DETERMINATION OF TOXICITY AND ANTIOXIDANTS ON WHITE EDIBLE BIRD NEST (*Aerodramus fuciphagus*) FROM DIFFERENT DISTRICT IN KELANTAN

ANISH AMEERA SHAHERAN

UNIVERSITI SAINS MALAYSIA

2018

DETERMINATION OF TOXICITY AND ANTIOXIDANTS ON WHITE EDIBLE BIRD NEST (*Aerodramus fuciphagus*) FROM DIFFERENT DISTRICT IN KELANTAN

by

ANISH AMEERA BINTI SHAHERAN

Thesis submitted in fulfilment of the requirements
for the degree of
Master of Science (Biomedicine) Mixed Mode

January 2018

ACKNOWLEDGEMENTS

In the name of Allah S.W.T, The Most Gracious and The Most Merciful

Assalamualaikum w.b.t

Alhamdulillah, I am thankful to Allah for his blessings and mercy because I have managed to complete this thesis and fulfilled the entire requirements within the timeframe allocated. It is undeniable that it was quite difficult and challenging for me to start writing this thesis at the beginning but Allah's will, I have successfully completed it. This thesis has been a result for contributions from various people who deserve special acknowledgements. Therefore, I would like to express my gratitude to those who had helped me for this completion of this study. First, special gratitude to my beloved supervisor, Dr. Mohd Dasuki Sul'ain for giving me valuable guidance, infinite support, thoughtful comments assistance throughout my research studies. It has been an honour to be under his supervision and this study will not be successful without his continuous guidance. My special thanks to all the Biomedicine's Lecturers for their kindness, time, advice and involvement throughout my study. Special thanks also rewarded to all the laboratory assistants and staff of School of Health Sciences. Special gratitude is conveyed to my laboratory partner, Nik Amalia Nasratiena Mat Zaib who had helped throughout this research studies. Last but not least, my grateful thanks to my beloved family and friends for their endless support and care throughout my studies in this Master of Science (Biomedicine).

Thank you.

Anish Ameera Shaheran

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABRREVIATIONS AND SYMBOLS

%	Percentage
µg/mL	Microgram per milliliter
µL	Microliter
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
ATCC	American Type Culture Collection
BSLA	Brine shrimp lethality assay
CO_2	Carbon dioxide
DMEM	Dulbecco's Modified Eagle Media
DMSO	Dimethyl sulfoxide
DPPH	2.2-diphenyl-1-picrylhydrazyl
EBN	Edible bird nest
EDTA	Ethylenediaminetetraacetic acid
FBS	Fetal bovine serum
FDA	Food and Drug Administration
FRAP	Ferric Reducing Antioxidant Power
g	Gram
GC-MS	Gas Chromatography Mass Spectrometry
HCl	Hydrochloric acid
IARC	International Agency for Research on Cancer
IC_{50}	50% inhibition concentration
ICP-MS	Inductively coupled plasma mass spectrometry
kg	Kilogram
L	Liter
LC_{50}	50% lethal concentration
LC-MS	Liquid Chromatography Mass Spectrometry
mL	Milliliter
mM	Millimolar
mM/L	Millimolar per litre

MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NaOH	Sodium hydroxide
NCI	National Cancer Institute
NCR	National Cancer Registry
ºC	Degree celcius
ORAC	Oxygen Radical Absorbance Capacity
PBS	Phosphate buffer saline
ppb	Part per billion
ppm	Part per million
SIRIM	Scientific and Industrial Research Institute of Malaysia
WHO	World Health Organization

PENENTUAN KETOKSIKAN DAN ANTIOKSIDAN PADA SARANG BURUNG PUTIH YANG BOLEH DIMAKAN (*Aerodramus fuciphagus*) DARI DAERAH YANG BERLAINAN DI KELANTAN

ABSTRAK

Sarang burung yang boleh dimakan (EBN) adalah salah satu produk semulajadi yang mempunyai terapeutik potensi yang tinggi dan sifat farmakologi. Oleh itu, kajian ini bertujuan untuk menentukan aktiviti ketoksikan dan antioksidan pada sarang burung putih yang boleh dimakan (*Aerodramus fuciphagus*) dari tiga daerah (Tanah Merah, Tumpat dan Jeli) di Kelantan. Sampel diekstrak menggunakan tiga pelarut iaitu air suling, natrium hidroksida dan asid hidroklorik. Ketoksikan sampel EBN dengan pelarut yang berbeza telah diuji menggunakan ujian MTT terhadap kanser (HeLa dan MCF-7) dan sel bukan kanser (SVG p12). Kandungan galian dalam EBN telah dikaji menggunakan spektrometri jisim plasma yang digabungkan secara induktif (ICP-MS) dan ujian ketoksikan yang lain adalah ujian kematian udang air garam (BSLA) untuk menentukan nilai LC_{50}. Aktiviti antioksidan diuji pada ekstrak sampel EBN menggunakan pengujian pemotongan radikal DPPH dan ujian pengurangan kuasa besi (FRAP). Ketiga-tiga ekstrak sampel EBN menunjukkan aktiviti perencatan yang lebih tinggi terhadap sel MCF-7. Sementara itu, ekstrak sampel EBN menunjukkan percambahan sel induksi pada sel SVG p12 kerana nilai IC_{50} lebih daripada 100%. Kandungan galian dalam semua sampel EBN adalah di bawah pengawalseliaan maksimum seperti yang dicadangkan oleh garis panduan FDA, WHO dan SIRIM kecuali kandungan natrium yang sedikit tinggi daripada 2 ppm. Keputusan menunjukkan tiada nilai LC_{50} yang ditentukan pada EBN terhadap udang air garam. Aktiviti antioksidan yang menggunakan DPPH dan FRAP assay menunjukkan bahawa ekstrak NaOH dari tiga sampel EBN mempunyai nilai IC_{50} terendah dan kuasa

pengurangan tertinggi. Oleh itu, penemuan hasil menunjukkan bahawa sampel EBN ekstrak boleh dianggap sebagai agen pencegahan kanser dan selamat untuk dimakan. Ekstrak sampel EBN adalah boleh dianggap sebagai agen antioksidan. Walau bagaimanapun, pengesahan selanjutnya perlu dilakukan untuk memahami sebatian aktif yang memberi kesan terhadap keselamatan, ketoksikan dan antioksidan EBN.

DETERMINATION OF TOXICITY AND ANTIOXIDANTS ON WHITE EDIBLE BIRD NEST (*Aerodramus fuciphagus*) FROM DIFFERENT DISTRICT IN KELANTAN

ABSTRACT

Edible bird nest (EBN) is one of the natural products that have high therapeutic potential and pharmacological properties. Therefore, this study aimed to determine the toxicity and antioxidants activity on white edible bird nest (*Aerodramus fuciphagus*) from different district in Kelantan (Tanah Merah, Tumpat and Jeli). The samples were extracted using three solvents which are distilled water, sodium hydroxide and hydrochloric acid. The toxicity of the EBN samples with different solvent of extraction were tested using MTT assay against cancerous (HeLa and MCF-7) and non-cancerous cell line (SVG p12). Minerals content in EBN was studied using inductively couple plasma mass spectrometry (ICP-MS) and the other toxicity test was brine shrimp lethality assay (BSLA) for LC_{50} values. The antioxidants activity was tested on EBN samples extracts using DPPH radical scavenging assay and Ferric reducing power assay (FRAP). EBN samples extracts showed higher inhibition activity against MCF-7 cell line. Meanwhile, three samples of EBN extracts induced cell proliferation on SVG cell line as the IC_{50} value more than 100%. Minerals content in all EBN samples were below the maximum regulatory limit as suggested by FDA, WHO and SIRIM guidelines except sodium content that slightly higher than 2 ppm. The results showed no LC_{50} value was determine on EBN against brine shrimp. The antioxidants activity using DPPH and FRAP assay showed that NaOH extracts from three sample EBN had the lowest IC_{50} values and the highest reducing power. Thus, the results finding showed that the EBN samples extracts can be considered as cancer-preventive agents and are relatively safe to be consumed. Extracts of EBN samples can be considered as

antioxidants agent. However, further validation have to be performed to understand the active compounds that poses safety, toxicity and antioxidants activity of EBN.

CHAPTER 1

INTRODUCTION

1.1 Background study

Edible bird nest (EBN) refers to the nest built from the swiftlet's saliva which usually found in Malaysia. The size of swiftlet is about that of a sparrow and wingspan wider than that of a pigeon around 9 to 12 cm. There are a few different swiftlet species produces edible bird nest. They are in the genus *Aerodramus* and *Collocalia* (Liu *et al*., 2012). There are two main species of *Aerodramus* which are *Aerodramus fuciphagus* (white nest) and *Aerodramus maximus* (black nest). The *A.fuciphagus* species structured their nest from the dried saliva which is secreted by the swiftlet's two sublingual glands. Meanwhile, the *A.maximus* species structured their nest from the saliva simultaneously with the birds' feathers (Nakagawa *et al.,* 2007).

The weights of swiftlet's sublingual salivary glands increase from time to time and then reach their top level secretory activity during nesting and breeding season (Medway, 1962). The swiftlet may take about 35 days of the construction process (Marcone, 2005). The distribution of swiftlet's house will be influenced by the process of harvesting the edible bird's nest, elevation above the ground or water bed, and other interrelated factors.

EBN consists of nutrients such as carbohydrates, high valued of glycoproteins and rich with calcium, sodium, amino acids and potassium (Zainab *et al.,* 2013). Based on research done by Marcone (2005), the nutrients composition of EBN from the lowest to the highest from the genus *Aerodramus* includes lipid (0.14-1.28%), ash (2.1%),

carbohydrates (25.62-27.26%) and protein (62-63%). EBN has been proven to have a number of health promoting qualities and rich in carbohydrate, amino acid and mineral salts (Zainab *et al.,* 2013). There are many EBN's consumer whose do not know or lacking information about the safety and toxicity of EBN even though there are a few researchers had studied about the nutritional contents of EBN. Thus, the further investigation has to be performed on EBN to detect or identify the toxic elements that would be hazard to human's life.

Recently, people or usually industrial workers are easily exposed to many pollutions such as air pollution, chemicals in beauty products as well as in unhealthy food preservatives and also carcinogen. This situation will lead to various deadly diseases such as the formation of cancer. Thus, researcher and scientist were preferred the natural products such as medicinal plants which are rich in antioxidants properties that could lower the various incidence of human diseases. Natural products such as EBN could be an alternative for synthetic antioxidants as they give high beneficial effects to human health (Zainab *et al*., 2013).

A past few decades, traditional people consumed EBN products for health, power and prestige. It is also incorporated as a medication for malnutrition, stimulation of the immune system as well as to increase the body's metabolism. EBN in Chinese community was considered as super meals and potent medicine as well as inner and outer of beauty enhancer (Yeap, 2002). According to Yeap (2002), it was recorded the earliest legend of EBN trading can be detected in the year 1589. In recent times, the bird nests have been used as a component of cosmetic products in the market worldwide (Zainab *et al.,* 2013).

Therefore, this research study was conducted to determine the toxicity and antioxidants of white edible bird nest (*A.fuciphagus*) from different district in Kelantan. Moreover, there are three different solvents used in this study. Thus, this study is being conducted to determine the toxicity between EBN samples from different district in Kelantan as well as their antioxidant properties.

1.2 Problem statement

There has been a large demand in studying toxicity and antioxidants of natural products due to their health promoting properties. Currently, natural products are widely used as medicinal purposes and they have been evaluated for their therapeutic potential and their pharmacological properties like toxicity and antioxidants effects (Lai *et al.*, 2010).

The swiftlets are insectivores and usually they feed at places that have abundant of insects or plants (Langham, 1980). Plants have known to take up minerals from the soil (Tangahu *et al*., 2011), when the swiftlets fed the plants, the traces elements could possibly transfer to their salivary product which after that will be consumed by humans in the form of soup. The construction of bird house is another source of heavy metal contamination in EBN such as rusty iron bars, lead-based paints or mercury tainted water supply (Chen *et al*., 2014). Moreover, there was one case reported in Vietnam, the patient was affected organic arsenic intoxication after consumption of EBN soup (Luong and Nguyen, 1999). These sources of toxicity could be toxic to the nests as well as hazards to the humans. EBN has highly demand from the other countries such as China, Vietnam and Hong Kong thus, the sellers that exported the EBN will used preservatives such as potassium nitrile or

sodium nitrile to maintain the quality of EBN. These common preservatives are usually used in foods but the excess consumption of these chemicals may result in cancer (Ward *et al.*, 2000). Thus, South East Asia countries banned from importing the EBN to protect consumers in China (Ramli & Azmi, 2012). In addition, there was questionable when there were untested claims made regarding the benefits of EBN effects on patients suffering from cancer (Lim & Cranbrook, 2002). Thus, it is important to determine the level of toxicity of the EBN samples in order to evaluate whether it is safe to be consumed. The test could be accessed on toxicity of EBN samples through cytotoxicity evaluation (MTT assay), mineral analysis and also brine shrimp lethality assay.

The antioxidants properties of any natural products have been widely studied as they could bring benefit especially in minimizing free radicals that can promote damage to biomolecules such as proteins and lipids. Therefore, antioxidants play the important role against oxidative stress diseases such as cancer, diabetes, skin damaged and aging. In this study, the EBN samples were originally harvested from different district in Kelantan, Malaysia to determine the antioxidants activity. The different district samples had chosen in this study because it has not been studied yet. According to Guo and co-workers (2003), the antioxidant activity could be influenced by the geographical origin, cultivar and harvest storage time. This showed that different EBN samples could have different antioxidant properties. In addition, there are not intensively studied on type of solvent extraction in the investigation of toxicity and antioxidant activities of EBN samples. Therefore, this study aimed to determine the toxicity and antioxidants of white edible bird nest (*A.fuciphagus*) from different district in Kelantan.

1.3 Rationale of study

Nowadays, people are getting aware about the risk of taking modern drugs which give side effects towards health. Therefore, they prefer the alternative drugs or natural products to cure their diseases. Thus, by discovering new research study about potential medicinal activity in natural products such as EBN, it might be replaced the modern drugs, supplements for health or can included in synthetic drugs.

Even though the price of EBN are higher and difficult to attract the swiftlets to be nested in the bird house, the demand of EBN from the other countries such as China, Hong Kong and Taiwan are larger as they contained a lot of nutritional contents including carbohydrates, proteins and others which are important to human's health. Therefore, more scientific evidences are needed to substantiate the various health claims associated with its consumption.

However, EBN are yet well known among Malaysians if compared to the Chinese. The bird nests are not only a pleasant food to be consumed but also it is traditionally believed to improve health benefits to the consumers such as speed up digestion, alleviating asthma and stomach ulcer, promote growth and others. Furthermore, EBN also can improve skin complexion and slow the aging effect (Chan, 2006; Hobbs, 2004; Kong *et al.,* 1987).

Thus, this research study will be important to produce health products for consumers and also can give a guideline for EBN processors for the production of quality and safe EBN for human consumption. These studies also serve as a collection of database for future studies in Malaysia.

1.4 Objectives of the study

1.4.1 General objective

This study aims to determine the toxicity and antioxidants properties on white edible bird nest (*Aerodramus fuciphagus*) from different district (Tanah Merah, Tumpat and Jeli) in Kelantan.

1.4.2 Specific objectives

1. To determine the toxicity of three EBN samples (Tanah Merah, Tumpat, and Jeli) extracts using MTT assay and brine shrimp lethality assay (BSLA).
2. To identify the minerals content of three EBN samples (Tanah Merah, Tumpat, and Jeli) using inductively coupled plasma mass spectrometry (ICP-MS).
3. To determine the effect of acid-base extractability of antioxidant properties on three EBN samples (Tanah Merah, Tumpat, and Jeli) by using different methods, DPPH assay and FRAP assay.

CHAPTER 2

LITERATURE REVIEW

2.1 Cancer

Cancer is a disease which can lead to the destruction of tissues in the body because of the rapidly abnormal growth of the cells or cells grow out of control (Gennari *et al.*, 2007). In Greek, the term cancer known as “carcinoma” and “Karakinos” which describes as tumor. The term was first used by Hippocrates, father of western medicine (Nobili *et al.*, 2009).

One defining feature of cancer is the abnormal cells which rapidly grow without control and normally forms as a solid tumor and spreads to other organs. However, like leukemia cases they do not form as tumor. The leukemia cells involve the blood and circulate them through other tissues where they grow. There are two classification of cancer which are benign and malignant. Benign tumors are not cancerous and they do not grow or spread to other parts of the body as well as do not harm life. Meanwhile, malignant tumors are cancerous and they spread to other body parts through bloodstream or lymphatic system. They also can harm life if not treated early.

The early detection of cancer screening used was the Pap test. The test was developed by George Papanicolaou to understand on how the menstrual cycle begins and to identify the early detection of cervical cancer (Tan and Tatsumura, 2015). The development of mammography in 1960s was used for the identification of breast cancer. Afterward, the early detection of cervical cancer, breast cancer, colon cancer, skin cancer,

lymph nodes cancer, testes cancer and ovaries cancers were identified and practiced in clinic (Sudhakar, 2009).

According to the National Cancer Registry of Malaysia (NCR), about 21,773 Malaysians were diagnosed with cancer however there were almost 10,000 cases unregistered every year. The ratio was estimated that one in four Malaysians (1:4) will develop cancer by 75 years old. Mostly cancer occur in females than in males with a ratio of male to female 1:1.2. Table 2.1 showed the ten most common cancers in Malaysia. However, there are 5 top cancer affected by both males and females in Malaysia which are breast, colorectal (bowel), lung, lymphoma and nasopharyngeal. Figure 2.1 showed that the breast cancer was leading about (17.7%) cases, followed by colorectal cancer (13.2%), lung cancer (10.2%), lymphoma (5.2%) and nasopharynx cancer (4.9%). In Malaysia, it has been considered that cancer was the 3rd leading cause of premature death, however, it has been found that only 30-40% of all deaths consequence to cancer are medically certified (National Cancer Society Malaysia, 2015).

Table 2.1: Ten most common cancers in Malaysia (Adapted from: Malaysian National Cancer Registry, 2007-2011).

ICD-10	Sites	Number	%
C50	Breast	18,343	17.7
C18-C21	Colorectal	13,693	13.2
C33-C34	Trachea, Bronchus, Lung	10,608	10.2
C81-C85,C96	Lymphoma	5,374	5.2
C11	Nasopharynx	5,090	4.9
C91-C95	Leukaemia	4,573	4.4
C53	Cervix Uteri	4,352	4.2
C22	Liver	4,128	4.0
C56	Ovary	3,472	3.4
C16	Stomach	3,461	3.3
	Others	30,413	29.4
	Total	**103,507**	**100.0**

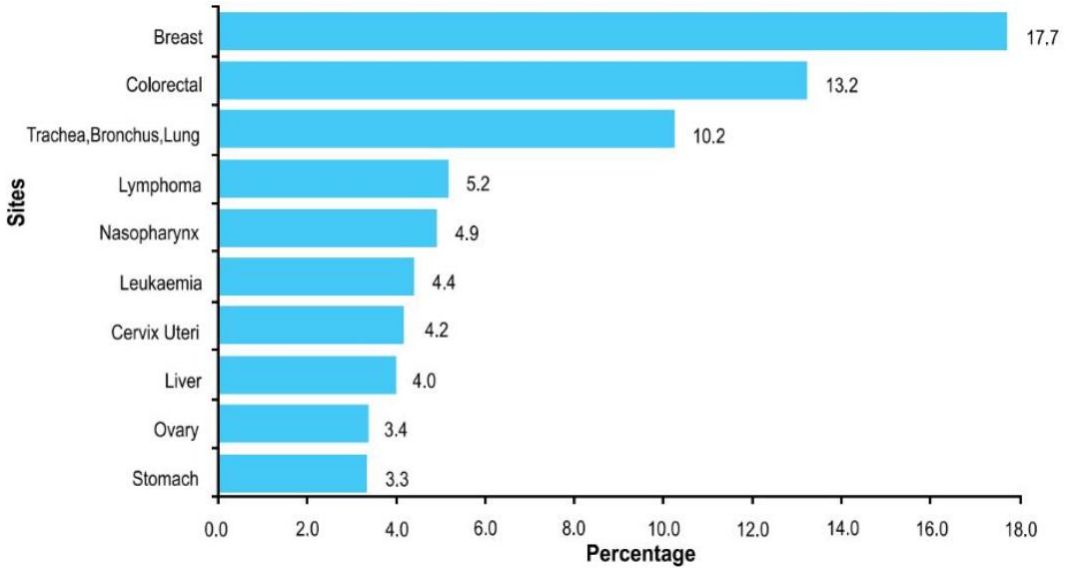

Figure 2.1: Percentage of ten most common cancers in Malaysia (Adapted from: Malaysian National Cancer Registry, 2007-2011).

The substances or chemicals can be toxic to living organisms as they have the abilities to give adverse effects. Toxic is a substance, compounds or any chemicals that can cause hazards to living organisms. However, the toxic levels depend on several conditions, for instance, the dose of the substances or chemicals can be determined whether it can be toxic, non-toxic or beneficial. Besides doses, other factors such as the routes of entry, frequency of exposure, variations of different species and variations among species may influence the toxicity in living organisms. There are four main routes of exposures that can enter the body including inhalation, absorption, ingestion and injection (Michael and Candace, 2015). These exposures will lead to cancer and slowly affected life as well as lead to death.

For example, nitrite and nitrate can be toxic as they are inorganic compounds that naturally present in the environment such as water and soil. Nitrite is widely used in food industry as food additives to prevent spoilage and also to maintain the color of meat such as bacon, ham, corned beef, hamburger and smoked fish, however nitrite contents is associated with cancers (Chan *et al*., 2013). The reaction of the nitrite with secondary amines and amides can affect human's health in the gastrointestinal tract and this cause the formation of carcinogenic N-nitrosamines which can lead to stomach cancer (Larsson *et al*., 2006; Butt *et al*., 2001; Wolff and Wasserman, 1972). According to Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2002, they suggested that the acceptable human daily intake amount of nitrite should not exceed 0.07 mg/kg of body weight (Chan *et al*., 2013). Nitrate also can be hazardous to health if taken in high portion because nitrate is a potential source of nitrite through bacterial or microbial reduction. However, nitrite is more toxic than nitrate because it can cause blood disorder and

breathing difficulty in human when its' intermediate with blood pigment to produce methemoglobinemia (Chan *et al*., 2013; Wolff and Wasserman, 1972).

There are many ways to prevent cancer from spreading rapidly. One of the main cause is smoking. There were nearly 80% of the 1 billion smokers in the world live in low and middle-income countries (World Health Organization, 2017). Tobacco is one of the largest cancer mortality and kills about 6 million people each year. There are 7000 chemicals and approximately 250 chemicals are known to be harmful and more than 50 chemicals can cause cancer. Different dietary habits, physical activity and lack of exercises are other important factors which can lead to cancer. These factors must be concerned and control in daily life as they can lower the risk of cancer. In addition, the increasing of consumption alcohol in 2010 onwards become worst and there was estimated about 337,400 deaths worldwide, predominantly among men (Stewart and Wild, 2014). The other factor which causes the cancer in 2012, is the healthy lifestyle or by the immunization causing by infections such as human papilloma virus (HPV), hepatitis B virus (HBV) and hepatitis C virus (HCV). Nowadays, high exposure to chemicals in working environment are very serious. The chemical exposure can be different in the workplace because the variety of occupations. For instance, the exposure comes from the pollution, carcinogenic and ionizing radiation. The most common cancer death is lung cancer and it was estimated about 3.2 million premature deaths occur in 2012. Although 40% of all cancers are preventable including lung, colorectal and cervical cancers, the improvement in early detection and treat adequately must be strengthen to help better survival rates for people with cancer (World Health Organisation, 2017; National Cancer Society Malaysia, 2015).

Cancer can be cured if they are detected at early stage by surgery, chemotherapy, hormonal therapy, and radiation therapy. Surgery is done by removing the entire tumors, however the cancer cells would usually come back and spread from primary tumor to other places through blood stream or lymphatic system. Surgery combine with chemotherapy and radiation therapy for cancer treatment give successful treatment result of many types of cancers for years. In addition, the hormonal therapy, immunotherapy and adjuvant therapy are another therapy that can be used for cancer treatment (Sudhakar 2009).

2.2 Antioxidant

Oxygen, or O_2, an element that are very essential for all living life and sometimes under certain circumstances can be harmful to the human body. Oxidation is crucial in many living organisms for the production of energy to fuel biological processes. Oxidation is a loss electron and act as oxidizing agent. It can cause another reactant to be oxidized and another substance that accepts electron. The uncontrolled production of oxygen derived free radicals that possess in the onset of many diseases such as cancer, rheumatid arthritis, artherosclerosis as well as in degenerative processes associated with ageing (Halliwell and Gutteridge, 1989). The free radicals can cause oxidative damage to lipids, proteins and nucleic acids.

Oxidative damage can be defined as a state of uncontrolled production and imbalanced formation of reactive oxygen species (ROS) which can result in many diseases and accelerate ageing (Vichitra *et al*., 2015; Bylka and Matlawaska, 2007). Examples of reactive oxygen species (ROS) are superoxide molecule, hydroxyl molecule, peroxyl and hydroperoxyl molecule as well as non-radical species such as hydrogen peroxide molecule and hydrochlorous acid (Bylka and Matlawaska, 2007). Furthermore, ROS that are overproduced can result in peroxidation of membrane lipids under pathological conditions or oxidative stress and can lead to the accumulation of lipid peroxides. Lipid peroxides could shorten the shelf life of foods and lead to deterioration where oxidation process occur (Wanasundara and Shahidi, 2005). However, this condition can be removed by antioxidant defense mechanisms (Vichitra *et al*., 2015).

The substances called antioxidants can protect the cells from damage by the unstable molecules known as free radicals. The antioxidants can interact with and stabilize the free radicals and at the same time prevent some of the damage free radicals might otherwise cause. They will trigger security against excessive free radicals by their preventive mechanisms, repair mechanisms, physical defenses and antioxidant defenses (Sen *et al.,* 2010). The effects of free radicals also may result in cancer disease. Whole grains, fruits and vegetables are the primary sources of naturally occurring antioxidants. Other antioxidants include beta-carotene, vitamins C, E, A, lycopene and other substances (Sies, 1997). Antioxidants are widely used as ingredients in dietary supplements in the hope of maintaining health and preventing disease such as cancer and coronary heart disease. Even though the previous studies suggested that antioxidants can promote health later, the clinical trials did not detect any benefit and that excess supplementation may be harmful (Bjelakovic *et al.,* 2007).

Other than these uses of natural antioxidants in medicine, these substances have many industrial uses such as preservation of food and cosmetics and preventing the degradation of rubber and gasoline. For many years, chemists have known that free radicals cause oxidation which can be controlled or prevented by a range of antioxidants substances (Bjelakovic *et al.*, 2007).

Antioxidants are important in human bodies as they act as defense system to neutralize the excessive levels of reactive oxygen species. Figure 2.2 shows the flowchart consists of enzymatic and non-enzymatic antioxidants. The antioxidant enzymes that provide a protection against ROS are superoxide dismutase, catalases and glutathione peroxidases meanwhile for the non-enzymatic small molecules such as glutathione,

tocopherol (vitamin E), vitamin C, β-carotene and selenium which are distributed widely in the biological system and capable of scavenging free radicals. In plant material, polyhydroxy flavones, flavanones, flavanols, isoflavones, chaconnes and many members are proved to have high degree of antioxidant activity (Rajani, 2004). The high content of polyphenols showed the antioxidant characteristics in plant derived materials (Andrea *et al*., 2003).

2.2.1 Types of Antioxidants

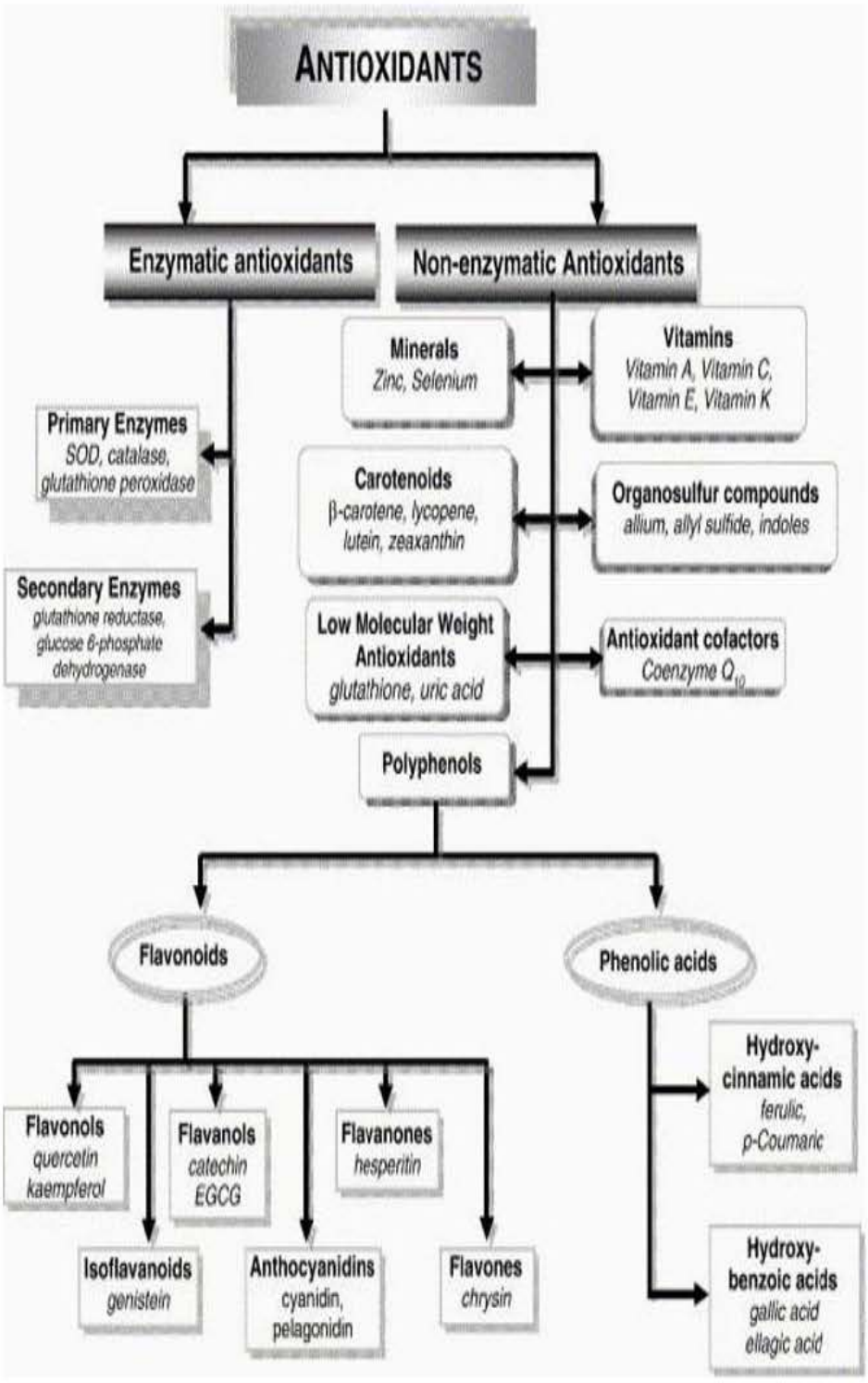

Figure 2.2: Types of antioxidants (Adapted from: Shalaby and Shanab, 2013).

Antioxidants can give direct scavenging ROS or inhibit cell proliferation secondary through protein phosphorylation to decrease oxidative stress induced carcinogenesis. Immuno-enhancement of β-carotene can act as protector against cancer through its antioxidant function. It may also have anti-carcinogenic effect that can alter the liver metabolism effects of carcinogens (Lobo *et al.,* 2010). β-carotene can be found in foods which is orange in color such as carrots, squash, apricots, pumpkins and mangoes. They also rich in some green and leafy vegetables such as spinach, kale and collard greens (Borek, 1991).

According to Sokol (1988), vitamin C or known as ascorbic acid is one of the antioxidants that may be helpful in preventing cancer. The mechanisms of vitamin C on antioxidants can affect carcinogenesis such as blocking formation of nitrosamines, enhancement of the immune response and acceleration of detoxification of liver enzymes. According to Antioxidants and Cancer Prevention (2007), vitamin C can be found in many fruits and vegetables as well as cereals, beef, poultry and fish (Hamid *et al.,* 2010).

Vitamin E which is also known as alpha-tocopherol usually abundance in almonds, in many oils including wheat germ, safflower, corn and soybean oils. Vitamin E also can be found in mangoes, nuts and broccoli (Herrera and Barbas, 2001). Vitamin E play a role as immune-competence by increasing humoral antibody protection, resistance to bacterial infections, cell-mediated immunity, blocking micro cell line formation and also useful in cancer prevention as well as inhibit carcinogenesis by the stimulation of the immune system (Sokol, 1988).

Selenium is a mineral and known as a component of antioxidant enzymes. The major dietary sources in most countries are from plant foods like rice and wheat. The amount of selenium in soil varies in each region so that the amount of selenium in the foods grown in soil are also different. Selenium has been proven to play a role in prevention of cancer. People makes selenium as supplements because it can repair nutritional deficiencies to one of pharmacological intervention especially in cancer chemoprevention and also can control of heart failure (Hamid *et al*., 2010).

Therefore, proper intake of antioxidants will help people to quench the naturally free radicals in the body, thus improving their health by lowering the risk of various deadly diseases such as cancer (Hamid *et al.,* 2010).

2.3 Edible Bird's Nest (EBN)

Edible bird nest (EBN) also known as *Yan Wo* in Chinese, Sarang Wallet in Indonesian and Enso in Japanese. It is most highly valued products of South East Asia including Malaysia (Ibrahim *et al.,* 2009). EBN refers to the nest made by the secretion of saliva of two specific swiftlets, namely *Aerodramus fuciphagus* and *Aerodramus maximus* (Goh *et al*., 2001). The swiftlets have more than 24 species, but only several species produced nests that are deemed edible (Babji *et al.,* 2015). According to Zainab and co-workers (2013), the nests are made by a few different swiftlet species in the genus of *Aerodramus* and *Collocalia* and they are mainly built by male swiftlets. Swiftlet is a bird with the plain feathers and usually in black and grey color. Figure 2.3 shows the swiftlet in the cave meanwhile Figure 2.4 shows the swiftlet in the bird house.

Several species of genus *Aerodramus* swiftlets build edible nests that can be eaten by humans, as a medicinal food. Many people who consumed the nests can be symbolized as wealth, power and prestige by the previous dynasty. The edible bird nests were also being used as Chinese traditional medicine since the Tang (618-907 AD) and Sung (960-1279 AD) dynasties (Koon and Cranbrook, 2002). The famous and well-known of EBN come from two exploited species which are the white nest swiftlet (*A.fuciphagus*) and the black nest swiftlet (*A.maximus*). The habitats are ranged from the Nicobar Islands in the Indian Ocean to the sea caves in the coastal regions of Thailand and the Palawan Islands in the Philippines (Koon and Cranbrook, 2002; Koon, 2000). However, the white nest usually has the highest price in the market compared to the black nest because of the consumer's perceptions that white nest has higher nutritional and beneficial values. The color of bird nest from *A.fuciphagus* species are from white or ivory for house nests as

shown in Figure 2.5, while for cave nests are the blackish-brown color as shown in Figure 2.6. The color of the bird nest influences the quality and also the acceptability to the consumer towards the nests (Lawless and Heymann, 1999). The male swiftlets usually built their nest within 35 days. The material used to build the nests is glutinous material found in saliva secreted from the swiftlet's two sublingual salivary glands (Goh *et al.*, 2001). EBN that had been harvested is observed to be in a half bowl shape. The major ingredient of the edible bird nest is salivary nest cement and it is the world's most expensive food ingredients (Koon and Cranbrook, 2002).

However, further investigations are needed about the exact compositional and nutritional properties of EBN for collection data base in future studies. When consumers are lacking information about the properties in EBN, the sellers can take advantage on the price of contaminated EBN. As the previous study reported that the number of recorded and conjectured cases of white bird nest contamination with cheaper materials has risen drastically over the past few years (Goh *et al.*, 2001; Law and Melville, 1994).

Figure 2.3: Swiftlet in the cave
(Retrieved from: http://www.google.com)

Figure 2.4: Swiftlet in the bird house
(Retrieved from: http://www.google.com)

Figure 2.5: White nest
(Retrieved from: http://www.google.com)

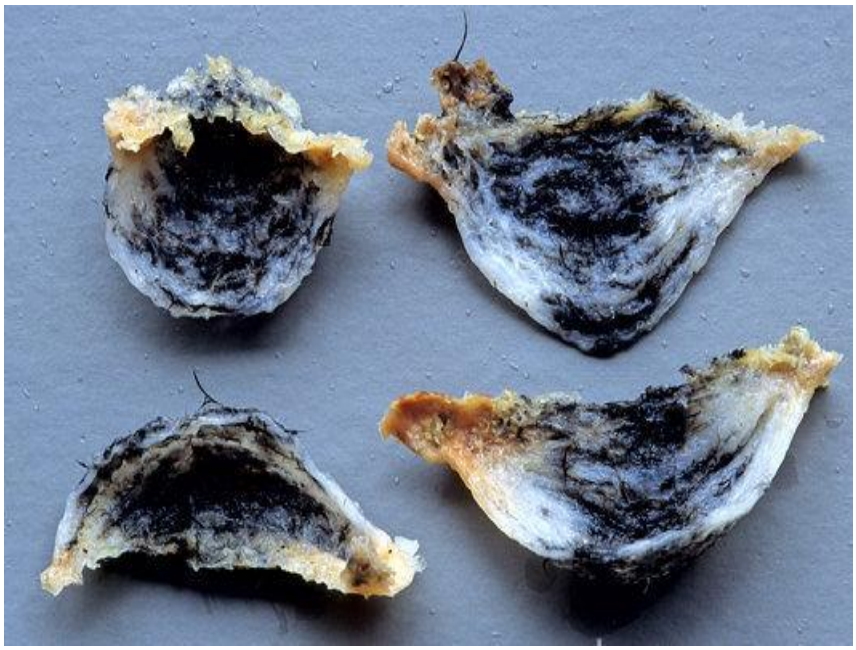

Figure 2.6: Black nest
(Retrieved from: http://www.google.com)

Nowadays a lot of swiftlet house has been devised with a simple method for the conservation of swiftlets in houses where existing structures of the non-commercial swiftlet nest are developed and renovated or new structures are built so as to attract the swiftlet. By using sound system that recorded the bird-call from the original sound in the cave, the swiftlets were attracted to go inside the house. It is proven that swiftlet's voice is very effective to attract the other swiftlet to be nested in the bird house (Siti Nurzalikha *et al.,* 2013). The suitable conditions of the swiftlet house are important to urge the swiftlet to nest. Four main factors to be concerned in providing suitable condition for swiftlet house are air temperature, relative humidity, air velocity and light intensity (Ibrahim *et al.,* 2009). The suitable range of temperature for swiftlet house is between 25 °C to 30 °C (Kuan and Lee, 2005). The productivity of swiftlet nest would be reduced at low air temperature. According to Nasir Salekat (2009), the built of the house must avoid direct sunlight exposure which will cause the increment of internal temperature and the light intensity. According to Anuar and co-workers (2013), relative humidity must be high to maximize the productivity of swiftlet's nest. The level of dampness of swiftlet house is between 80 to 95% of relative humidity. Air ventilation is vital in swiftlet house in order to provide air movement in the building. These factors or conditions are important to attract more swiftlets to be nested in the bird house and also get the standard and quality of EBN.

2.3.1 Nutritional Content of EBN

The white edible bird nest built by *A.fuciphagus* was usually sold at higher price than the black edible bird nest from *A.maximus*. The perceptions of past consumers', the white EBN have the higher nutritional values than the black EBN. According to Marcone (2005), EBN composed of lipid (0.14-1.28%), ash (2.1%), carbohydrate (25.62-27.76%) and protein (62-63%). The major nutrient components of EBN are glycoproteins (Kathan and Weeks, 1969), amino acids, carbohydrate, calcium, sodium and potassium (Norhayati *et al.,* 2010) and also sialic acid-containing sugar chains (Kakehi *et al.,* 1994). According to Colombo and co-workers (2003), the sialic acid may be beneficial to the neurological and intellectual advantages in infants. Moreover, sialic acid can act as an excellent immune system moderator such as defend the flow resistance of mucus which in turn repels bacteria, viruses and other harmful microbes. It also reduces the low-density lipoprotein (LDL), preventing influenza A and B strains, increasing fertility and controlling blood coagulation (Aswir and Nazaimoon *et al.,* 2011).

There are a few glyconutrients found in the form of glycoproteins include 7.2% N-acetylgalactosamine (galNAc), 5.3% N-acetylglucosamine (glcNAc), 16.9% galactose and 0.7% fucose (Dhawan and Kuhad, 2002). The function of galNac normally involved in synapses, the nerve's cell junction and also deficiency which can cause severe problems (Argueso *et al*., 2003). Whereas, glcNac is an amino acid and act as precursor for glycosaminoglycans, which is a major component of joint cartilage. Extra supplement of glucosamine may help to avoid cartilage degeneration and alleviate symptoms associated with arthritis (Pasztoi *et al*., 2009). Galactose and fucose can give impact on brain