05 ± 0.53	14.44 ± 0.54	6.51 ± 0.35
	K. Samarahan	76.56 ± 0.21	16.20 ± 0.35	7.25 ± 0.04
	Sibu	76.22 ± 0.07	17.31 ± 0.08	6.49 ± 0.01
	Mukah	75.50 ± 0.25	16.34 ± 0.10	8.16 ± 0.20
A. odoratissimus	Kuching	37.55 ± 0.74	22.36 ± 1.08	40.08 ± 0.75
	K. Samarahan	34.30 ± 0.48	24.33 ± 0.96	41.37 ± 2.41
	Sibu	42.24 ± 0.44	14.88 ± 0.46	42.87 ± 3.11
	Miri	36.49 ± 0.54	20.88 ± 0.10	42.63 ± 1.84

This study revealed various composition of fatty acid in the kernel oils for different fruit samples. *L. garciae* kernel oils are rich in SFA while *A. odoratissimus* contained more unsaturated fatty acids than SFA. However, *C. odontophyllum* kernel oils contain a balance amount of SFA and the unsaturated analogues. The fatty acid value of these seed oils could be justified for cosmetic and industrial applications. It will also provide a new source and low-cost feedstock which will increase the value of agricultural products. In addition, the high level of SFA and PUFA in kernel oils of *L. garciae* and *A. odoratissimus* respectively, make them desirable in terms of nutrition. Kernel oil of *A. odoratissimus* might be a suitable substitute for PUFA-rich oils such as sunflower and soybean oil while kernel oil of *L. garciae* might be a suitable substitute for virgin coconut oil, coconut oil and palm kernel oil.

4.2.5 Composition of individual fatty acid in kernel oils

4.2.5.1 *Canarium odontopyllum*

Table 4.12 presents the composition of fatty acids in kernel oils of *C. odontopyllum* collected from different districts of Sarawak. The number of individual fatty acids detected in the kernel oils is lower than those in the pulp oils. A total of 9 different fatty acids have been identified in the kernel oils with major compounds detected were palmitic acid and oleic acid. The highest abundance of palmitic acid was detected Miri sample (43.13±2.18%), followed by Kapit (42.59±1.92%), Sibu (39.95±1.43%) and Mukah (35.94±0.29%). The abundance of oleic acid in *C. odontopyllum* kernel oils ranged from 36.56 to 46.16% is slightly higher compared to the one reported by Azlan *et al.* (2010) which was 35.11±0.04%. Myristic acid (C14:0) was only detected in kernel oils from Mukah and Miri with percentage of abundance of 0.12±0.01% and 0.29±0.01%, respectively.

Table 4.12: Fatty acid compositions (%) in kernel oils of *C. odontopyllum*.

	Sibu	Mukah	Kapit	Miri
SFA				
C14:0	-	0.12 ± 0.01	-	0.29 ± 0.01
C16:0	39.95 ± 1.43	35.94 ± 0.29	42.59 ± 1.92	43.13 ± 2.18
C17:0	0.15 ± 0.01	0.32 ± 0.01	0.16 ± 0.05	0.17 ± 0.02
C18:0	8.11 ± 0.60	17.46 ± 1.25	9.92 ± 0.25	12.63 ± 1.07
C20:0	1.48 ± 0.03	1.14 ± 0.02	-	0.80 ± 0.04
Total SFA	**49.69 ± 0.52**	**54.98 ± 0.32**	**52.67 ± 0.74**	**57.02 ± 0.66**
MUFA				
C16:1	0.53 ± 0.02	0.89 ± 0.06	0.66 ± 0.01	0.37 ± 0.03
C18:1n9c	46.16 ± 9.42	37.74 ± 0.56	42.21 ± 2.16	36.56 ± 7.19
C20:1	0.08 ± 0.01	0.27 ± 0.00	0.19 ± 0.02	4.11 ± 0.04
Total MUFA	**46.77 ± 3.15**	**38.90 ± 0.21**	**43.06 ± 0.73**	**41.04 ± 2.75**
PUFA				
C18:2n6c	3.53 ± 0.01	6.12 ± 0.06	4.28 ± 0.12	1.93 ± 0.00
Total PUFA	**3.53 ± 0.01**	**6.12 ± 0.06**	**4.28 ± 0.12**	**1.93 ± 0.00**

4.2.5.2 *Litsea garciae*

A total of 11 different fatty acids were identified in the kernel oils of *L. garciae*. In contrast to the pulp oils, the kernel oils are rich in SFA which represented mostly by lauric acid and myristic acid with total accounted more than 50%. These acids, particularly lauric acid, have the preventive effect on prostatic hyperplasia development due to their 5α-reductase inhibitory activity (Veeresh Babu *et al*., 2010; Raynauld *et al*., 2002). The amount of lauric acid in the oils is comparable to lauric acid in virgin coconut oil (45.51%) and coconut oil (43.55%) as reported by Nevin and Rajamohan (2008). Oleic acid was identified

in all samples with the highest abundance detected in Sibu sample (16.68±0.13%), followed by Kota Samarahan (14.99±1.02%), Mukah (15.09±0.22%), and Kuching (13.17±1.58%). As shown in Table 4.13, linoleic acid was the only PUFA identified in the kernel oils with percentage between 6.49 to 8.16%.

Table 4.13: Fatty acid compositions (%) in kernel oils of *L. garciae*.

	Kuching	K. Samarahan	Sibu	Mukah
SFA				
C10:0	4.74 ± 0.69	4.52 ± 0.26	8.89 ± 0.19	6.13 ± 0.14
C11:0	0.62 ± 0.04	0.64 ± 0.03	0.66 ± 0.00	0.64 ± 0.01
C12:0	44.79 ± 0.89	40.37 ± 0.65	38.64 ± 0.07	39.10 ± 0.41
C13:0	0.60 ± 0.02	0.62 ± 0.04	0.43 ± 0.01	0.65 ± 0.02
C14:0	20.11 ± 1.38	18.99 ± 0.34	19.59 ± 0.04	20.05 ± 0.25
C16:0	7.02 ± 0.63	7.28 ± 0.01	6.56 ± 0.01	8.07 ± 0.06
C18:0	1.17 ± 0.01	4.14 ± 0.12	1.88 ± 0.02	0.86 ± 0.12
Total SFA	**79.05 ± 0.53**	**76.56 ± 0.21**	**76.22 ± 0.07**	**75.50 ± 0.25**
MUFA				
C16:1	0.65 ± 0.03	0.61 ± 0.02	-	0.62 ± 0.00
C18:1n9c	13.17 ± 1.58	14.99 ± 1.02	16.68 ± 0.13	15.09 ± 0.22
C20:1	0.62 ± 0.01	0.60 ± 0.00	0.63 ± 0.01	0.63 ± 0.02
Total MUFA	**14.44 ± 0.54**	**16.20 ± 0.35**	**17.31 ± 0.08**	**16.34 ± 0.10**
PUFA				
C18:2n6c	6.51 ± 0.35	7.25 ± 0.04	6.49 ± 0.01	8.16 ± 0.20
Total PUFA	**6.51 ± 0.35**	**7.25 ± 0.04**	**6.49 ± 0.01**	**8.16 ± 0.20**

4.2.5.3 *Artocarpus odoratissimus*

Fatty acids composition in kernel oils of *A. odoratissimus* is presented in Table 4.14. Kernel oils of *A. odoratissimus* consist the highest number of fatty acids compared to the other two fruit samples, where 13 fatty acids have been successfully identified. Similar to the pulp oils, the predominant component of *A. odoratissimus* kernel oils is linoleic acid with the highest percentage of abundance was observed in Sibu sample (40.74±6.19 %), followed by Miri (40.65±3.63 %), Kuching (37.36±1.39%) and Kota Samarahan (37.30±4.62%). The significance amount of PUFA in both pulp (38.02 – 46.07%) and kernel (40.08 – 42.87%) oils of *A. odoratissimus* are comparable to those in the seed oil of *A. camansi* which was reported at 30.81% by Adeleke and Abiodun (2010).

Palmitic acid and oleic acid occured in all kernel oils of *A. odoratissimus*. The Sibu sample contains the highest abundance of palmitic acid (15.74±0.99%) but the lowest amount of oleic acid (11.79±0.39%) compared to other kernel oils of *A. odoratissimus* from different districts of Sarawak. An unusual long chain saturated fatty acid - lignoceric acid (C24:0) has been identified in the kernel oils which accounted for 12.11 to 13.26% of the total fatty acids. The presence of lignoceric acid has been reported in the seed oils of *Washingtonia filifera* (Nehdi, 2011), *Cucurbita pepo* (Schinas *et al*., 2009) and *Phoenix canariensis* (Nehdi *et al*., 2010) with the percentage of abundance of 0.094%, 0.06% and 0.02%, respectively. Nervonic acid (C24:1) was also identified in all kernel oil samples at low percentage of abundance between 0.34 to 1.12%.

Table 4.14: Fatty acid compositions (%) in kernel oils of *A. odoratissimus*.

	Kuching	K. Samarahan	Sibu	Miri
SFA				
C16:0	14.66 ± 0.45	14.63 ± 0.30	15.74 ± 0.99	15.10 ± 0.58
C17:0	0.07 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.07 ± 0.01
C18:0	0.94 ± 0.03	0.57± 0.01	0.19 ± 0.02	0.72 ± 0.03
C20:0	1.48 ± 0.02	0.79 ± 0.04	2.14 ± 0.08	1.38 ± 0.02
C22:0	7.96 ± 0.90	6.18± 0.12	11.69 ± 0.38	5.96 ± 0.13
C24:0	12.44 ± 3.02	12.11 ± 2.40	12.46 ± 1.09	13.26 ± 2.47
Total SFA	**37.55 ± 0.74**	**34.30 ± 0.48**	**42.24 ± 0.44**	**36.49 ± 0.54**
MUFA				
C16:1	0.02 ± 0.00	-	-	0.02 ± 0.00
C18:1n9c	17.94 ± 4.23	20.88 ± 2.46	11.79 ± 0.39	16.82 ± 0.29
C20:1	3.49 ± 0.06	2.57 ± 0.42	2.75 ± 0.13	2.92 ± 0.04
C24:1	0.91 ± 0.03	0.88 ± 0.01	0.34 ± 0.07	1.12 ± 0.06
Total MUFA	**22.36 ± 1.08**	**24.33 ± 0.96**	**14.88 ± 0.46**	**20.88 ± 0.10**
PUFA				
C18:2n6c	37.36 ± 1.39	37.30 ± 4.62	40.74 ± 6.19	40.65 ± 3.63
C22:6n3	2.72 ± 0.01	4.07 ± 0.09	2.13 ± 0.03	1.98 ± 0.04
Total PUFA	**40.08 ± 0.75**	**41.37 ± 2.41**	**42.87 ± 3.11**	**42.63 ± 1.84**

4.3 Composition of Mineral in Pulp Samples

4.3.1 *Canarium odontophyllum*

The concentration of minerals in the pulps of *C. odontophyllum* is presented in Table 4.15. Results of the analysis were reported in mg/kg of dry weight. The highest concentration of potassium (K) detected in *C. odontophyllum* from Miri (10572.05±700.89 mg/kg) while the lowest was detected in Sibu sample (6116.47±70.85 mg/kg). The concentration of K in the

pulp of *C. odontophyllum* is higher than those in the pulp of date palm from Saudi Arabia, reported at 2896 to 5120 mg/kg by Assirey (2015). Significant amounts of Ca and Mg were also detected in *C. odontophyllum* with concentrations ranged between 2160.23-3735.45 and 867.91-1978.78 mg/kg, respectively. The concentrations of major minerals in *C. odontophyllum* are higher compared to the data reported by Chew *et al*. (2011). Micro minerals such as Zn, Cu, Fe and Mn were detected at considerable amounts with concentration less than 6.18 mg/kg.

Table 4.15: Mineral concentration in *C. odontophyllum* (mg/kg).

	Sibu	Mukah	Kapit	Miri
Ca	2160.23 ± 140.71	3002.46 ± 77.04	2703.56 ± 168.72	3735.45 ± 141.78
K	6116.47 ± 70.85	10814.84 ± 877.23	8487.08 ± 643.15	10572.05 ± 700.89
Mg	867.91 ± 12.51	1978.78 ± 91.80	1440.02 ± 97.67	1828.43 ± 140.31
Pb	0.08 ± 0.00	0.13 ± 0.00	0.17 ± 0.00	0.16 ± 0.00
Na	31.89 ± 0.55	30.63 ± 2.13	30.89 ± 3.32	31.14 ± 3.24
Zn	3.53 ± 0.05	4.03 ± 0.17	4.45± 0.24	4.53 ± 0.47
Cu	2.74 ± 0.03	2.08 ± 0.24	2.94 ± 0.50	4.10 ± 0.72
Fe	6.17 ± 0.51	4.51 ± 0.03	4.18 ± 0.45	3.78 ± 0.70
Mn	2.42 ± 0.05	2.67 ± 0.17	3.32 ± 0.38	5.64 ± 0.45

4.3.2 *Litsea garciae*

The highest concentration of K in *L. garciae* was detected in Kota Samarahan sample (6467.12±564.44 mg/kg), followed by Kuching (5790.78±956.32 mg/kg), Mukah (5494.63±534.07 mg/kg) and Sibu (5292.16±89.59 mg/kg). *L. garciae* are also rich in Ca and Mg, accounted for 366.08-471.80 mg/kg and 403.72-468.04 mg/kg, respectively. It can also

be inferred in Table 4.16 that Na was detected in the range between 45.71 to 53.13 mg/kg, while the concentrations of other micro minerals were less than 8.65 mg/kg.

Table 4.16: Mineral concentration in *L. garciae* (mg/kg).

	Kuching	K.Samarahan	Sibu	Mukah
Ca	433.82 ± 41.89	471.80 ± 37.44	366.08 ± 23.04	387.08 ± 46.34
K	5790.78 ± 956.32	6467.12 ± 564.44	5292.16 ± 89.59	5494.63 ± 534.07
Mg	451.34 ± 91.82	431.89 ± 86.82	403.72 ± 72.89	468.04 ± 10.62
Pb	0.29 ± 0.00	0.38 ± 0.00	0.34 ± 0.05	0.30 ± 0.00
Na	45.71 ± 7.23	53.13 ± 10.21	45.02 ± 2.25	46.20 ± 7.67
Zn	8.33 ± 0.90	8.26 ± 0.18	8.45 ± 1.44	8.64 ± 2.26
Cu	1.35 ± 0.72	1.22 ± 0.69	1.78 ± 0.48	1.82 ± 0.20
Fe	4.45 ± 0.26	3.76 ± 0.97	3.74 ± 0.37	4.31 ± 0.45
Mn	2.04 ± 0.11	2.35 ± 0.21	2.17 ± 0.30	2.07 ± 0.00

4.3.3 *Artocarpus odoratissimus*

Mineral concentration in fruit pulp of *A. odoratissimus* is summarized in Table 4.17. Potassium is also the main element in *A. odoratissimus* with the highest concentration detected in Sibu sample (2990.89±162.77 mg/kg) while the lowest concentration detected in Miri sample (2244.01±108.68 mg/kg). This result is in agreement with the data reported by Tang *et al.* (2013). However, the concentration of Ca in the range between 324.92 to 358.31 mg/kg was significantly high compared to the data reported by Tang *et al.* (2013) (0.5-1.4 mg/100 g) and Galang (1955) (17 mg). Mg is the third most abundant element in *A. odoratissimus* with concentrations ranged between 232.51 to 254.62 mg/kg, while the

concentration of Na was ranged from 25.45 to 43.11 mg/kg. Other trace elements such as Zn, Cu, Fe and Mn have been detected as low as 0.22 mg/kg.

Table 4.17: Mineral concentration in *A. odoratissimus* (mg/kg).

	Kuching	K.Samarahan	Sibu	Miri
Ca	343.89 ± 2.92	358.31 ± 4.05	324.92 ± 5.94	333.58 ± 3.04
K	2985.94 ± 195.67	2738.44 ± 98.37	2990.89 ± 162.77	2244.01 ± 108.68
Mg	254.62 ± 40.11	250.82 ± 17.56	232.51 ± 20.04	254.57 ± 20.03
Pb	0.36 ± 0.02	0.40 ± 0.00	0.43 ± 0.00	0.47 ± 0.00
Na	37.35 ± 7.14	40.07 ± 2.45	43.11 ± 2.76	25.45 ± 1.10
Zn	0.62 ± 0.02	0.75 ± 0.11	1.02 ± 0.25	0.86 ± 0.17
Cu	0.30 ± 0.00	0.42 ± 0.00	0.22 ± 0.04	0.48 ± 0.00
Fe	4.56 ± 0.35	3.36 ± 0.12	2.68 ± 0.02	1.53 ± 0.21
Mn	1.33 ± 0.06	2.06 ± 0.01	1.89 ± 0.23	2.88 ± 0.28

4.3.4 Distribution of minerals in pulp samples

The composition of mineral in fruit samples slightly varied according to locations. The variation in the element content between the fruit samples and the variation within each fruit sample from different origin is expected since they are belonged to different families and grown in different soil and climatic conditions. It has been known that the mineral content in fruit is influenced by genetic factors, soil and weather conditions, the use of fertilizers, and the state of the fruit's maturity at harvest (Sanchez-Castillo *et al*., 1998).

Potassium is the major element in every sample. Ca, K, Mg and Na are the major dietary minerals that are required at more than 100 mg per day by an adult (Macrae *et al.*, 1993). Ca and Mg have been detected highest in *C. odontophyllum* and lowest in *A.*

odoratissimus. Nonetheless, the concentrations of Ca and Mg in *A. odoratissimus* are comparable with those detected in breadfruit and passion fruit from Colombia (Leterme *at al*., 2006). Similarly, *C. odontophyllum* contained the highest amount of K compared to other fruits and the K concentration is comparable to those detected in apple, cranberry and sea buckthorn from Finland (Ekholm *et al*., 2007). K is an essential dietary nutrient and comprises about 70% of the positive ions in cells and it is important for the regulation of acid-base and water balance of the cells (Macrae *et al.*, 1993). In addition, *L. garciae* contained significant amount of Na and the concentration is comparable to those detected in avocado, mango and plum from Mexico (Sanchez-Castillo *et al*., 1998).

The concentrations of Zn (8.26-8.64 mg/kg) and Cu (1.22-1.82 mg/kg) in *L. garciae* are comparable with those in strawberry from Mersin, Turkey that has been reported at 8.09±0.96 and 1.65±0.41 mg/kg, respectively by Ozcan and Haciseferogullari (2007). Zn is an important element for the normal functioning of various enzyme systems. Deficiency in Zn particularly in children may lead to loss of appetite, weakness, growth retardation and stagnation of sexual growth (Saracoglu et al., 2009). Iron was detected in all samples and the concentrations of Fe in the three fruit samples are comparable to those in apple, grapes, orange, papaya and pineapple from Mexico (Sanchez-Castillo *et al*., 1998). This essential metal transports oxygen from the lungs to the body's tissues such as the muscles and the brain via the haemoglobin in red blood cells (Konczak and Roulle, 2011). The fruit samples were also analyzed for the abundance of lead (Pb), cadmium (Cd) and selenium (Se). However Cd and Se were not detected in any of fruit sample. Pb has been detected with concentrations less than 0.047±0.00 mg/100 g.

4.4 Total Phenolic Content and Antioxidant Activity in Methanol Extract

4.4.1 Total phenolic content in extracts from pulp samples

Gallic acid standard ranged between 0 to 100 ppm was analysed. A linear calibration curve produced r^2 value of 0.998 (see Figure 4.8). The total phenolic content is expressed as mg gallic acid equivalent in g dry weight (mg GAE/g dw) basis. As presented in Table 4.18, *C. odontophyllum* contained the highest amount of phenolic with the highest abundance detected in the extract from Mukah sample (37.09±0.20 mg GAE/g dw), followed by Sibu (33.1±0.37 mg GAE/g dw), Miri (27.02±0.43 mg GAE/g dw) and Kapit (19.33±0.63 mg GAE/g dw). These results are well-corresponded with the data reported by Chew *et al*. (2011) who found 9.50-33.21 mg GAE/g dw in *C. odontophyllum* collected from four different districts (Kanowit, Kapit, Sarikei and Song).

It can also be inferred from Table 4.18 that there is only a minor difference in the concentration of phenolic content in *L. garciae* of different origin. Phenolic was detected at highest concentration in Mukah sample (5.06±0.04 mg GAE/g dw) and the lowest abundance in Kuching sample (3.26±0.10 mg GAE/g dw). Ali Hassan *et al.* (2013a) have reported total phenolic content in fruit pulp of *L. garciae* at 2.65±0.11 mg GAE/g dw. The total phenolic content in the methanol extract of *A. odoratissimus* was quite low which accounted only between 0.97 to 1.06 mg GAE/g dw.

The significant amount of phenolic content in *C. odontophyllum* extracts compared to the other fruits might be due to the colour pigmentation. The skin of this fruit is dark purple colour while *A. odoratissimus* pulp is creamy white. A study in grapes showed that purple juice has higher total phenolic content compared to white juice (Dani *et al.*, 2007).

Anthocyanins are thought to be responsible for the colour of several plants particularly in the skin of fruits. In addition, other factors such as maturity, variety and different agronomic conditions can also influence the phenolic content in fruits (Romani *et al*., 2002).

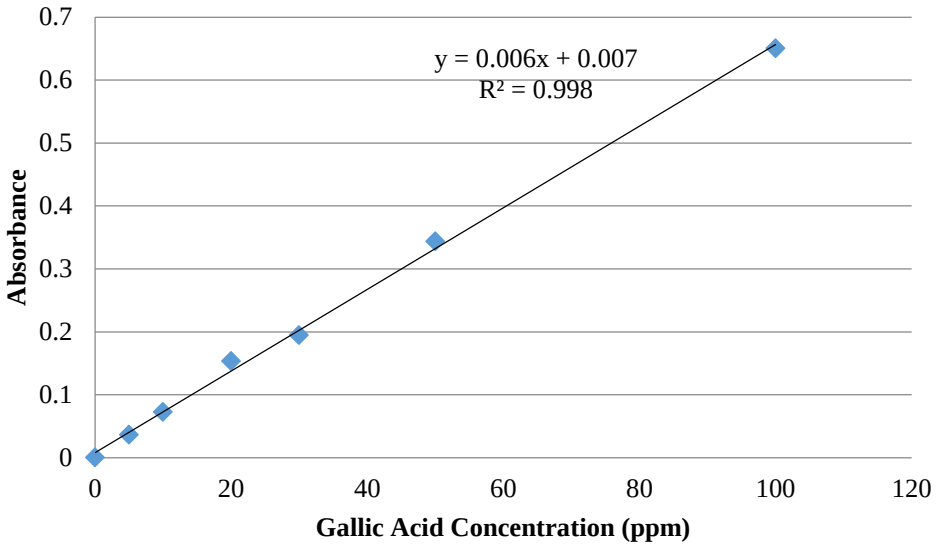


Figure 4.8: Calibration curve of gallic acid standard for the absorbance at 765 nm.

Table 4.18: Total phenolic content in methanol extracts of fruit samples collected from different districts of Sarawak.

Fruit	Location	Phenolic content (mg GAE/g dw)
C. odontophyllum	Sibu	33.18 ± 0.37
	Mukah	37.09 ± 0.20
	Kapit	19.33 ± 0.63
	Miri	27.02 ± 0.43
L. garciae	Kuching	3.26 ± 0.10
	K.Samarahan	4.82 ± 0.04
	Sibu	3.60 ± 0.19
	Mukah	5.06 ± 0.04
A. odoratissimus	Kuching	1.04 ± 0.10
	K.Samarahan	1.03 ± 0.05
	Sibu	1.06 ± 0.02
	Miri	0.97 ± 0.01

4.4.2 Antioxidant activity

The results of the DPPH scavenging assay are presented in Table 4.19. It was found that the antioxidant compounds in the extracts have the free radical scavenging ability. There is only a slight difference observed for the antioxidant activity in the extracts for each fruit sample between different origins. In Table 4.19, the results were presented as the percentage of scavenging at 10 µg/mL and the concentration at 50% inhibition (IC_{50}). A lower IC_{50} signifies a stronger ability of the methanol extract to scavenge DPPH.

Table 4.19: Antioxidant activity in the methanol extracts of fruit samples collected from different districts of Sarawak.

Fruit	Location	S (%)[a]	IC_{50} (µg/mL)[b]
C. odontophyllum	Sibu	16.67 ± 0.14	109.76 ± 3.81
	Mukah	15.56 ± 0.49	119.50 ± 1.09
	Kapit	17.06 ± 0.36	95.18 ± 0.89
	Miri	22.29 ± 0.15	87.58 ± 0.86
L. garciae	Kuching	22.02 ± 0.23	135.22 ± 1.37
	K.Samarahan	18.05 ± 0.14	142.50 ± 1.09
	Sibu	17.75 ± 0.29	164.68 ± 2.45
	Mukah	17.06 ± 0.33	190.43 ± 0.96
A. odoratissimus	Kuching	14.96 ± 0.86	185.32 ± 0.58
	K.Samarahan	17.15 ± 0.18	115.55 ± 2.07
	Sibu	6.55 ± 0.20	290.08 ± 1.19
	Miri	10.42 ± 0.06	230.67 ± 1.45
Ascorbic Acid		32.51 ± 0.11	32.05 ± 0.84

[a] Percent of scavenging at 10 µg/mL as mean triplicate experiments
[b] IC_{50} is defined as the concentration sufficient to obtain 50% of the maximum scavenging activity

The extracts of *C. odontophyllum* possessed the highest antioxidant activity with IC_{50} of between 87.58-119.50 µg/mL. The methanol extract of *L. garciae* from Kuching had the strongest free radical scavenging ability with IC_{50} of 135.22± 1.37 µg/mL and the least active is from Mukah (190.43±0.96 µg/mL). *A. odoratissimus* extract shows high value of $IC_{50,}$ ranged from 115.55 to 290.08 µg/mL.

The constituents in mixture of phenolic compound such as anthocyanins, flavonoids and phenolic acids play important roles as antioxidant in fruits, thus extracts with higher phenolic content generally exhibit higher antioxidant ability (Johnny *et al*., 2011). Flores *et al*. (2015) documented a study on antioxidant activity of seven edible guava cultivars that varied in colour from white to pink. The results using DPPH assay showed the pink-pulp guavas have higher activity than the white pulp cultivars. The correlation between total phenolic content and antioxidant activity of different fruit species studied was shown in Figure 4.9. It can be inferred from the figure that methanol extracts of *C. odontophyllum* has the highest total phenolic compounds and the lowest IC_{50}, compare to the other two species.

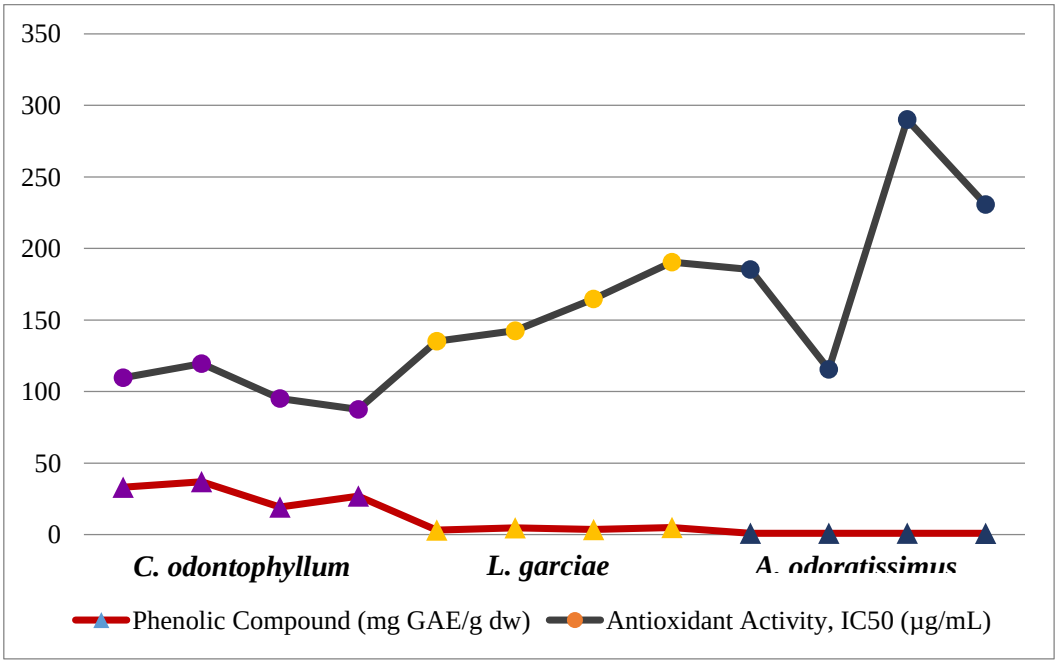


Figure 4.9: Correlation graph between total phenolic compounds and antioxidant activity of methanol extracts from different fruit species.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Fruits of *C. odontophyllum*, *L. garciae* and *A. odoratissimus* were successfully analysed for their nutritional properties. Proximate composition such as moisture content, ash content, crude fat, crude fiber and vitamin C were examined and the results suggested that these fruits are good sources of necessary nutrients for human diet. Mineral contents in the pulp samples were also determined using AAS. The fruits are rich in essential major minerals including K, Ca, Mg and Na with K as the most abundant element in all samples. Pulp of *C. odontophyllum* contained the highest amount of K (6116.47–10814.84 mg/kg) compared to the other two species. Micro minerals including Zn, Cu, Fe and Mn were also detected in all studied fruits at considerable amounts.

The pulp and kernel oils of these fruits have been successfully extracted using Soxhlet extraction apparatus and then esterified into FAMEs prior to GC-MS analysis. Pulp oils of all fruit samples contain high amount of palmitic, oleic and linoleic acid. The highest abundance of oleic acid was detected in the pulp oils of *L. garciae* (45.25-51.36%) among the three species, while pulp oils of *C. odontophyllum* contain the highest amount of palmitic acid (40.93-55.75%). Significant amount of linoleic acid in pulp oils of *A. odoratissimus* was detected in the range between 38.02 to 45.60%. The composition of fatty acid in the kernel oils varied from different fruit species. Kernel oils of *L. garciae* are rich in SFA (75.50-79.05%) while kernel oils of *A. odoratissimus* contain more unsaturated fatty acids than SFA,

accounted for more than 60% of the total fatty acids. The amount of SFA in kernel oils of *C. odontophyllum* is almost balance with the unsaturated analogues. Fatty acids analysis showed the potential of both pulp and kernel oils of these fruits in cosmetic and industrial applications, as well as for human consumption. MUFAs are the best components for biodiesel when considering the oxidative stability and low temperature fluidity. Thus, high MUFA content in both pulp and kernel oils of *C. odontophyllum* and pulp oils *L. garciae* have the potential for biodiesel. In addition, high level of SFA in kernel oils of *L. garciae* might be a suitable substitute for virgin coconut oil and palm kernel oil while kernel oil of *A. odoratissimus* might be a suitable substitute for PUFA-rich oils such as sunflower and soybean oil.

The fruit pulps were also successfully extracted with methanol for the determination of phenolic content and antioxidant activity. *C. odontophyllum* extracts had the highest phenolic content among all the three fruits, ranging between 19.33 to 37.09 mg GAE/g dw, while *A. odoratissimus* had the lowest (0.97-1.06 mg GAE/g dw). The difference might be due to the colour pigmentation. The skin of *C. odontophyllum* fruit is dark purple in colour, while the pulp of *A. odoratissimus* is creamy white coloured. The antioxidant activity using DPPH scavenging assay showed that antioxidant compounds in all studied extracts possess the free radical scavenging ability. The extracts of *C. odontophyllum* possessed the highest antioxidant activity compare to the other two fruits with IC_{50} of between 87.58 and 119.50 µg/mL.

5.2 Recommendation for Future Studies

The study on the chemical compositions in these underutilized edible fruits should be extended to provide more information because they are largely consumed by the local people during the fruits' season. Proximate analysis including protein, energy and carbohydrates should be further studied. Both the pulp and kernel oils are rich in essential fatty acids and can be justified in cosmetic, industrial and agricultural applications. Thus, the quality of the oils are needed to be further analysed so that the oils could be exploited and utilized as new natural sources of feedstock. Antioxidants have different chemical and physical characteristics thus they may respond in a different manner to different radical or oxidant sources. Since this study only focused on one assay for the determination of antioxidant activity, further studies using other assays such as ferric-reducing ability of plasma (FRAP) assay and 2,2'-Azino-bis(3-ethylbenzo-thiazoline-6-sulphonic acid) (ABTS) decolourisation assay are recommended. In addition, other chemical analysis for instance sugar, cholesterol and vitamins can be further investigated as additional information for the nutritive values in these underexploited fruits.

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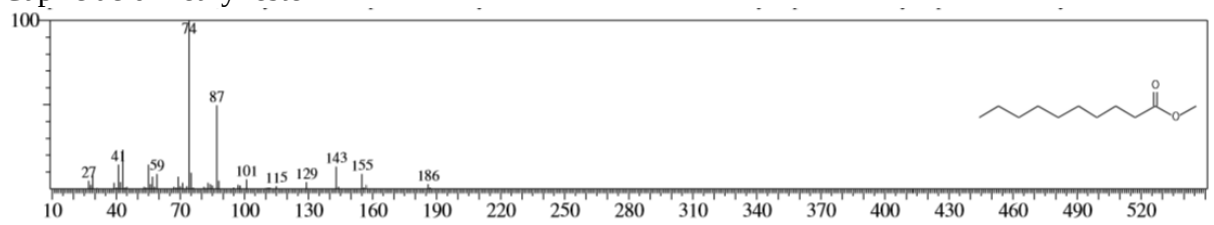
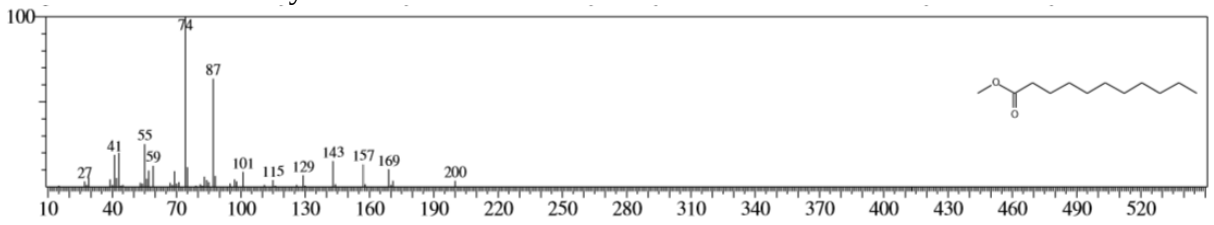
APPENDICES

Appendix A: Mass spectra of several FAMEs identified in esterified pulp and kernel oils.

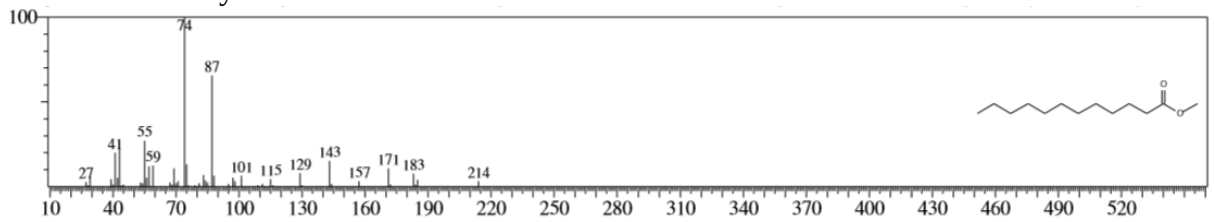
Capric acid methyl ester



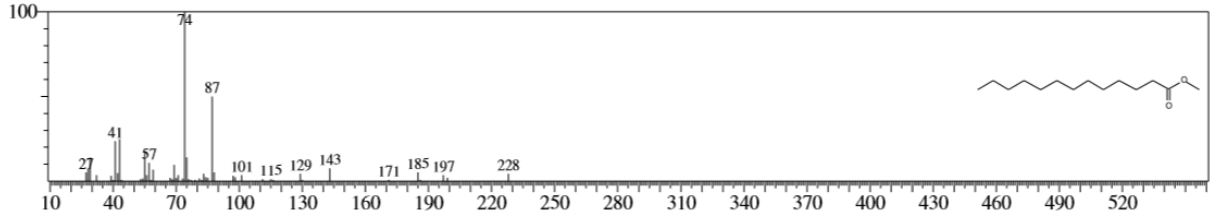
Undecanoic acid methyl ester



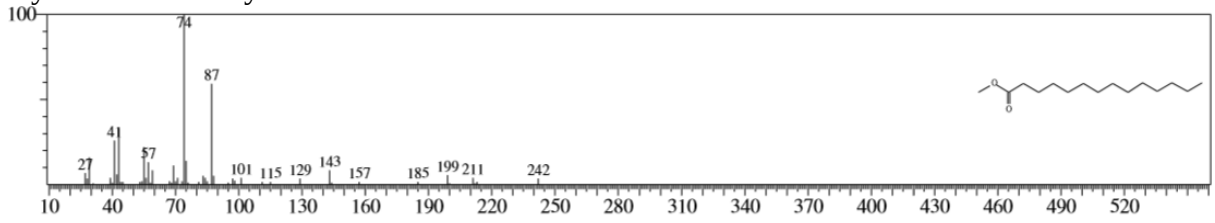
Lauric acid methyl ester



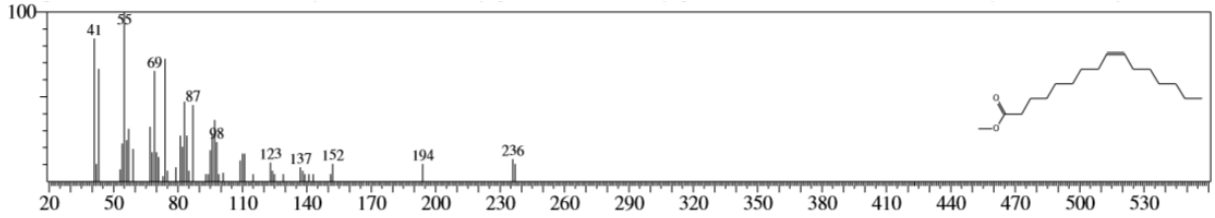
Tridecanoic acid methyl ester



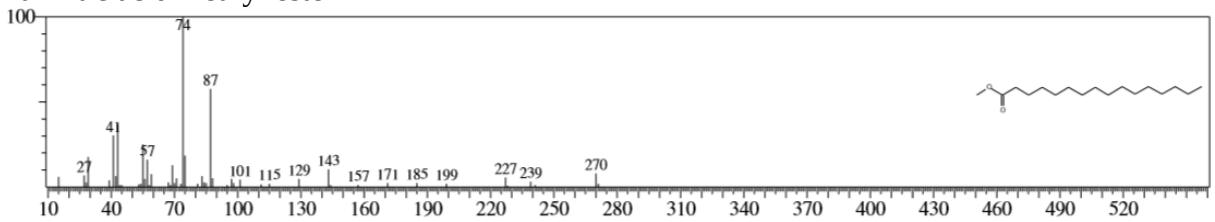
Myristic acid methyl ester



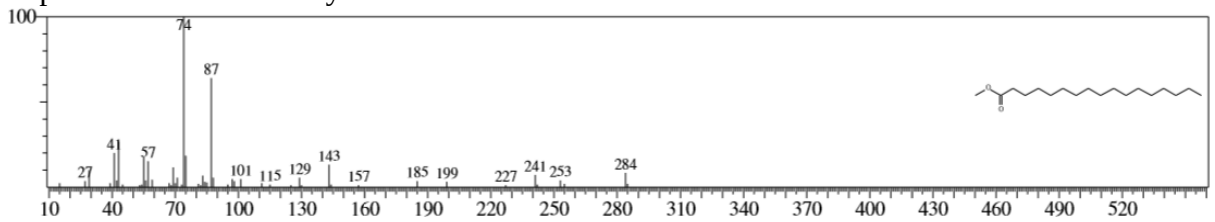
Palmitoleic acid methyl ester



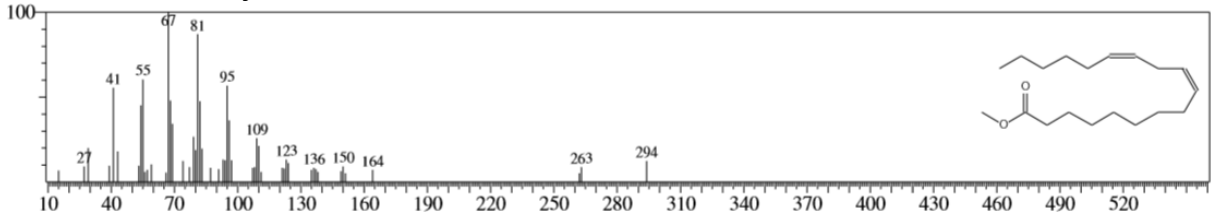
Palmitic acid methyl ester



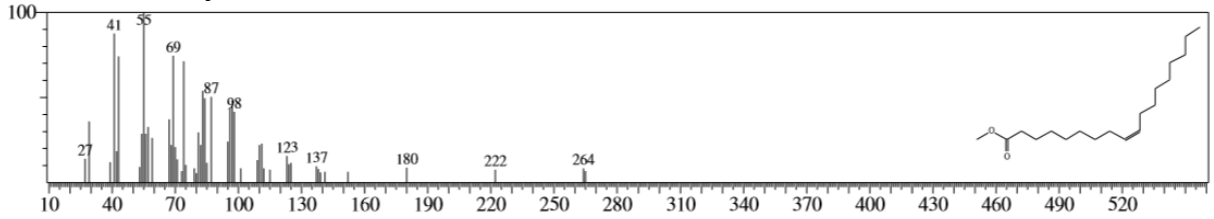
Heptadecanoic acid methyl ester



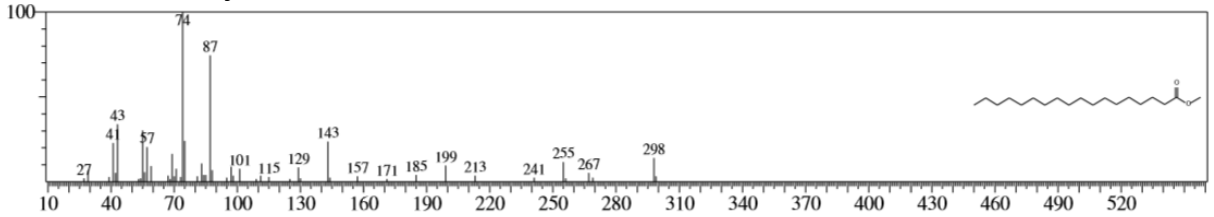
Linoleic acid methyl ester



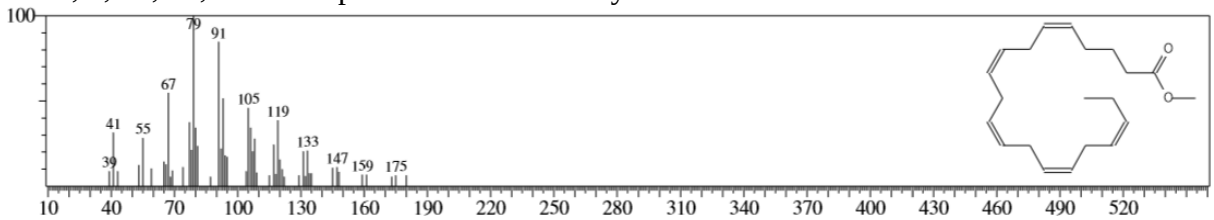
Oleic acid methyl ester



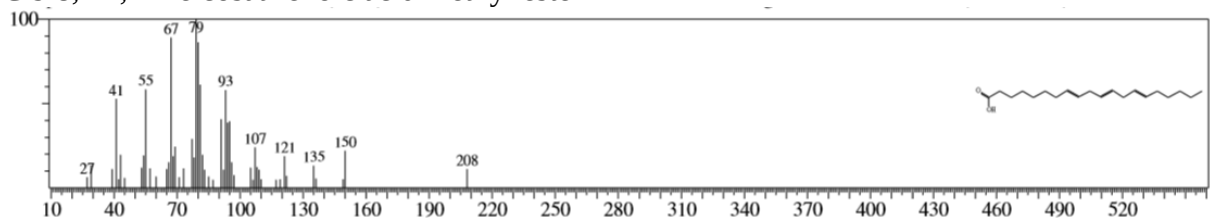
Stearic acid methyl ester



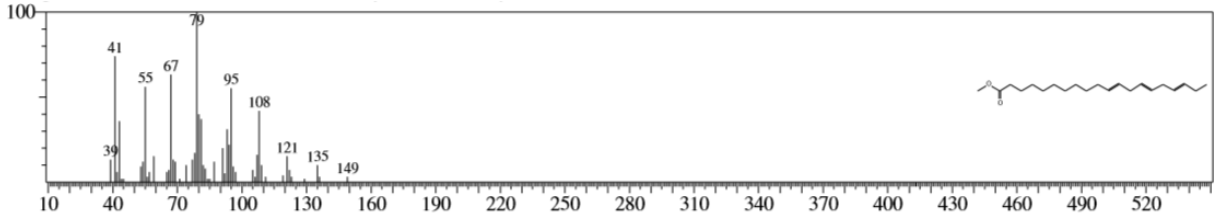
Cis-5, 8, 11, 14, 17-eicosapentaenoic acid methyl ester



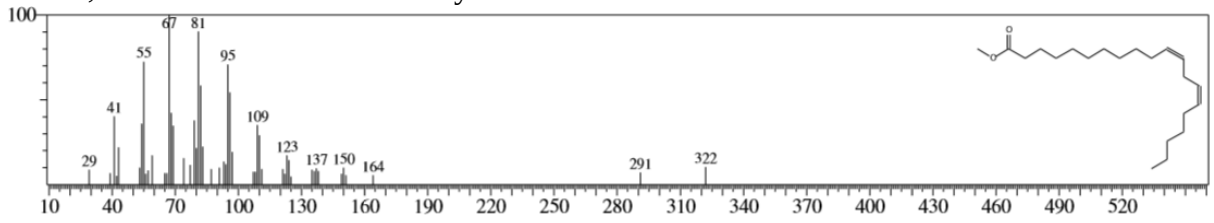
Cis-8, 11, 14-eicosatrienoic acid methyl ester



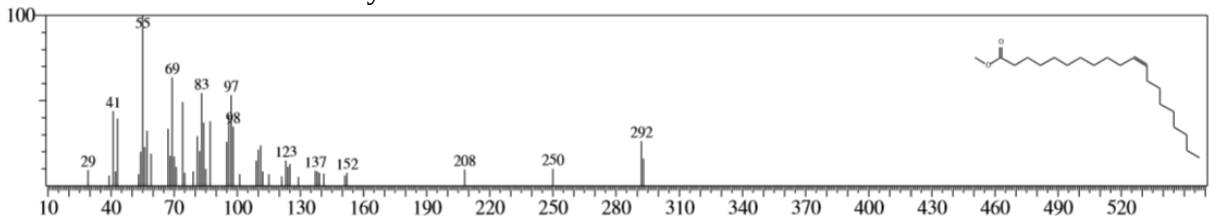
Cis-11, 14, 17-eicosatrienoic acid methyl ester



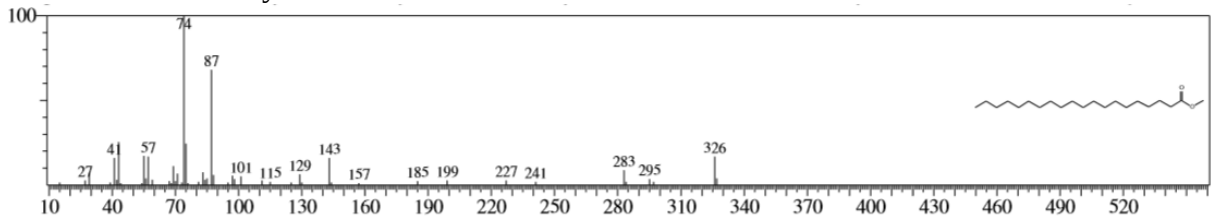
Cis-11, 14-eicosadienoic acid methyl ester



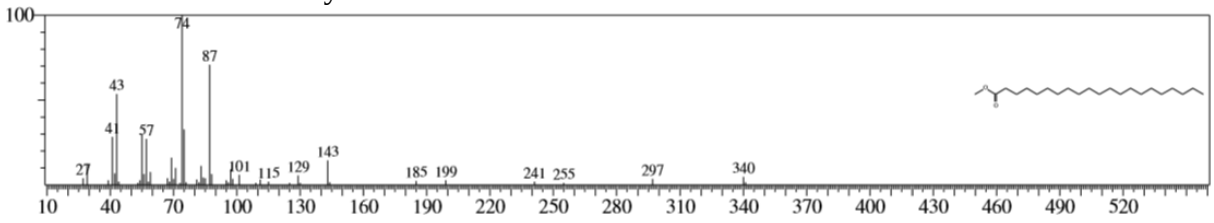
Cis-11-eicosenoic acid methyl ester



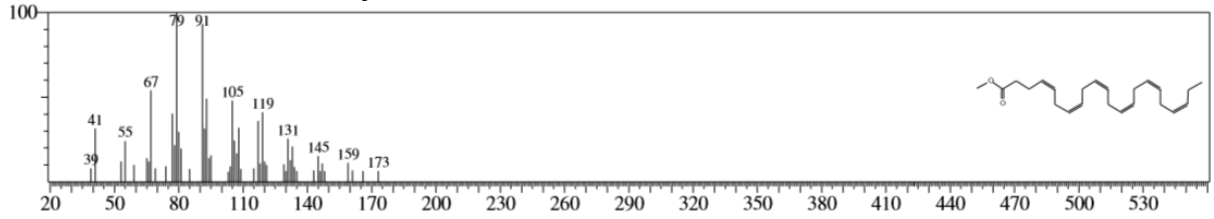
Arachidic acid methyl ester



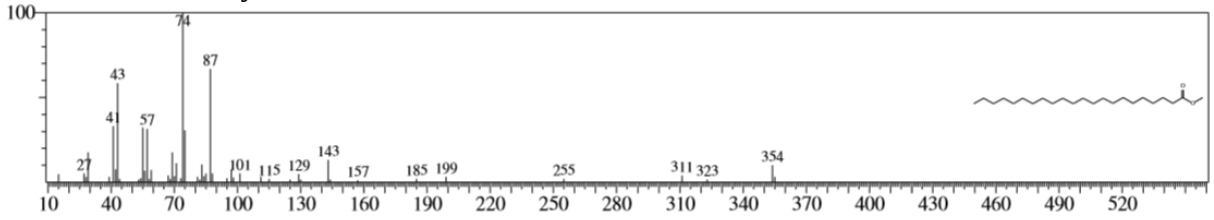
Heneicosanoic acid methyl ester



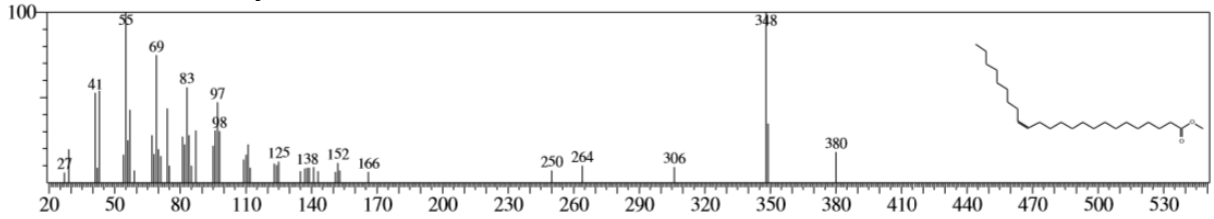
Docosahexaenoic acid methyl ester



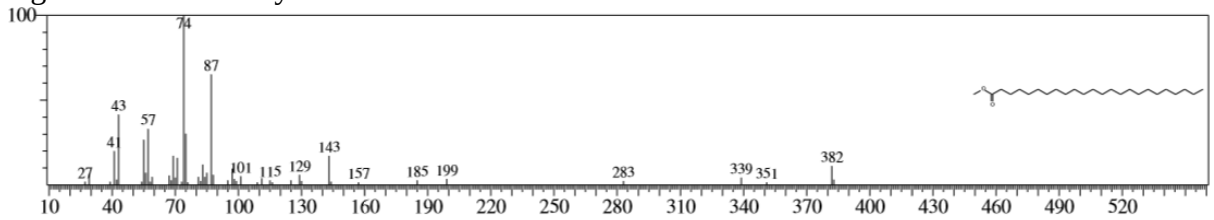
Behenic acid methyl ester



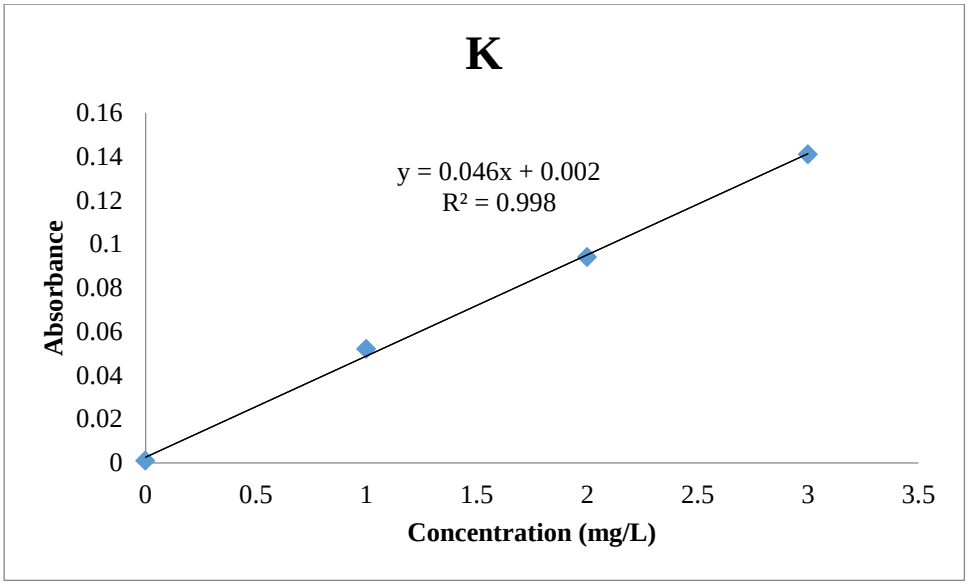
Nervonic acid methyl ester



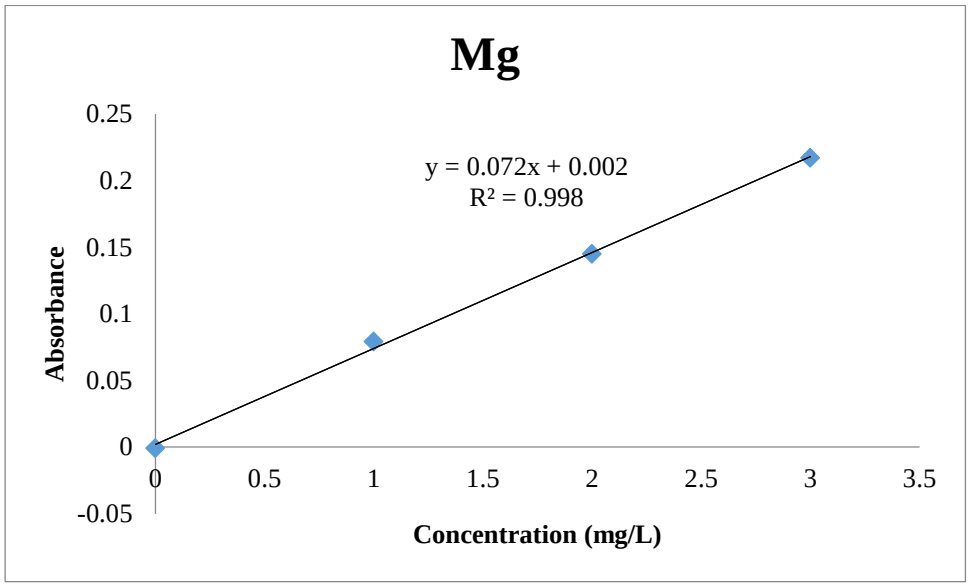
Lignoceric acid methyl ester



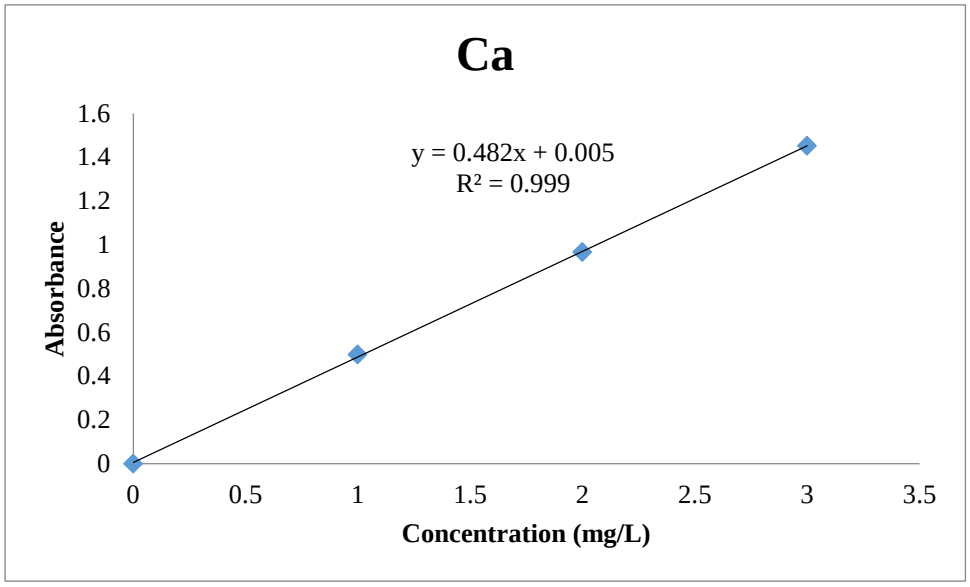
Appendix B: Calibration curves of mineral standards.



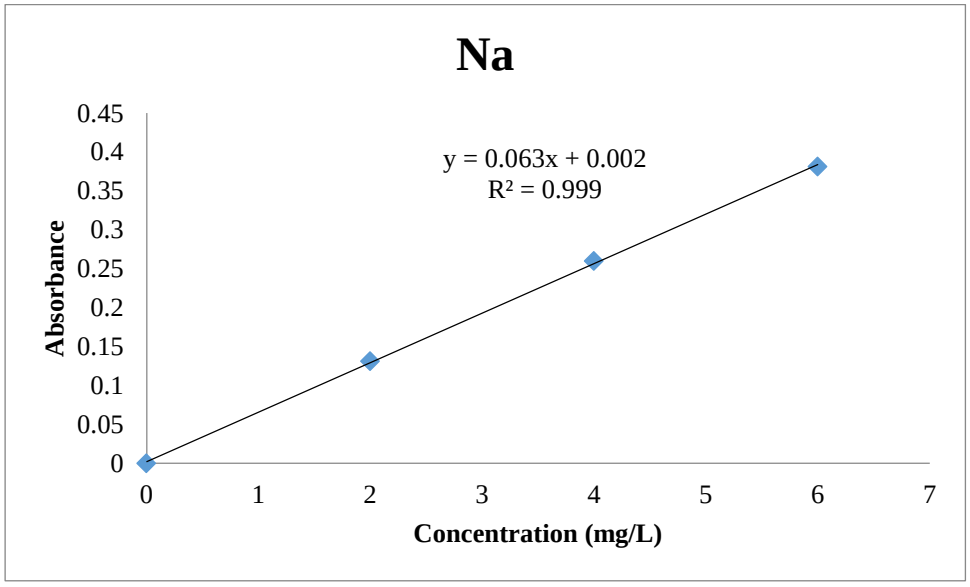
Appendix B1: Calibration curve of potassium.



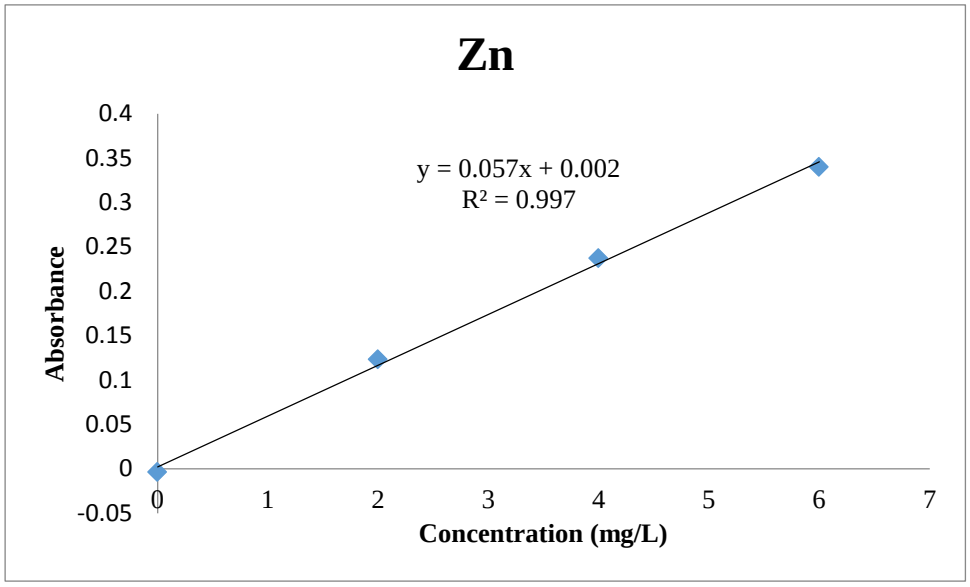
Appendix B2: Calibration curve of magnesium.



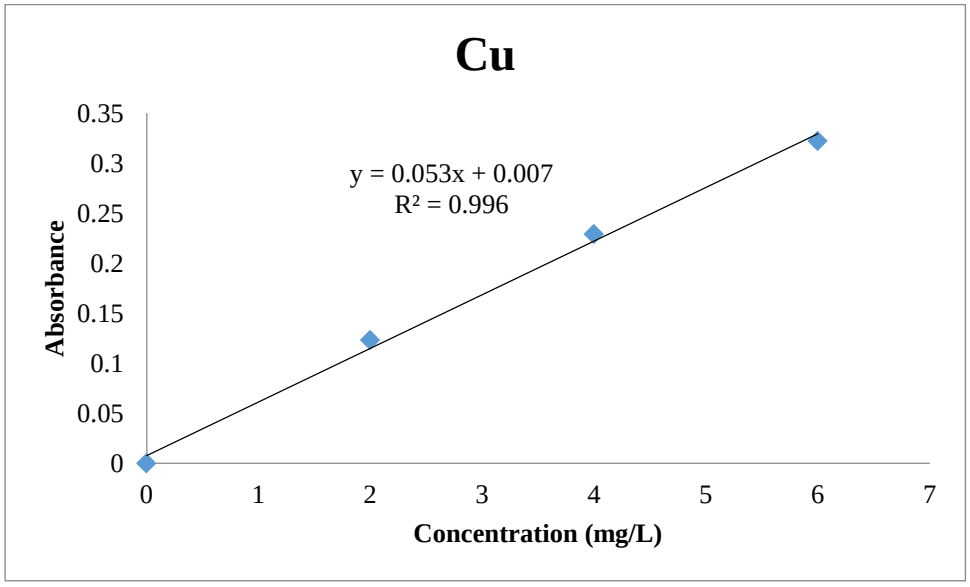
Appendix B3: Calibration curve of calcium.



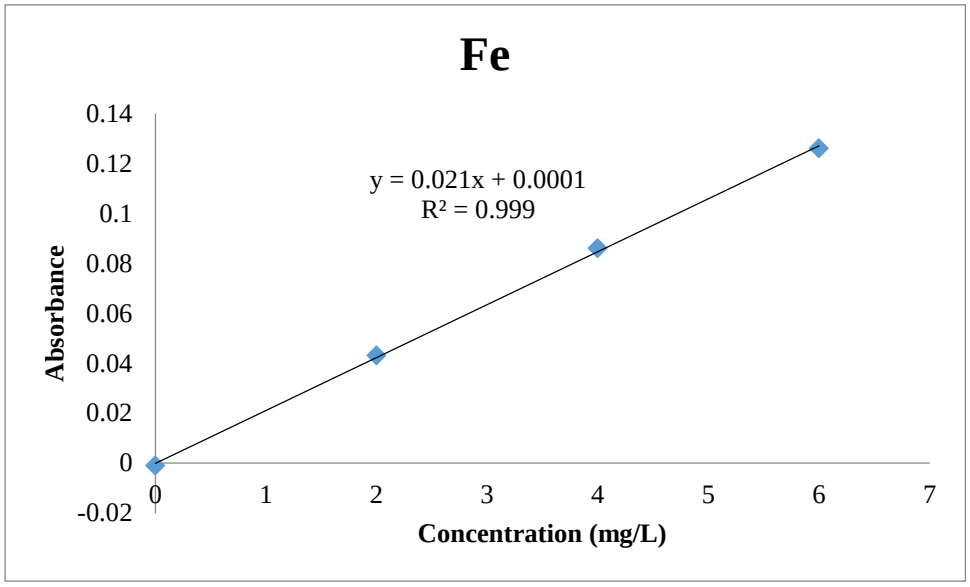
Appendix B4: Calibration curve of sodium.



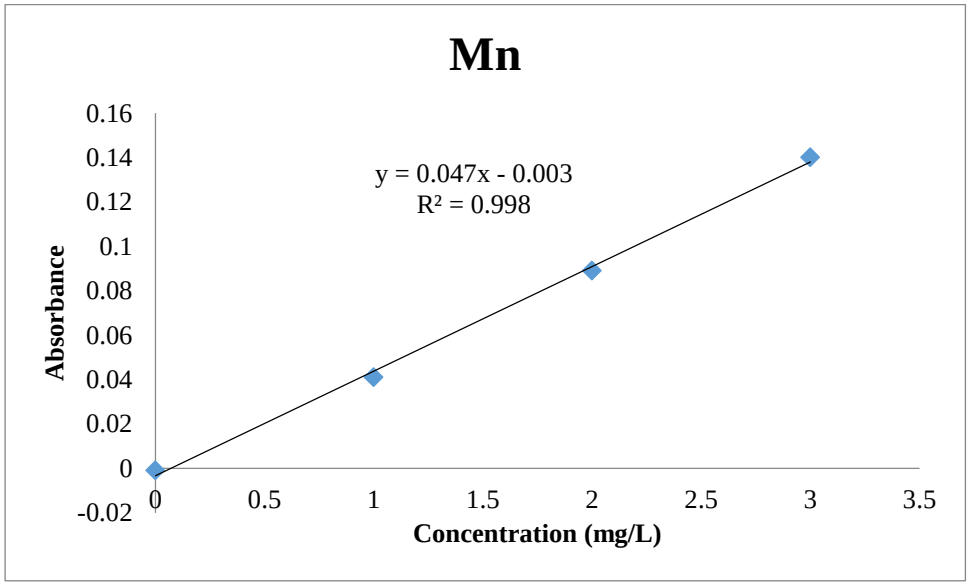
Appendix B5: Calibration curve of zinc.



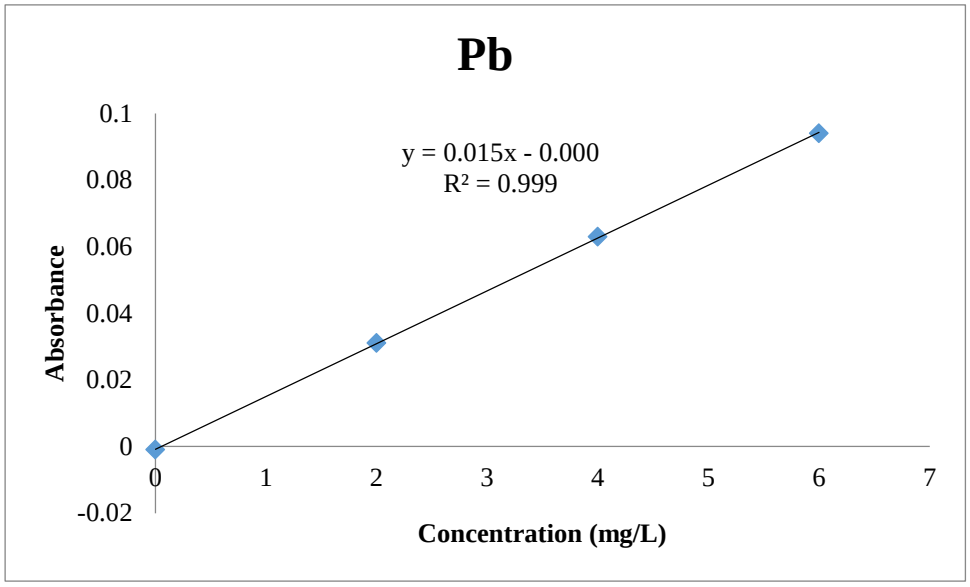
Appendix B6: Calibration curve of copper.



Appendix B7: Calibration curve of iron.



Appendix B8: Calibration curve of manganese.



Appendix B9: Calibration curve of lead.