Faculty of Resource Science and Technology

FATTY ACID COMPOSITION AND ANTIOXIDANT PROPERTIES OF SEED OIL FROM *Canarium* spp.

NOOR MUHAZAH BINTI MAZLAN

(35045)

Bachelor of Science with Honours

(Program of Resource Chemistry)

2015

FATTY ACID COMPOSITION AND ANTIOXIDANT PROPERTIES OF SEED OIL FROM *Canarium* spp.

By:

NOOR MUHAZAH BINTI MAZLAN

STUDENT NUMBER: 35045

This Final Year Project report is submitted in partial fulfillment of the requirement of Bachelor of Science with Honours

(Program of Resource Chemistry)

Department of Chemistry
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak
2015

ACKNOWLEDGEMENTS

All praise to Allah, the Almighty and the Merciful for giving me strength and ability to learn and complete this Final Year Project.

The successful completion of this report might never be possible in time without the help some persons whose inspiration and suggestion made it happen. First and foremost, I would like to express my gratitude to my supervisor, Prof Dr Zaini Bin Assim who gives me this opportunity to work on this report and learn something beneficial in my practical experience.

I wish to extend my earnest and sincere thanks to assistants of Analytical Chemistry Laboratory, Mr Rajuna Tahir and also Mr. Benedict for their help and guidance during my practical session. Without their help, it would be difficult for me to complete this final year project. I also would like to thank all my labmates Khairunnisa, Diana, Syafiqah, Asma, Zulfadhli, Aiza, Afiffah, Joanna, Jevinna, Atikah and Laily, my friends; Jenny, Soo Lian Zye, Afrina and Rasyidah for helping me to complete this thesis by sharing information and knowledge. Not only that, big thanks to the postgraduate students; Badrunnaim, Nadia, Rafaiza, Latifah, Afina, Gloria and Sam for their opinion and help in completing this thesis.

Finally, I would like to express my heartfelt thanks to my parents, Mazlan Bin Dali and Latifah Binti Md Yassin and my family because of their sincere prayers, support and motivation which always an inspiration for me. Thank you also to all who involved directly or indirectly during the completion of this study. May Allah S.W.T reward them with the highest reward.

UNIVERSITI MALAYSIA SARAWAK

Grade: _____

Please tick (√)

Final year report (   )

Masters (   )

PhD (   )

DECLARATION OF ORIGINAL WORK

This declaration is made on the ____ day _____ 2015

Student's declaration:

I, NOOR MUHAZAH BINTI MAZLAN, 35045, FACULTY OF SCIENCE AND TECHNOLOGY hereby declare the work entitled, FATTY ACID COMPOSITION AND ANTIOXIDANT PROPERTIES OF SEED OIL FROM *Canarium* spp. is my original work. I have not copied from any other student's work or from any other sources except when due reference or acknowledgment, is made explicitly in the text, nor has been any part been written for me by another person.

____________________   ____________________

Date submitted   NOOR MUHAZAH BINTI MAZLAN

Supervisor's declaration:

I, PROF. DR. ZAINI BIN ASSIM, hereby certify the work entitled, FATTY ACID COMPOSITION AND ANTIOXIDANT PROPERTIES OF SEED OIL FROM *Canarium* spp. was prepared by the above named student, and was submitted to the "FACULTY OF SCIENCE AND TECHNOLOGY" as a full fulfillment for the conferment of BACHELOR OF SCIENCE WITH HONOURS (RESOURCE CHEMISTRY), the aforementioned work, to the best of my knowledge, is the said student's work.

Received for examination by: ____________________   ____________________

PROF DR ZAINI BIN ASSIM   Date

I declare this Project/Thesis is classified as (Please tick (√)):

( ) CONFIDENTIAL (Contains confidential information under the Official Secret Act 1972)*****

( ) RESTRICTED (Contains restricted information as specified by the organization where research was done)

( ) OPEN ACCESS

Validation of Project/Thesis

I therefore duly affirmed with free consent and willingness declared that this said Project/Thesis shall be placed officially in the Centre for Academic Information Services with the abide interest and rights as follows:

- This Project/Thesis is the sole legal property of Universiti Malaysia Sarawak (UNIMAS).
- The Centre for Academic Information Services has the lawful right to make copies for the purpose of academic and research only and not for other purpose.
- The Centre for Academic Information Services has the lawful right to digitise the content to for the Local Content Database.
- The Centre for Academic Information Services has the lawful right to make copies of the Project/Thesis for academic exchange between Higher Learning Institute.
- No dispute or any claim shall arise from the student himself / herself neither third party on this Project/Thesis once it becomes sole property of UNIMAS.
- This Project/Thesis or any material, data and information related to it shall not be distributed, published or disclosed to any party by the student except with UNIMAS permission.

Student's signature: ___________________ Supervisor's signature: _________________

( ) ( )

Current Address:

NO. 540, JALAN RAJAWALI, TAMAN TG. HARMONI, TG. AGAS, 84000 MUAR, JOHOR

TABLE OF CONTENT

LIST OF ABBREVIATIONS

BHA	: Butylated hydroxyanisole
BHT	: Butylated hydrotoluene
DNA	: Deoxyribonucleic acid
FDA	: Food and Drug Administration
GC-FID	: Gas chromatography-flame ionization detector
HDL	: High density lipoprotein
ICP-OES	: Inductively-coupled plasma atomic emission spectrocopy
LDL	: Low density lipoprotein
MUFAs	: Monounsaturated fatty acids
PUFAs	: Polyunsaturated fatty acids
ROS	: Reactive oxygen species
SFA	: Saturated fatty acid
UPS	: Underutilized plant species
UV	: Ultraviolet
WHO	: World Health Organisation

LIST OF TABLES

LIST OF FIGURES

Fatty Acid Composition and Antioxidant Properties of Seed Oil from *Canarium* spp.

Noor Muhazah Binti Mazlan

Resource Chemistry Programme
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak

ABSTRACT

Seed from *Canarium odontophyllum* and *C. megalanthum* were analyzed for proximate composition, mineral content, fatty acid compositions and antioxidant properties. Results obtained showed the percentage moisture contents as 12.84% and 3.17%, ash as 4.05% and 2.65%, organic matter as 95.56% and 97.35% and fat content as 50.03% and 79.10% for *C. odontophyllum* and *C. megalanthum*, respectively. K, Mg, Ca and Na were analyzed as the macrominerals, Mn, Zn, Fe and Cu were identified as a microminerals and Pb and Cd as toxic minerals. The fatty acid compositions in both seed oil were analyzed using gas chromatography-flame ionization detector and each component was identified based on fatty acid methyl ester (FAME) content. The fatty acid compositions in seed oil from *C. odontophyllum* and *C. megalanthum* was found to be 66.80% and 72.44% of saturated fatty acid, 32.04% and 27.58% monounsaturated fatty acid and 0.97% and 0.00% polyunsaturated fatty acid respectively. The antioxidant activity was assessed by 1, 1-diphenyl-2-picrylhydrazil (DPPH) scavenging activity assay and show that both seed oil from *Canarium* spp. has lower scavenging activity compared to L-ascorbic acid.

Keywords: *Canarium odontophyllum*, *C. megalanthum*, proximate, mineral, fatty acids, fatty acid methyl ester (FAME), antioxidant activity, oxidative stability

ABSTRAK

Biji daripada *Canarium odontophyllum* dan *C. megalanthum* telah dianalisis untuk komposisi proksimat, kandungan mineral, komposisi asid lemak dan sifat antioksidan. Peratusan keputusan menunjukkan kandungan kelembapan 12.84% dan 3.17%, abu 4.05% dan 2.65%, bahan organik 95.56% dan 97.35% dan kandungan lemak 50.03% dan 79.10% dalam *C. odontophyllum* dan *C. megalanthum*. K, Mg, Ca dan Na dianalisis sebagai mineral makro, Mn, Zn, Fe dan Cu telah dikenal pasti sebagai mineral mikro dan Pb dan Cd sebagai mineral bertoksik. Komposisi asid lemak dalam kedua-dua minyak biji dianalisis menggunakan gas kromatografi pengesan nyalaan dan setiap komponen dalam minyak biji telah dikenal pasti berdasarkan kandungan asid lemak metil ester. Komposisi asid lemak dalam minyak biji daripada *C. odontophyllum* dan *C. megalanthum* didapati 66.80% dan 72.44% daripada asid lemak tepu, 32.04% dan 27.58% asid lemak monotidaktepu dan 0.97% dan 0.00% asid lemak politidaktepu. Aktiviti antioksidan ditentukan dengan assel 1, 1-Diphenyl-2-picrylhydrazil aktiviti memerangkap dan menunjukkan kedua-dua minyak biji daripada spesies *Canarium* adalah rendah berbanding acid askorbik.

Kata kunci*: Canarium odontophyllum, C. megalanthum*, proksimat, mineral, asid lemak, asid lemak metil ester, sifat antioksidan, kestabilan oksidatif

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Fruits are very crucial in our life as they provide essential vitamins, dietary antioxidants, fibre and other beneficial effects on human health. Experts recommend that at least 5.7 g of fruits need to be consumed in daily diet in addition to vegetables, milk, pulse and cereals (Gordon, 1999). Since fruits are vital to maintain human health, nutritionists realize that fruits are a necessity instead of luxury (Kocchar, 1981). Consumers who pose greater health problems and high purchasing power are very keen to take part in any initiative to stay healthy (Jimenez-Colmenero *et al.*, 2001)

Malaysia has a wide diversity of underutilized fruits which can be found in the regions of Peninsular Malaysia, Sabah and Sarawak (Umi Kalsum and Mirfat, 2014). There are species that are only used in small communities where they are found, so, little attention has been given to them and they are underutilized. Azlan *et al*. (2009) stated that the issues of underutilized plant species (UPS) has been debated in academic, national and international circles mainly in research area. UPS is defined as species that are lack in terms of utilization, consumption and production, and not fully important to contribute to the national economy (Lawrence *et al*., 2007). However, according Azlan *et al.* (2009), UPS has potential in medicine, industry and agriculture and not only functioning as food source to minimize poverty and. Globally, more than 7000 species are cultivated and harvested from the wild (Owalarafe *et al*., 2007; Rafiee *et al*., 2006). Those species are there for some purposes and contribute significantly to nutrition, household income, food security and agro-biodiversity (Azlan *et al*., 2009). However, gradually, some of these underutilized plant species are being endangered, due to some main causes which are mining activities, displacement by improved plant, infrastructure development, overgrazing and bushfires, including lack in technology, science, research and development (Lawrence *et al*., 2007).

*Canarium i*s a large genus in the family of *Burseraceae*, comprising about 75 species distributed throughout Pacific Islands, tropical Asia and Asia (Evans, 1992). The fruits are harvested by the local farmers and sold in their market. Several studies have shown plant oil is a good source of dietary lipid because it provides lower level of saturated fat and also important dietary antioxidants like phenolic compounds and vitamin E (Foster *et al*., 2009). Processed plant oils are often used in salad dressing, frying, recipes, production of spreads and margarine (Brown, 2008).

However, there is still lack of published data regarding fatty acid composition, antioxidant properties of seed oil from *Canarium* species. Therefore, in this study, fatty acid composition, antioxidant properties and oxidative stability of seed oil from *Canarium* species will be determined. The new findings from this study are crucial to evaluate the potential of *Canarium* species as a new source of edible oil.

1.2 Problem Statement

Vegetable oils are broadly used in pharmaceutical, chemical industries, cooking and alimentary and cosmetic and previous year's show that their consumption has been increasing due to their cholesterol reducing effect, thus preventing against cardiovascular pathologies (Dugo *et al*., 2007). With time, today's dietary trend has changed from animal fat to vegetables including countries where people conventionally consume fat mainly from animal in order to have healthier lifestyles (Tuberoso *et al*., 2007). Because of the increasing demand of vegetable oil, research activities have been focused on investigating and characterizing new sources of edible oils from less familiar plant resources. In Malaysia, there are abundant of underutilized plant species which can be source of nutrients has not been extensively. They are characterized by the fact that they are locally rich but globally rare, and the scientific information and knowledge about them is limited (Gruere *et al*., 2009). For instance, little attention has been paid to extract oil from the seed especially the wild fruits like *Canarium.* Previous studied by Azlan *et al*. (2010a) reported that dabai kernel and pulp oils have good fatty acid composition and a great potential to be developed into healthy cooking oils.

1.3 Objectives of the Study

The objectives of this project were as following:

a. To evaluate the proximate data such as moisture, ash content, organic matter and fat content from seed of *Canarium* spp.

b. To study the mineral composition of seed oil from *C. odontophyllum* and *C. megalanthum*.

c. To determine fatty acid composition of seed oil from several species of *Canarium.*

d. To assess the anti-oxidant activities of seed oil from several species of *Canarium.*

CHAPTER 2

LITERATURE REVIEWS

2.1 Seed oil

Seed oil have been part of the diet for a long time and their manufacturing has shown significant increase in the last few decades due to the increasing use of edible fats as vegetable oil and their products of transformations such as shortenings, margarine and functional foods (Tuberoso *et al*., 2007). Edible oil is a vital nutrient and an essential source of energy providing 9 kcal/g and oil in the diet are available to the body as fatty acids, which are great sources of dietary calorie intake (Chowdhury *et al.*, 2007). Oils from plant seeds, called vegetable oil, are mostly edible and used in food preparation. These seed are generally produced on annually plants (crops) or large trees such as olive, coconut, olive and others (Nasiruddin *et al.*, 2012). Oil seed meals are high in protein which most with over 40% (Young, 1982). They also contain about 10% carbohydrates and some fat (1-6%) depending on method used for extraction and oil seed (Nasiruddin *et al*., 2012).

Oilseeds and the products made from them, mostly vegetable oils and spreads, have a role in a healthy balanced diet even though they contain high proportion of fat and energy dense (McKevith, 2002). Oils contain higher amount of unsaturated fatty acids while solid fats contain more saturated fatty acids which increased the low density lipoprotein (LDL) level of the blood, which is considered unsafe for human health (Lucas, 2000). Thus, vegetable oil is the preferable choice in food preparation.

2.2 Genus of *Canarium*

Lemmens *et al.* (1995) reported 16 genera and about 550 altogether species under Burseraceae, occurring naturally in the subtropics and tropics. In Borneo, there are 8 genera consist about 59 species such as *Trioma* sp. (1 sp.), *Protium* sp. (1 sp.), *Scutinanthe* sp. (1 sp.), *Garuga* sp. (1 sp.), *Santiria* spp. (16 sp.), *Haplolobus* spp. (6sp.), *Canarium* spp. (27 sp.) and *Dacryodes* spp. (12 sp.). *Canarium* has the largest number of species under Burceraceae family. The main centre of diversity lies in the Malaysia area where most species occur in the moister parts, hence in Sumatra, Peninsular Malaysia and New Guinea in the east and Borneo in the west (Sabah Forestry Department, 2008).

Sabah Forestry Department (2008) reported that *Canarium* are commonly found in the lowland mixed dipterocarp forest but rarely in submontane forest, rocky coast and kerangas forests. They can occur up to 1800 m altitude, on alluvium, clay-rich and friable fertile soils, and yellow sandy soils and particularly on basic volcanic rocks. Besides, *Canarium* wood is light-weight to medium-weight and moderately soft to moderately hard.

Some of the fruits are edible and popularly consumed for many purposes such as *C. littorale* (its kernel is edible), *C. megalanthum* (seeds eaten raw or roasted after removal), *C. pilosum* (seeds are sweet and edible, resins used to close wounds), *C. patentinervium* (seeds edible, resins used to close wound) (Sabah Forestry Department, 2008). Koudou *at al*. (2005) reported that the essential oil of *Canarium* species can be used as painkiller. The resin of "Manila-elemi" (*C. luzonicum*) is used in ointments and plasters in pharmacy and also as a constituent of cellulose lacquers (Burkill, 1966).

2.3 *Canarium odontophyllum*

The tropical rain forest in Sarawak has rich source of *C. odontophyllum* or commonly known as 'dabai' or 'Borneo olives' which has purple skin colour and have a thin, edible skin. The fruits are oblong in shape with 3 to 4 cm in length and 10 to13 g in weigh (Prasad, 2010). The fruit is indigenous to Sarawak and can be found along the riverbank in Sarikei, Sibu and Kapit divisions (Azlan *et al*., 2009).

C. odontophyllum has female and male flowers borne on different trees and the flesh is either yellow or white which covers a large three-angled seed (Umi Kalsum and Mirfat, 2014). The fruit is delicious with a unique flavor and oily texture just like an avocado (Dayang *et al*., 2012). Recently, the Agriculture Department of Sarawak has promoted 'dabai' as a specialty fruit due to the nutritional quality of the fruit (Kunding, 2007). The department believes that the fruits can be internationally marketed after discovering a breakthrough in the method to extend its shelf life (Jackson, 2008).

The dabai tree is tall, medium-sized, and with a straight trunk which only starts to branch at a height about 2-3 m above ground (Kueh, 2003). According to Yahia (2011), the tree grows more than 20 m tall with a stem girth of more than 150 cm and its bark is grey-brown. The crown is rounded and dense. The leaves are spirally arranged, 3-8 pairs of leaflets, pinnate with a terminal leaflet. Leaflets are oblong to lanceolate with 9.5-28 cm in length and 4-11 cm in width. Kocchumen (1995) reported that *C. odontophyllum* flowers in May, June and November and fruits in March and August. Dabai is highly nutritious as it contain carbohydrate (22.17%), protein (3.8%), energy (339 kcal) and fat (26.2%) (Voon and Kueh, 1999). Shakirin *et al*. (2010) reported that the skin is major source of antioxidants.

2.4 *Canarium megalanthum*

According to Slik (2009), the tropical plant of *C. megalanthum* also commonly known as Kedondong Keruing, Baab, Kamatoa, Mantus, Meritus, Ngaling, Ngela and Rarawa dammar. It is distributed in Peninsular Malaysia, Sumatra, Brunei and Borneo. In Brunei, this species is cultivated for its edible seeds which are among the largest of this genus.

Philip (1926) stated that the tree is large up to 40 m high without buttresses and its trunk can be 60-70 cm across. The small branches are 0.5-1 cm thick. They have rusty hairs. The leafy structures (stipules) on the leaf stalks are 0.5 cm from the base and deeply 3-4 lobed. The leaves have 4-5 pairs of leaflets. The leaflets are oval or long and 9-20 cm long by 4-8 cm wide. They are leathery and the base is wedge shaped while the tip tapers to a point. There are 13-19 pairs of faint slightly curved veins. The flower clusters are near the ends of branches. These are 25 cm long for female clusters and 25-30 cm long for male flower clusters. Male clusters have about 10 flowers while female clusters have few flowers. The fruit clusters are 25 cm long and with 4-5 fruit. The fruit are oval and triangular in cross section. The fruit can be 5 cm long while the seed are thin, long and 1.5 cm wide. *C. megalanthum* is reported to flower in May and fruits in May and September (Kocchumen, 1995).

2.5 Fatty Acids

Fatty acids represent 30–35% of total energy intake in many industrial countries and the most vital dietary sources of fatty acids are dairy products, vegetable oils, grain, meat products and fish oils or fatty fish (Rustan and Devron, 2005). Fatty acids are carbon chains with a methyl group at one end of the molecule and a carboxyl group at the other end (Figure. 2.1). The carbon atom next to the carboxyl group is called the α carbon, and the subsequent one the ß carbon. The letter n is also often used instead of the Greek ω to indicate the position of the double bond closest to the methyl end. There are two types of fatty acids which are saturated fat and unsaturated fat. Saturated fat has no double bond in the structure while unsaturated bond contain one or more double bond. Saturated fat commonly found in butter and margarine while unsaturated fat can be found in olive oil and sunflower oil. The main constituent of vegetable oils and animal fat is triglyceride which is also called as triacylglycerol. It is a chemical compound formed from three fatty acids and one molecule of glycerol. Triglycerides have low density compared to water and also it can either be solid or liquid at room temperature. When solid, they are called “fats” while when liquid, they are called “oil”.

$$\underset{\omega}{CH_3} — (CH_2)_n — \underset{\beta}{CH_2} — \underset{\alpha}{CH_2} — COOH$$

Figure 2.1: Structure of fatty acid (Rustan and Drevon, 2005)

According to Rustan and Devron (2005), most saturated fatty acids have straight hydrocarbon chains with an even number of carbon atoms and most common fatty acids contain 12 to 22 carbon atoms. Unsaturated fatty acid consist of two types which are monounsaturated and polyunsaturated. MUFAs have one carbon–carbon double bond, which can occur in different positions while in PUFAs the first double bond may be found between the third and the fourth carbon atom from the ω carbon and it is called ω-3 fatty acids. If the first double bond is between the sixth and seventh carbon atom and they are called ω-6 fatty acids. The methylene grouping separates the double bonds in PUFAs from each other. There are several important fatty acids as presented in Table. 2.1. Essential fatty acid such as alpha-linolenic acid (omega-3 fatty acids) and linolenic acid (omega-6 fatty acids) is a long chain PUFAs are important for cellular function and only be consumed through diet. According to Azlan *et al*. (2010b), the plant oil with its fatty acids constituents with the minor components are accountable for its functionality and health promoting properties.

Table 2.1: Several important fatty acids (Eğe, 2004)

Name	Carbons	Structure	Melting point (ºC)
Saturated			
Palmitic	16	$CH_3(CH_2)_{14}COOH$	62.9
Stearic	18	$CH_3(CH_2)_{16}COOH$	69.6
Unsaturated			
Oleic acid	18	$CH_3(CH_2)_7CH= CH(CH_2)_7COOH$ (cis)	13
Linoleic acid	18	$CH_3(CH_2)_4CH=CHCH_2CH =CH(CH_2)_7COOH$ (cis, cis)	-5
Linoleic acid	18	$CH_3(CH_2)_4CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7COOH$ (cis, cis)	-16
Arachnidonic acid	20	CH3(CH2)4(CH=CHCH2)4CH2CH2COOH (all cis)	-50

2.6 Antioxidant Properties

Reactive oxygen species (ROS) are free radicals that able to also cause damage to DNA, heart disease, carcinogenesis and various health problems led to advancing age (Cadenas and Davies, 2000; Marnett, 2000; Uchida, 2000). Antioxidant is a chemical that interact with and neutralize free radicals and prevent them from causing harm. According to its action mode, antioxidants can be categorized as chelators of metallions, free radical terminators or oxygen scavengers in which react with oxygen in closed system (Shahidi and Naczk, 2004). Antioxidant can be gained through endogenous or exogenous. Endogenous is antioxidant that produced naturally by human body while exogenous antioxidant is consumed through diet like fruits, vegetables and others. Fruits, vegetables and medicinal herbs are the richest sources of antioxidant compounds (Sies *et al*., 1992).

The antioxidants are essential for food additives either in naturally or synthetic occurring forms (Lim and Murtijaya, 2007). Synthetic antioxidant such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been banned in foods because they are carcinogenic (Madavi and Salunkhe, 1995). Thus, the synthetic antioxidants should be replaced with natural antioxidants (Madsen and Bertelsen, 1995). Natural antioxidants are safer and posses anti-inflammatory, anti-viral, anti-tumor, anti-cancer, anti-mutagenic and hepatoprotective properties (Lim and Murtijaya, 2007). Therefore, natural antioxidants such as vitamin C, Vitamin E, lycopene and beta carotene from plants and vegetables are preferable. Consumption of vegetables and fruits has been shown to lower the risk and incidence of certain types of cancer, cardiovascular diseases, diabetes and ability to act as anti-Alzheimer’s agents (Siti Hawa *et al.*, 2013). In addition, the search for natural antioxidants from plants has greatly increased in recent years (Loliger, 1991). Thus, underutilized plant can be evaluated as a new source of natural antioxidants.

CHAPTER 3

MATERIALS AND METHODS

3.1 Sample Collection and Preparation

The fruits of *Canarium* used in this study were collected from various places around in Sarawak. Prior to analysis, the flesh and seed of the fruits were separated using a knife. Since the kernel was enclosed by hard shell, seed was cracked in order to obtain the kernel. The size of kernel was reduced using high speed blender and sieved.

3.2 Chemical composition Analysis

3.2.1 Proximate Composition Analysis

Moisture content was determined according to method described by Karl-Fischer (AOAC, 1990). In this method, a clean crucible was dried in an oven at 80ºC for 30 min, cooled in a desiccator and weighed (W_1). About 4 g of sieved *Canarium* fruit powder was poured into the weighed crucible and accurately reweighed (W_2). The sample and the crucible were dried in an oven at 105ºC for 14 hours. It was quickly transferred to a desiccator to cool and then reweighed again with minimum exposure to atmosphere. The procedure of drying for 3 h was repeated for each subsequent drying, until constant weight (W_3) was obtained. Triplicate determination on the sample were carried out and calculated as:

$$\text{Moisture (\%)} = \frac{\text{loss in weight due to drying}}{\text{weight of sample taken}} \times 100 \qquad \text{Equation 3.1}$$